Neuropsychology
2007, Vol. 21, No. 1, 1–8

0894-4105/07/$12.00 DOI: 10.1037/0894-4105.21.1.1

APOE Genotype and Cognitive Functioning in a Large Age-Stratified Population Sample

Anthony F. Jorm
University of Melbourne and Australian National University

Karen A. Mather, Peter Butterworth, Kaarin J. Anstey, Helen Christensen, and Simon Easteal
Australian National University

There is evidence that the cognitive effects of Alzheimer's disease can be seen decades before disease diagnosis. If this is the case, then the *apolipoprotein E* (*APOE*)**E4* allele might be expected to have effects on cognitive functioning earlier in the life span. To assess such effects, the authors examined data on the **E4* allele and cognitive functioning from a population sample of 6,560 Caucasians covering the age groups of 20–24, 40–44, and 60–64 years. Participants were assessed on tests of episodic memory, working memory, mental speed, reaction time, and reading vocabulary. Although performance on all tests except reading vocabulary declined across age groups, there was no effect of the *APOE *E4* allele at any age. These results indicate that *APOE *E4* does not have preclinical effects early in the life span on these cognitive functions. Cognitive aging effects between the ages of 20 and 64 years must not be due to preclinical Alzheimer's disease.

Keywords: APOE, memory, vocabulary, reaction time, aging

There is evidence from several prospective studies that Alzheimer's disease (AD) has a very long preclinical phase, with cognitive differences evident decades before the disease can be diagnosed. In the first study of this type, La Rue and Jarvik (1987) followed a sample of aging twins. Those who were diagnosed with dementia at a mean age of 85 years were found to have poorer performance 20 years earlier on a range of tests covering verbal and nonverbal abilities. Although specific dementia-related diseases were not diagnosed, it is likely that the majority of participants with dementia had AD. Similar findings emerged from a 22-year follow-up of the Framingham cohort at 65–94 years of age (Elias et al., 2000). Those who developed AD were found to have poorer performance at baseline on tests of verbal episodic memory and abstract reasoning.

Although these studies show preclinical effects in middle age, there is also evidence that cognitive differences exist even earlier in adulthood. Snowdon et al. (1996) examined whether language ability in early adulthood predicted AD in late life. They studied a group of 93 nuns who were 75–95 years of age, 14 of whom had neuropathologically confirmed AD. Language ability was assessed retrospectively by analyzing autobiographies the nuns had written at a mean age of 22 years. Those who later developed AD were found to have a lower density of ideas in their autobiographies written almost 60 years previously. Consistent with the evidence on early cognitive effects of AD, there is neuropathological evidence that amyloid deposits and neurofibrillary changes occur in middle-aged adults (Braak & Braak, 1997). However, preclinical AD may not be the only factor in these early cognitive differences. Whalley et al. (2000) have reported that persons diagnosed with late-onset dementia had lower mean scores on a test of general cognitive ability administered at 11 years of age. Such childhood differences are unlikely to represent preclinical AD. It is more probable that higher cognitive ability protects against dementia either through associated lifestyle factors or because a greater initial cognitive reserve allows for compensation against disease-related losses.

Taking all the evidence together, one can conclude that people who develop AD show cognitive differences many decades before the onset of the disease. However, the cause of these differences is not established. There are possible roles for preclinical disease, protective effects of cognitive reserve, and lifestyle factors associated with cognitive ability that affect risk for AD.

Given that these early differences exist, which cognitive functions are most affected? Bäckman, Jones, Berger, Laukka, and Small (2005) carried out a meta-analysis of studies that assessed cognitive functioning in a sample without dementia and then followed the sample to see who developed AD and who did not. Looking at various kinds of cognitive tests, they found the largest effect sizes (>1 standard deviation unit) for tests of global cognitive functioning, episodic memory, speed, and executive functioning. Tests of verbal ability, attention, and spatial ability were also found to discriminate. The only domain that did not discriminate

Anthony F. Jorm, ORYGEN Research Centre, University of Melbourne, Victoria, Australia, and Centre for Mental Health Research, Australian National University, Canberra, Australia; Karen A. Mather, Peter Butterworth, Kaarin J. Anstey, and Helen Christensen, Centre for Mental Health Research, Australian National University; Simon Easteal, Centre for Bioinformation Science and John Curtin School of Medical Research, Australian National University.

Funding was provided by National Health and Medical Research Council Program Grant 179805. We thank Susan Tan and Karen Nunweek for technical assistance and Trish Jacomb, Karen Maxwell, Bryan Rodgers, and the team of Centre for Mental Health Research interviewers for their assistance with this study.

Correspondence concerning this article should be addressed to Anthony F. Jorm, ORYGEN Research Centre, University of Melbourne, Locked Bag 10, Parkville, Victoria, Australia 3052. E-mail: ajorm@unimelb.edu.au

was short-term memory, which included digit span. The pattern of results was similar for studies with longer as opposed to shorter follow-ups.

If there are very early preclinical effects of AD, then it would be expected that risk factors for AD would be related to cognitive functioning earlier in the life span. One of the most consistently replicated risk factors for AD is the *apolipoprotein E* (*APOE*) genotype. The **E4* allele is known to increase risk for AD and the **E2* allele to decrease risk (Farrer et al., 1997; Rubinzstein & Easton, 1999). Furthermore, the **E4* allele increases risk for ischemic cerebrovascular disease (McCarron, Delong, & Alberts, 1999), which may also contribute to age-related cognitive deficits. The **E4* allele has also been reported to be more frequent in cases of mild cognitive impairment, which fall short of satisfying diagnostic criteria for dementia (Collie & Maruff, 2002). It might therefore be expected that the *APOE* genotype would be related to cognitive functioning in persons without dementia and that this association would become more apparent with age as the risk of preclinical AD increases.

A number of previous studies have looked for associations of the *APOE* genotype with cognitive functioning in samples without dementia. Anstey and Christensen (2000) have reviewed the evidence from longitudinal studies of cognitive change in older individuals. They found fairly consistent evidence that the **E4* allele predicts decline in memory and processing speed but not in crystallized or fluid abilities. However, the results are complicated by the fact that some people who were included in these studies later developed dementia, whereas other studies excluded them.

There are fewer studies looking at the effect of the *APOE* genotype on cognitive functioning in young and middle-aged adults. The **E4* allele does not appear to be related to lower scores on intelligence tests in either children (Deary et al., 2002) or adults who are in their 20s (Yu, Lin, Chen, Hong, & Tsai, 2000). Given that episodic memory is affected early in AD, it might be expected that *APOE* genotype would affect memory tasks earlier in life. However, the evidence from middle-aged samples is largely negative. Flory, Manuck, Ferrell, Ryan, and Muldoon (2000) found that, in a healthy community sample of adults with a mean age of 46 years, the **E4* allele was associated with poorer episodic memory performance. However, negative results on episodic memory tasks have been reported in several other studies (Caselli et al., 1999; R. M. Cohen, Small, Lalonde, Friz, & Sunderland, 2001; Greenwood, Sunderland, Friz, & Parasuraman, 2000; Nilsson, Nyberg, & Bäckman, 2002; Rosen, Bergeson, Putnam, Harwell, & Sunderland, 2002).

More promising results have emerged from studies of divided attention. Rosen et al. (2002) found that middle-aged individuals carrying the **E4* allele performed worse on a working memory task requiring divided attention. Similarly, Greenwood et al. (2000) found middle-aged carriers to have deficits in components of visuospatial attention. In both of these studies, **E4* carriers did not exhibit episodic memory deficits. A possible reason for the negative results in many of the studies of episodic memory in middle age is small sample size, particularly with **E4* homozygotes. Most of these studies involved 50 or fewer **E4* carriers and would only be able to detect medium-to-large effect sizes (J. Cohen, 1992).

Two factors thought to interact with the *APOE* genotype and cognitive performance are head trauma and alcohol consumption. Studies of patients after traumatic brain injury have shown that *APOE *E4* is associated with poorer performance on memory measures (Crawford et al., 2002), poorer general neuropsychological test performance 3 weeks after traumatic brain injury (Liberman, Stewart, Wesnes, & Troncoso, 2002), longer periods of coma (Friedman et al., 1999), and late traumatic seizures (Diaz-Arrastia et al., 2003). However, all these studies involved small clinical samples, and it is still unclear whether an interaction between *APOE* and head trauma is evident at the population level. Several studies indicate that there may be an interaction of alcohol consumption with APOE in relation to cognition and dementia, but the results are mixed. A recent case-control study of 373 patients with dementia and 373 controls found that heavy drinking was associated with increased risk of dementia associated with *APOE *E4* (Mukamal et al., 2003). By contrast, another case-control study of 758 outpatients and 557 controls found that the association between alcohol consumption and AD was not affected by *APOE* genotype (Tanaka, Asada, Kinoshita, Yamashita, & Uno, 2002). However, a recent prospective study of 589 war veterans found that *APOE *E4* increased both the protective effect of light drinking and the harmful effect of heavy drinking on cognitive performance (Carmelli, Swan, Reed, Schellenberg, & Christian, 1999). Another prospective study of older people found that drinking was associated with an increased risk of cognitive decline in **E4* carriers but with a decreased risk in noncarriers (Dufouil et al., 2000). There are no data available on the interactive effects of *APOE *E4* and alcohol consumption on cognitive performance in population-based samples of young or middle-aged adults.

In the present article, we report data from a very large community sample grouped according to the following ages: 20–24, 40–44, and 60–64 years of age. This sample is large enough to detect small effect sizes and involves considerable numbers of homozygotes. The study included tests of episodic memory and speed of the sort that Bäckman et al. (2005) found were affected in preclinical AD. Given that AD neuropathology increases with age, we predicted an Age × Genotype interaction, with cognitive deficits becoming more apparent in **E4* carriers as age increased. The study also looked for interaction effects involving history of head trauma and of alcohol abuse.

Method

Participants

The sample came from the PATH Through Life Project (Jorm, Anstey, Christensen, & Rodgers, 2004), a large community survey concerned with the health and well-being of people who are 20–24, 40–44, and 60–64 years of age and who live in the city of Canberra, Australia, or in the neighboring town of Queanbeyan. Each cohort is to be followed up every 4 years over a total period of 20 years. Results presented here concern the first-wave interviews with 20- to 24-year-olds (conducted in 1999–2000), 40- to 44-year-olds (conducted in 2000–2001), and 60- to 64-year-olds (conducted in 2001–2002). Participants had to be in their respective age group on the January 1 of either 1999 (for 20- to 24-year-olds), 2000 (for 40- to 44-year-olds), or 2001 (for 60- to 64-year-olds). The sampling frames were the Electoral Rolls for Canberra and Queanbeyan. Registration on the electoral roll is compulsory for Australian citizens. Because the Australian Electoral Commission would only release decade age ranges for research purposes,

we wrote to 12,414 persons recorded as being 20–29 years of age on the electoral roll and asked for participation from those who were 20–24 years of age.

Of these individuals, 5,058 were found to be out of the required age range, 1,061 were known to have moved out of the area, 2,190 could not be found, 1,701 refused, and 2,404 were interviewed. The participation rate of those who were located and were in the required age range was 58.6%. Similarly, for the 40- to 44-year-olds, 9,033 persons were sent letters, 4,222 were out of the required age range, 280 had moved, 612 could not be found, 1,389 refused, and 2,530 were interviewed (64.6% of those found and in age range). For the 60- to 64-year-olds, there was a change to the law allowing the Australian Electoral Commission to release more specific age group information. Letters were sent to 4,832 persons, 34 were out of the required age range, 182 had moved, 28 were dead, 209 could not be found, 1,827 refused or their English was too poor to allow an interview, and 2,551 were interviewed (58.3% of those found and in age range). The gender breakdown of the sample was 1,163 men and 1,241 women at ages 20–24, 1,193 men and 1,337 women at ages 40–44, and 1,319 men and 1,232 women at ages 60–64.

Survey Procedure

Persons selected at random from the electoral roll were sent a letter informing them of the survey and saying that an interviewer would contact them soon to see if they wanted to participate. If a person agreed to participate, the interviewer arranged to meet them at some convenient location, usually the participant's home or the Centre for Mental Health Research at the Australian National University. Most of the interview was self-completed on a Hewlett-Packard 620LX palmtop personal computer using the Surveycraft software for computer-assisted personal interviewing (SPSS, 2006). However, testing by the interviewer was required for the physical tests, for some of the cognitive tests, and for a cheek swab from which DNA could be extracted. The components of the interview relevant to the present article are described in the following sections.

Ethics Approval

Ethics approval was obtained from the Australian National University's Human Research Ethics Committee.

Cognitive Tests

Reading vocabulary was assessed with the Spot-the-Word Test Version A, which asks participants to choose the real words from 60 pairs of words and nonsense words (Baddeley, Emslie, & Nimmo-Smith, 1992). Working memory was assessed with the Digits Backwards subtest of the Wechsler Memory Scale (Wechsler, 1945), which presents participants with series of digits at the rate of one per second and asks them to repeat the digits backwards. Mental speed was measured with the Symbol–Digit Modalities Test, which asks the participant to substitute as many digits for symbols as possible in 90 s (A. Smith, 1982). Immediate and delayed recall were assessed with the first trial of the California Verbal Learning Test (Delis, Kramer, Kaplan, & Ober, 1987), which involves recalling a list of 16 nouns. The interval between immediate and delayed recall was occupied by a test of grip strength. To measure reaction time (RT), we had participants hold a small box with both hands; they could use the index fingers to depress the left and right buttons on the top of the box. The front of the box had three lights: one red stimulus light under both the left and right buttons and a green "get ready" light in the middle beneath. There were four blocks of 20 trials measuring simple RT, followed by two blocks of 20 trials measuring choice RT. Means for simple and choice RT were calculated after removing outliers, as described by Jorm et al. (2004).

Data on long-term stability of most of these tests were available from a 4-year follow-up of 1,497 of the 20- to 24-year-old participants. These follow-up data came from the second wave of the PATH Through Life Project, which is currently incomplete. The correlations across 4 years were as follows: .75 for Spot-the-Word, .62 for Digits Backwards, .74 for Symbol–Digit Modalities, .50 for immediate recall, and .55 for delayed recall. Data are presently not available for the stability of RT. However, the correlation between simple and choice RT at the initial interview was .67 for the whole sample.

Individuals in the 60- to 64-year-old cohort were also given the Mini-Mental State Examination (Folstein, Folstein, & McHugh, 1975) and the Purdue Pegboard (both hands; Tiffin & Asher, 1948). Participants in this cohort were suspected of possible dementia if they fulfilled one or more of the following criteria: (a) scored 25 or below on the Mini-Mental State Examination, (b) were below the 5th percentile on the immediate or delayed recall of the California Verbal Learning Test, or (c) performed below the 5th percentile on two or more of the following tests: RT, Purdue Pegboard, or Symbol–Digit Modalities.

Assessment of Head Trauma and Alcohol Consumption

Experience of head trauma was determined by a question asking respondents whether they had ever had a serious head injury that had caused them to become unconscious for more than 15 min. The analyses focused on those who could respond with certainty about such previous experience. Around 4.0% of respondents were uncertain about prior experience of a serious head injury (likely due to lack of clarity about their duration of unconsciousness) and were excluded from analysis involving the head injury measure. A further 28 respondents had missing data to this item (0.4%) and were therefore excluded from these head injury analyses.

The survey included three items measuring current alcohol consumption from the Alcohol Use Disorders Identification Test (Saunders, Aasland, Babor, de la Fuente, & Grant, 1993). Respondents with hazardous or harmful levels of drinking (over 28 standard drinks per week for men and over 14 standard drinks per week for women, with upward adjustment of estimated drinking level to take binge drinking into account; National Health and Medical Research Council, 2001) were identified. There were 37 respondents (0.5%) with missing data (or implausible combinations of responses) who were excluded from the analyses. Questions included in the survey that assessed previous highest sustained drinking levels enabled a similar classification of individuals with previous hazardous or harmful levels of alcohol consumption (this classification did not take account of previous binge drinking as this aspect of previous consumption was not assessed). There were 59 respondents with missing data that pre-

vented the calculation of previous drinking levels and who were therefore excluded from analysis (0.8%).

Genotyping

Genomic DNA was extracted from buccal swabs using QIAGEN DNA Blood kits (#51162; QIAGEN, Hilden, Germany). To determine the *APOE* genotype (*APOE *E2, APOE *E3, APOE *E4* alleles), we genotyped two single-nucleotide polymorphisms (SNPs; NCBI SNPs *rs429358* and *rs7412*) using TaqMan assays (Applied Biosystems [ABI], Foster City, CA).

DNA (1μl) was added to each well of a 384-well clear optical reaction plates (ABI #4309849) using a liquid handling robot and was dried down at 60° C for 30 min. These plates were then stored at −20° C until required.

Two separate TaqMan assays were performed: one for SNP *rs429358* and the other for SNP *rs7412*. Each *APOE* TaqMan assay contained 2.0 μl of TaqMan 2× universal polymerase chain reaction (PCR) master mix (ABI #4304437), 0.0625 μl of the appropriate 80× assay mix containing the SNP-specific primers and probes (TaqMan genotyping assays), and H_2O to a total volume of 5 μl. A liquid-handling robot dispensed this mix into each well containing the dried-down DNA. Plates were then sealed with optical adhesive covers (ABI #4311971), spun briefly (4,000 rpm for 2 min), and placed into an 7900HT real-time PCR machine (ABI). The cycling program was as follows: 95° C for 10 min, followed by 40 cycles of 95° C for 15 s and 60° C for 1 min. Allelic discrimination was automated using the manufacturer's software (Applied Biosystems, 2004).

Positive controls, consisting of DNA of each of the six possible *APOE* genotypes (**E2/*E2, *E2/*E3, *E2/*E4, *E3/*E3, *E3/*E4, *E4/*E4*), were included on each genotyping plate. These six controls were genotyped using an alternative genotyping method. In this method, a fragment of the *APOE* gene was amplified using PCR and then digested with the restriction endonuclease *Cfo1* (Hixson & Vernier, 1990). The resulting digested products were resolved on an agarose gel, and the *APOE* genotypes were deduced from the observed combinations of different-sized fragments. Genotype scorers were blinded to the identity of the samples.

Analyses

There was one extreme outlier in the latency data from the two RT tasks whose data were excluded from the analysis. Because there are ethnic differences in the frequency of *APOE* genotypes, only data from participants who described themselves as "Caucasian/White" were used ($N = 6{,}560$). Associations of *APOE* genotype and age group with cognitive performance were assessed with a series of two-factor analyses of variance. These analyses were repeated when those who were suspected of possible dementia ($n = 165$) were excluded.

The possibility of associations between the environmental factors examined in this study (experience of head injury, current hazardous or harmful alcohol consumption, previous hazardous or harmful alcohol consumption) and the *APOE* genotype on the various measures of cognitive performance was assessed by a series of four-factor analyses of variance. The factors were experience of the environmental hazard, age group, **E4* carrier status, and gender (included in the analysis given significant gender differences in the prevalence of these environmental factors). Although the sample was very large, some of the cells created by the combination of these factors were very small or empty, particularly those representing the combination of homozygotic **E4* and positive exposure to the relatively uncommon environmental hazards. As such, the **E4* carrier status variable examined in these analyses contrasted **E4* carriers with noncarriers, combining homozygotic and heterozygotic **E4* carriers. An alpha of .05 was used for all analyses, but exact probability values are reported.

Results

APOE genotyping results were available for 99.9% of the provided DNA samples. The validity of the genotyping is indicated by the concordance of the allele frequency distribution with Hardy–Weinberg equilibrium for each of the two SNPs: *rs429358*, $\chi^2(1, N = 6{,}560) = 0.23$, $p > .25$, and *rs7412*, $\chi^2(1, N = 6{,}560) = 1.09$, $p > .25$. In addition, the frequency of the *APOE* alleles for Caucasians are consistent with previously published studies of Caucasians (e.g., Farrer et al., 1997; Henderson et al., 1995; Martins et al., 1995). Furthermore, the six positive controls were consistently called correctly across all of the genotyped sample. The observed genotype frequencies are shown in Table 1. There were no significant genotype differences across the three age groups, $\chi^2(4, N = 6{,}560) = 3.29$, $p = .51$.

The means and standard deviations on the cognitive tests according to genotype and age group are shown in Table 2. Participant numbers vary slightly because of missing data. Analyses of variance with the factors of age group and genotype showed significant effects of age group on all tests, with the 60- to 64-year-olds showing the worst performance on all tests

Table 1
APOE Genotype Sample Sizes and Frequencies Within Age Groups for Caucasians

		**E4⁺/*E4⁺* genotype		**E4⁺/*E4⁻* genotype		**E4⁻/*E4⁻* genotype	
Age group	*n*	*n*	%	*n*	%	*n*	%
20–24	2,097	56	2.7	517	24.6	1,524	72.7
40–44	2,182	45	2.1	566	25.9	1,571	72.0
60–64	2,281	48	2.1	564	24.7	1,669	73.2
Total	6,560	149	2.3	1,647	25.1	4,764	72.6

Note. *APOE* = *apolipoprotein E.*

Table 2
*Performance on Cognitive Tests According to Age Group and APOE*E4 Genotype*

n	Age	$*E4^+/*E4^+$ genotype *M* (*SD*)	$*E4^+/*E4^-$ genotype *M* (*SD*)	$*E4^-/*E4^-$ genotype *M* (*SD*)	*p* value for genotype	*p* value for Genotype × Age interaction
			Symbol–Digit Modalities Test			
2,068	20–24	64.60 (10.21)	64.19 (10.37)	63.82 (10.05)	.58	.87
2,176	40–44	59.47 (9.46)	60.38 (9.01)	60.36 (9.28)		
2,269	60–64	50.69 (10.53)	50.46 (9.05)	50.00 (9.59)		
			Immediate Recall Test[a]			
2,096	20–24	8.32 (2.08)	7.93 (2.14)	8.08 (2.17)	.87	.54
2,175	40–44	7.91 (2.58)	7.96 (2.09)	7.89 (2.23)		
2,281	60–64	7.17 (2.33)	7.23 (2.26)	7.19 (2.23)		
			Delayed Recall Test[a]			
2,096	20–24	7.71 (2.64)	7.18 (2.39)	7.34 (2.36)	.91	.17
2,175	40–44	6.47 (2.79)	7.13 (2.46)	7.07 (2.46)		
2,281	60–64	6.21 (2.63)	6.29 (2.52)	6.23 (2.46)		
			Digits Backwards Test[b]			
2,096	20–24	5.68 (1.86)	5.35 (2.27)	5.36 (2.31)	.72	.40
2,175	40–44	4.91 (2.29)	5.40 (2.29)	5.22 (2.32)		
2,277	60–64	4.98 (2.14)	4.92 (2.21)	4.94 (2.25)		
			Spot-the-Word Test			
2,096	20–24	48.13 (4.68)	47.99 (5.15)	47.67 (5.21)	.38	.21
2,165	40–44	50.20 (5.44)	50.91 (5.37)	50.98 (5.27)		
2,246	60–64	53.81 (3.93)	52.27 (5.54)	52.06 (5.73)		
			Simple RT (ms)			
2,040	20–24	216 (26)	218 (30)	219 (31)	.45	.78
2,087	40–44	236 (33)	229 (36)	232 (37)		
2,240	60–64	248 (39)	254 (59)	255 (59)		
			Choice RT (ms)			
2,030	20–24	267 (28)	265 (34)	266 (33)	.84	.64
2,075	40–44	293 (36)	287 (36)	289 (35)		
2,231	60–64	319 (59)	320 (45)	318 (46)		

Note. *APOE* = *apolipoprotein E;* RT = reaction time.
[a] Measured with the first trial of the California Verbal Learning Test. [b] A subtest of the Wechsler Memory Scale.

except for Spot-the-Word. On Spot-the-Word, this age group had the best performance. No effects of the *APOE* genotype and no Age × Genotype interactions were observed. When the analyses were performed after elimination of any individuals suspected of possible dementia (n = 165 from the 60–64 age group), the results did not change.

The remaining analyses considered the interaction between environmental factors and the *APOE* genotype on the various measures of cognitive performance. Given the hypotheses tested here, we do not report on main or interaction effects that do not include **E4* carrier status. However, it is important to note that the main effect of head injury and hazardous or harmful alcohol consumption were significant for several of the cognitive measures, supporting the validity and robustness of the operationalization of these constructs.

The analyses involving the head trauma variable showed no main effect of **E4* carrier status for any of the cognitive outcome measures, confirming the simple analyses. Only 2 of the 77 two-way, three-way, and four-way interactions involving the **E4* carrier variable were significant. For the delayed recall measure, the interactions between **E4* carrier status and age, $F(2, 6267) = 3.30$, $MSE = 5.69$, $p = .037$, and **E4* carrier status, age, and head injury status, $F(2, 6267) = 3.48$, $MSE = 5.69$, $p = .031$, were significant. The pattern of results was contrary to expectations. The two-way interaction reflected that only in the youngest age group did **E4* carriers show poorer delayed recall than noncarriers. The three-way interaction was due to the fact that **E4* carriers who reported head injury showed poorest performance on the delayed recall task within the youngest group but not within the other two age groups (in fact, the group representing **E4* carriers who reported head injury demonstrated the highest mean scores in the 40–44 year age group).

For the analyses examining current alcohol consumption, only the interaction between currently drinking status (hazardous or

harmful vs. other) and *E4 carrier status was significant for the measures of vocabulary: Spot-the-Word, $F(1, 6474) = 5.70$, $MSE = 528.89$, $p = .017$; RT (simple), $F(1, 6335) = 4.62$, $MSE = 0.0019$, $p = .032$; and RT (choice), $F(1, 6304) = 3.98$, $MSE = 0.0015$, $p = .04$. For each of these outcome measures, *E4 carriers consuming alcohol at hazardous or harmful levels demonstrated poorer performance (fewer items correct or slower mean latencies) than *E4 carriers with lesser levels of current alcohol consumption. In contrast, the effect of alcohol consumption on the performance of noncarriers was in the opposite direction, with those consuming alcohol at hazardous or harmful levels showing superior performance to others. Neither the main effect of carrier status nor any higher order interaction involving carrier status was significant at the $p < .05$ level. Finally, the analysis of previous highest levels of alcohol consumption found no significant main effects or interactions involving *E4 carrier status for any of the cognitive outcome measures.

A power analysis was carried out with nQuery Advisor (Statistical Solutions, 2000). Using a two-tailed alpha of .05 and a small effect size of 0.2 standard deviation units (J. Cohen, 1992), we found that there was 99% power to detect a difference between *E4 allele carriers and noncarriers for the whole sample and 98% power for a single age cohort. When homozygotes were compared with noncarriers, there was 68% power for the whole sample and 28% power for a single age cohort. A power analysis was also carried out for the effects of *APOE* *E4 in subgroups that had a history of head trauma or of hazardous or harmful alcohol consumption. Because of the complexity of doing a power analysis for two- and three-way interaction effects, the power analysis was simplified by separately considering the subgroups exposed to these insults. Taking the head trauma group separately, there was 38% power to detect an effect of being an *E4 carrier. For the subgroup with a history of hazardous or harmful alcohol consumption, the power was 92%, whereas for those with current hazardous or harmful consumption, it was 44%.

Discussion

All but one of the cognitive tests examined in the present study revealed lower performance in the oldest age group, which shows that the tests are sensitive to aging effects. It would be expected that if preclinical AD were involved in these age group differences, then there should be an effect of *APOE* genotype. However, we failed to find any effect of *APOE* genotype or any Age × Genotype interaction. The most likely reason for these negative findings is that cognitive aging effects observed between 20 and 60 years of age are not due to preclinical AD. The mechanisms by which *APOE* alters risk for AD are not fully understood, but it has been proposed to affect amyloid metabolism, neurite extension, tau phosphorylation, neuronal survival, and cerebrovascular changes (J. D. Smith, 2002). Such processes may underlie AD but not normal cognitive aging. There may be other processes responsible for the age group differences found in the present study and reported in many previous cross-sectional and longitudinal studies. AD processes may occur later in the life span and add to normal cognitive aging to produce a dementia syndrome, and consequently, *APOE* effects would only be found in old age.

Although some significant interactions were found between *APOE* status and hazardous or harmful alcohol consumption or head trauma, the number of significant effects is around the level expected under the null hypothesis. Furthermore, the significant effects showed little consistency across cognitive tests.

Several possible explanations for these negative findings must be considered. One explanation might be a lack of sensitivity of the cognitive tests. However, we were able to show that the tests had age group differences and were affected by head trauma and hazardous or harmful alcohol consumption, so there is no general lack of sensitivity. It is also possible that the tests are sensitive but do not tap the cognitive functions that are affected by *APOE* *E4*. Although changes in episodic memory functioning are one of earliest effects of AD, noneffects of *APOE* have been reported from normal middle-aged samples. More promising results have been seen for divided attention tasks, which were not used in the present study (Greenwood et al., 2000; Rosen et al., 2002).

A final possibility is that the effect of *APOE* genotype was too small to be detected in the present study, even though the study is the largest of its type. A power analysis showed excellent power (≥98%) to detect small differences (0.2 standard deviation units) between *E4 carriers and noncarriers, even within a single age cohort. Power was also satisfactory (68%) for detecting small differences between *E4 homozygotes and noncarriers in the whole sample, but it was low for detecting such differences in a single age group. Power was also excellent for detecting effects of the *E4 allele in the subgroup with a history of hazardous or harmful alcohol use (92%), but it was weaker for detecting an effect in those with a history of head trauma (38%). However, because the number of *E4 homozygotes was too small in these subgroups, we cannot dismiss the possibility that there is a small effect of one of these brain insults on homozygotes. For the planning of future studies on this issue, detecting a difference of 0.2 standard deviation units between *E4 homozygotes and noncarriers would require a total sample of approximately 8,000 persons (assuming the same allele frequency as the present study, Type I error rate of .05, two-tailed test, and 80% power). Detecting this difference between *E4 carriers and noncarriers would require approximately 1,000 participants. These same numbers would be required for subgroups of the population, such as those with a history of head trauma or heavy alcohol consumption.

The study has a number of potential weaknesses that must be acknowledged. First, the data were cross-sectional and did not include older age groups in which AD risk is highest. However, because the PATH Through Life Project is designed as a 20-year longitudinal study, such data will eventually become available. Second, there may have been biases in the sample due to the refusal rate. Comparing the sociodemographic characteristics of the sample with census information indicates that the sample is better educated than the population from which it is drawn. It is therefore likely that the refusers tended to have lower cognitive functioning. For the *APOE* genotype, there is unlikely to be any bias because the *APOE* allele frequencies were very close to other samples from comparable populations and did not differ across age groups. For the variables of head trauma and alcohol consumption, we have no way of knowing whether there was any bias. Despite these potential weaknesses,

the study has considerable strengths given its large population sample and the inclusion of three age groups covering young, middle, and advanced adulthood.

References

Anstey, K. J., & Christensen, H. (2000). Education, activity, health, blood pressure and apolipoprotein E as predictors of cognitive change in old age: A review. *Gerontology, 46,* 163–177.

Applied Biosystems. (2004). Sequence detection system (Version 2.2) [Computer software]. Foster City, CA: Author.

Bäckman, L., Jones, S., Berger, A. K., Laukka, E. J., & Small, B. J. (2005). Cognitive impairment in preclinical Alzheimer's disease: A meta-analysis. *Neuropsychology, 19,* 520–531.

Baddeley, A., Emslie, H., & Nimmo-Smith, I. (1992). *The Spot-the-Word Test.* Bury St. Edmunds, United Kingdom: Thames Valley Test Company.

Braak, H., & Braak, E. (1997). Frequency and stages of Alzheimer-related lesions in different age categories. *Neurobiology of Aging, 18,* 351–357.

Carmelli, D., Swan, G. E., Reed, T., Schellenberg, G. D., & Christian, J. C. (1999). The effect of apolipoprotein E epsilon4 in the relationships of smoking and drinking to cognitive function. *Neuroepidemiology, 18,* 125–133.

Caselli, R. J., Graff-Radford, N. R., Reiman, E. M., Weaver, A., Osborne, D., Lucas, J., et al. (1999). Preclinical memory decline in cognitively normal apolipoprotein ε4 homozygotes. *Neurology, 53,* 201–207.

Cohen, J. (1992). A power primer. *Psychological Bulletin, 112,* 155–159.

Cohen, R. M., Small, C., Lalonde, F., Friz, J., & Sunderland, T. (2001). Effect of apolipoprotein E genotype on hippocampal volume loss in aging healthy women. *Neurology, 57,* 2223–2228.

Collie, A., & Maruff, P. (2002). An analysis of systems of classifying mild cognitive impairment in older people. *Australian and New Zealand Journal of Psychiatry, 36,* 133–140.

Crawford, F. C., Vanderploeg, R. D., Freeman, M. J., Singh, S., Waisman, M., Michaels, L., et al. (2002). *APOE* genotype influences acquisition and recall following traumatic brain injury. *Neurology, 58,* 1115–1118.

Deary, I. J., Whiteman, M. C., Pattie, A., Starr, J. M., Hayward, C., Wright, A. F., et al. (2002, October 3). Cognitive change and the *APOE* ε4 allele. *Nature, 418,* 932.

Delis, D. C., Kramer, J. H., Kaplan, E., & Ober, B. A. (1987). *California Verbal Learning Test.* San Antonio, TX: Psychological Corporation/ Harcourt Brace Jovanovich.

Diaz-Arrastia, R., Gong, Y., Fair, S., Scott, K. D., Garcia, M. C., Carlile, M. C., et al. (2003). Increased risk of late posttraumatic seizures associated with inheritance of *APOE* epsilon4 allele. *Archives of Neurology, 60,* 818–822.

Dufouil, C., Tzourio, C., Brayne, C., Berr, C., Amouyel, P., & Alperovitch, A. (2000). Influence of apolipoprotein E genotype on the risk of cognitive deterioration in moderate drinkers and smokers. *Epidemiology, 11,* 280–284.

Elias, M. F., Beiser, A., Wolf, P. A., Au, R., White, R. F., & D'Agostino, R. B. (2000). The pre-clinical phase of Alzheimer's disease: A 22-year prospective study of the Framingham cohort. *Archives of Neurology, 57,* 808–813.

Farrer, L. A., Cupples, L. A., Haines, J. L., Hyman, B., Kukull, W. A., Mayeux, R., et al. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. *Journal of the American Medical Association, 278,* 1349–1356.

Flory, J. D., Manuck, S. B., Ferrell, R. E., Ryan, C. M., & Muldoon, M. F. (2000). Memory performance and the apolipoprotein E polymorphism in a community sample of middle-aged adults. *American Journal of Medical Genetics (Neuropsychiatric Genetics), 96,* 707–711.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). Mini-Mental State: A practical method for grading the cognitive status of patients for the clinician. *Journal of Psychiatric Research, 12,* 189–198.

Friedman, G., Froom, P., Sazbon, L., Grinblatt, I., Shochina, M., Tsenter, J., et al. (1999). Apolipoprotein E-epsilon4 genotype predicts poor performance in survivors of traumatic brain injury. *Neurology, 52,* 244–248.

Greenwood, P. M., Sunderland, T., Friz, J., & Parasuraman, R. (2000). Genetics and visual attention: Selective deficits in healthy adult carriers of the ε4 allele of the apolipoprotein E gene. *Proceedings of the National Academy of Sciences, USA, 97,* 11661–11666.

Henderson, A. S., Easteal, S., Jorm, A. F., Mackinnon, A. J., Korten, A. E., Christensen, H., et al. (1995). Apolipoprotein E allele ε4, dementia, and cognitive decline in a population sample. *Lancet, 346,* 1387–1390.

Hixson, J. E., & Vernier, D. T. (1990). Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hha1. *Journal of Lipid Research, 31,* 545–548.

Jorm, A. F., Anstey, K. J., Christensen, H., & Rodgers, B. (2004). Gender differences in cognitive abilities: The mediating role of health state and health habits. *Intelligence, 32,* 7–23.

La Rue, A., & Jarvik, L. F. (1987). Cognitive function and prediction of dementia in old age. *International Journal of Aging and Human Development, 25,* 75–89.

Liberman, J. N., Stewart, W. F., Wesnes, K., & Troncoso, J. (2002). Apolipoprotein E epsilon4 and short-term recovery from predominantly mild brain injury. *Neurology, 58,* 1038–1044.

Martins, R. N., Clarnette, R., Fisher, C., Broe, G. A., Brooks, W. S., Montgomery, P., & Gandy, S. E. (1995). ApoE genotypes in Australia: Roles in early and late onset Alzheimer's disease and Down's syndrome. *NeuroReport, 6,* 1513–1516.

McCarron, M. O., Delong, D., & Alberts, M. J. (1999). APOE genotype as a risk factor for ischemic cerebrovascular disease: A meta-analysis. *Neurology, 53,* 1308–1311.

Mukamal, K. J., Kuller, L. H., Fitzpatrick, A. L., Longstreth, W. T., Mittler, M. A., & Siscovick, D. S. (2003). Prospective study of alcohol consumption and risk of dementia in older adults. *Journal of the American Medical Association, 19,* 1405–1413.

National Health and Medical Research Council. (2001). *Australian alcohol guidelines: Health risks and benefits.* Canberra, Australia: Author.

Nilsson, L. G., Nyberg, L., & Bäckman, L. (2002). Genetic variation in memory functioning. *Neuroscience and Biobehavioral Reviews, 26,* 841–848.

Rosen, V. M., Bergeson, J. L., Putnam, K., Harwell, A., & Sunderland, T. (2002). Working memory and apolipoprotein E: What's the connection? *Neuropsychologia, 40,* 2226–2233.

Rubinzstein, D. C., & Easton, D. F. (1999). Apolipoprotein E genetic variation and Alzheimer's disease: A meta-analysis. *Dementia and Geriatric Cognitive Disorders, 10,* 199–209.

Saunders, J. B., Aasland, O. G., Babor, T. F., de la Fuente, J. R., & Grant, M. (1993). Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption II. *Addiction, 88,* 791–804.

Smith, A. (1982). *Symbol–Digit Modalities Test (SDMT) manual.* Los Angeles, CA: Western Psychological Services.

Smith, J. D. (2002). Apolipoproteins and aging: Emerging mechanisms. *Ageing Research Reviews, 1,* 345–365.

Snowdon, D. A., Kemper, S. J., Mortimer, J. A., Greiner, L. H., Wekstein, D. R., & Markesbery, W. R. (1996). Linguistic ability in early life and cognitive function and Alzheimer's disease in late life: Findings from the Nun study. *Journal of the American Medical Association, 275,* 528–532.

SPSS. (2006). Surveycraft [Computer software]. Retrieved May 4, 2006, from http://www.spss.com/surveycraft/

Statistical Solutions. (2000). *nQuery Advisor* (Version 4.0) [Computer software]. Los Angeles: Author.

Tanaka, N., Asada, T., Kinoshita, T., Yamashita, F., & Uno, M. (2002). Alcohol consumption and risk of dementia. *Lancet, 360,* 491.

Tiffin, J., & Asher, E. J. (1948). The Purdue Pegboard: Norms and studies of reliability and validity. *Journal of Applied Psychology, 32,* 234–247.

Wechsler, D. (1945). A standardized memory scale for clinical use. *Journal of Psychology, 19,* 87–95.

Whalley, L. J., Starr, J. M., Athawes, R., Hunter, D., Pattie, A., & Deary, I. J. (2000). Childhood mental ability and dementia. *Neurology, 55,* 1455–1459.

Yu, W. Y., Lin, C. H., Chen, S. P., Hong, C. J., & Tsai, S. J. (2000). Intelligence and event-related potentials for young female human volunteer apolipoprotein E e4 and non-e4 carriers. *Neuroscience Letters, 294,* 179–181.

Received October 14, 2003
Revision received May 17, 2006
Accepted May 30, 2006 ■