ORIGINAL CONTRIBUTION

Lower Cognitive Performance in Normal Older Adult Male Twins Carrying the Apolipoprotein E ε4 Allele

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Objective: Given the strong association of the apolipoprotein E (apoE) allele ε4 with late-onset Alzheimer dementia or multi-infarct dementia, we tested whether normal older adult men with at least one ε4 allele demonstrate subclinical changes in cognition and perform more poorly on tests of cognitive function compared with subjects without the ε4 allele.

Design: Matched-pair design of normal adult male (average age, 63 years) fraternal twins.

Setting: Subjects voluntarily participated on an outpatient basis at a research or medical center facility.

Participants: Members of the National Heart, Lung, and Blood Institute twin panel third examination previously genotyped for apoE.

Main Outcome Measure: Education-adjusted scores on several neuropsychological tests were compared in twins discordant for the apoE ε4 allele. Subjects with documented cerebrovascular disease were excluded.

Results: Among 20 fraternal twin pairs discordant for the presence of ε4, twins with the ε4 allele demonstrated poorer mean performance than their co-twins without the ε4 allele. This relationship was also noted cross-sectionally where age- and education-adjusted scores of 50 individual twin subjects with at least one ε4 allele demonstrated poorer performance compared with 138 individual twins without an ε4 allele.

Conclusions: The apoE ε4 allele may be associated with decreased cognitive function in discordant twin pairs. Our results suggest that ε4 may represent a potential marker for accelerated cognitive aging and such individuals may be at greater risk for development of late-onset Alzheimer dementia or multi-infarct dementia.

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FOLLOWING THE demonstration of an association of familial Alzheimer disease (FAD) with DNA markers on chromosome 19 including region 19q13.2 that contains the apolipoprotein E locus (apoE),[1-3] there is now ample evidence that individuals with at least one ε4 allele (coding for the E4 protein isoform) are found in significantly higher numbers of patients with late-onset FAD[4-8] and in late-onset sporadic AD.[5,8-12] In some families with early onset (<60 years) AD, particularly in those with a positive family history, ε4 may also be a risk factor for AD.[13-15] Allele ε4 frequency may also be increased in patients with multi-infarct dementia, and it is suggested that ε4 is a marker of both AD and arteriosclerotic dementia.[6,16]

Although memory loss and gradual decline in intellectual function are hallmarks of AD, other features include impairment in orientation, judgment and problem solving, language, and perception. Previously, we demonstrated significant heritability for a number of tests of neuropsychological function[17,18] and for decline in performance over time.[19] Some of the earliest subclinical changes associated with possible dementing illness may be reflected in poorer scores on neuropsychological tests.[20,21] Because of the strong association of ε4 with dementia, we hypothesized that older adults with ε4 should demonstrate poorer cognitive function than subjects without ε4 before any clinical onset of dementia. To test this hypothesis, we examined the association of ε4 with several measures of neuropsychologic function administered during the third examination of the National Heart, Lung, and Blood Institute (Bethesda, Md) twin panel.

See Subjects and Methods on next page

SUBJECTS AND METHODS

In 1986 through 1987, 622 subjects of the National Heart, Lung, and Blood Institute twin panel participated at the third examination.[22] At each of the five examination centers, informed consent statements were approved by institutional review committees. Twins were contacted by letter asking their participation in the third examination of this longitudinal study. Once agreeing to participate, all subjects read and signed informed consent statements before the third examination began. The mean age of the subjects at the third examination was 63 years (range, from 59 to a single pair aged 70 years). Of the 129 complete fraternal (dizygotic [DZ]) pairs who participated at the third examination, 100 were randomly selected for apoE genotyping from stored DNA.[23] The polymerase chain reaction (PCR) was used to amplify a 244–base pair fragment that contained variant amino acid residues 112 (cysteine → arginine = ε4 allele) and 158 (arginine → cysteine = ε2 allele). Polymerase chain reaction products were digested with the restriction enzyme HhaI and electrophoresed on an 8% polyacrylamide nondenaturing gel.[24] The most common ε3 allele is cut by HhaI at position 158; the ε4 allele is cut twice by the addition of a second restriction site at position 112; and the less frequent ε2 allele lacks either recognition site. Genotyping was completed on 197 of the 200 samples.

Several measures of cognitive and neuropsychological function were assessed, These were as follows:

1. The Iowa screening battery for mental decline[20,25] that includes: (a) the Benton visual retention test (BVRT)[26], Administration A, Form C that assesses short-term visual memory for designs, and (b) the controlled oral word association test[25] that requires oral production of words beginning with a given letter of the alphabet in a 1-minute period.

2. The Mini-Mental State Examination (MMSE) scored as prescribed by Folstein et al,[27] or in an alternate method using both serial subtraction and reverse spelling items. Maximum score on the former was 30, and on the latter it was 35. The MMSE provides brief questions of orientation, registration (verbal learning), attention and calculation, delayed recall, and language and visuoconstructive ability. The decision to administer the MMSE by including both subtraction and reverse spelling items was made shortly after the start of the third examination. Thus, some of the pairs seen early in the examination cycle were only scored using the Folstein protocol.

3. The digit symbol substitution test of the Wechsler Adult Intelligence Scale–Revised[28] as a measure of psychomotor speed that combines several cognitive and perceptual motor functions including attention, visual perception, and short-term memory.

Of the 197 subjects with apoE genotyping, 170 also took part in a 1990 through 1991 screening protocol for dementia using the Telephone Interview for Cognitive Status, modified for epidemiologic or survey work (TICS-m).[29] The TICS-m items included assessment of orientation, concentration, memory, naming comprehension, calculation, and reasoning. Any subject participating in the TICS-m screening protocol with follow-up testing leading to a rating of "demented"[30] was not included in the present study.

To further focus on AD, 10 individuals were omitted who had documented cerebrovascular accidents from medical records or in whom residual effects of a cerebrovascular accident were noted at the third examination. Following this exclusion, there were no significant differences in the proportion of subjects with at least one ε4 allele vs subjects lacking the ε4 allele for heart disease (11.6% vs 14.5%) or hypertension (30.2% vs 31.2%).

The neuropsychological data were analyzed using paired *t* tests in co-twins discordant for the presence of the ε4 allele. All test scores were adjusted for years of education via linear regression. Because of the matched pair design, there was no need to adjust for age or any other factor that may influence cognitive performance among unrelated individuals. Because of recent evidence suggesting that the ε2 allele is protective of AD,[31,32] the paired *t* test was repeated, excluding all pairs where one or both co-twins had an ε2 allele. We also analyzed the twins as individual subjects to compare mean neuropsychological scores between subjects with ε4 and without ε4 to see if the differences noted between discordant twin pairs appeared to be consistent across the total sample.

RESULTS

Allele frequencies calculated from genotypes for all 197 subjects were ε2, 0.09; ε3, 0.76; and ε4, 0.15, consistent with expected frequencies in white populations.[9,33,34] **Table 1** shows the results from the paired difference *t* tests in DZ twins discordant for the ε4 allele. All mean differences were negative, indicating poorer performance in the co-twin with ε4. Two of these differences, the Iowa battery and the BVRT scores, attained statistical significance ($P<.05$). To reinforce the consistent pattern of mean scores relative to ε4 status in the discordant twin pairs, three-way analysis of variance was performed. The three factors in the design were pairs as a random effect, ε4 status nested in pairs, and tests. All neuropsychological test scores were converted to standard scores, so all tests had a mean of zero and an SD of one. Because pairs needed to be complete for all tests, the MMSE using all items was omitted. In the remaining 18 discordant DZ pairs, there was a significant main effect of ε4 status on standardized test scores ($F[1,17] = 7.21, P=.013$, two tailed). Overall test means were positive in co-twins without an ε4 allele and were negative in co-twins having at least one ε4 allele. More importantly, there was no significant interaction between ε4 and tests indicating that all neuropsychological tests had a similar pattern of relationship with the ε4 status of the discordant co-twins.

The majority of discordant DZ twins were of genotypes ε3ε3 vs ε3ε4. There were too few individuals of the ε4ε4 and ε2ε4 genotypes to be analyzed separately. In the total sample, among subjects with at least one ε4 allele and omitting those with stroke, there were only five with the ε4ε4 genotype and seven with the ε2ε4 genotype. **Table 2** displays the same paired difference *t* test results omitting four pairs where one or both co-twins had an ε2 allele. In this table, subjects with the ε4 allele are compared with co-twins having the ε3ε3 genotype. Results were similar to those in Table 1, although the difference in TICS-m scores now approached statistical significance.

When the mean cognitive function test scores of individual twins with and without the ε4 allele in the total

Table 1. Mean Cognitive Function Scores in DZ Pairs Discordant for the Presence of the ε4 Allele*

Test	Pairs	Score, Mean (SD) ε4+ Co-twin	Score, Mean (SD) ε4− Co-twin	Average Difference, (ε4+)−(ε4−) (95% CI)	P†
TICS-m[29]	18	31.32 (3.91)	32.46 (3.09)	−1.14 (−2.64, 0.36)	.10
Digit symbol[28]	20	39.10 (8.07)	40.41 (8.55)	−1.31 (−4.22, 1.60)	.29
Iowa Battery[20]	20	2.54 (1.79)	3.52 (1.75)	−0.98 (−1.70, −0.24)	.016
BVRT[26]	20	5.29 (1.54)	6.08 (1.45)	−0.79 (−1.44, −0.14)	.024
COWA[25]	20	27.39 (11.4)	29.89 (9.11)	−2.50 (−6.14, 1.14)	.12
MMSE[27]	20	26.12 (2.25)	26.68 (2.10)	−0.56 (−1.37, 0.25)	.12
All items	17	29.57 (3.11)	30.47 (3.14)	−0.90 (−2.64, 0.46)	.13

DZ indicates dizygotic; ε4+ and ε4− apolipoprotein E allele ε4 present and absent, respectively; CI, confidence interval; TICS-m, Telephone Interview for Cognitive Status, modified for epidemiologic or survey work; COWA, controlled oral word association test; BVRT, Benton visual retention test; and MMSE, Mini-Mental State Examination.

†*One-tailed paired* t *test* P *value.*

Table 2. Mean Cognitive Function Scores in DZ Pairs Discordant for the Presence of the ε4 Allele*

Test	Pairs	Score, Mean (SD) ε4+ Co-twin	Score, Mean (SD) ε3ε3− Co-twin	Average Difference, (ε4+)−(ε4−) (95% CI)	P†
TICS-m[29]	15	30.58 (3.67)	32.19 (3.03)	−1.61 (−3.27, 0.05)	.06
Digit symbol[28]	16	37.90 (7.24)	37.95 (6.14)	−0.05 (−3.35, 3.25)	.46
Iowa Battery[20]	16	2.21 (1.55)	3.45 (1.47)	−1.24 (−2.06, −0.42)	.004
BVRT[26]	16	5.05 (1.43)	6.05 (1.32)	−1.00 (−1.73, −0.27)	.008
COWA[25]	16	26.44 (11.6)	29.30 (8.71)	−2.86 (−6.98, 1.26)	.13
MMSE[27]	16	25.99 (2.24)	26.36 (2.16)	−0.37 (−1.30, 0.58)	.25
All items	13	28.95 (3.07)	29.86 (3.32)	−0.91 (−2.48, 0.66)	.19

All subjects with ε2 allele were omitted. DZ indicates dizygotic; ε4+ and ε4−, apolipoprotein E allele ε4 present and absent, respectively; CI, confidence interval; TICS-m, Telephone Interview for Cognitive Status, modified for epidemiologic or survey work; COWA, controlled oral word association test; BVRT, Benton visual retention test; and MMSE, Mini-Mental State Examination.

†*One-tailed paired* t *test* P *value.*

sample were compared, the results were consistent with those observed in the discordant twin pairs. In all instances, mean scores adjusted for age and education were lower in the 50 subjects carrying at least one ε4 allele than in the 138 individuals without ε4. Several tests attained or approached statistical significance (MMSE all items, $P=.026$; TICS-m, $P=.035$; BVRT, $P=.056$; and Iowa battery, $P=.066$).

COMMENT

Despite small numbers in this normal older male population, twin pairs with at least one ε4 allele tended to perform less well on various education-adjusted measures of cognitive function than did their discordant co-twins without an ε4 allele. A similar pattern of poorer performance in individuals with ε4 was noted in the full sample for age- and education-adjusted test scores. The design using discordant twin-pairs controls for early-shared environmental influences, including socioeconomic status, which might influence measures of cognitive function. Individuals who had prior cerebrovascular accidents or clinical evidence of the residual effects of a cerebrovascular accident, which could influence neuropsychological function, were excluded. Over 86% of the participants were screened 3 to 5 years later for clinical dementia and judged not to be demented,[30] suggesting that our results are not likely to be explained by a few subjects showing early detectable signs of clinical dementia. In our full sample, the mean adjusted MMSE score, following the protocol by Folstein et al,[27] was approximately 27 (±2.0) comparable to recently reported population-based MMSE norms for the ages of our twins, even though the reported norms were generated using the better of the two performances on the serial subtraction and reverse spelling items.[35]

Scores on neuropsychological tests used in this study are moderately intercorrelated. They differ with respect to the emphasis each place on the assessment of memory. The MMSE, TICS-m, and Iowa battery were all developed to screen for cognitive impairment. As such, they were not designed to distinguish Alzheimer type dementia or multi-infarct dementia from other causes of loss of cognitive function. The BVRT and oral word association tests are part of the Iowa summary score. The BVRT forms a major portion of the Iowa score, and this visual memory test could reasonably be expected to be more sensitive to subclinical changes of dementia than verbal fluency.[36] The digit symbol substitution test measures psychomotor speed, and deficits in this element of function may be less detectable than tests with a stronger emphasis on assessment of memory.

Family history specifically concerning AD is not available for our twins. It is possible that some of the subjects may be from families with a history of FAD; at-risk unaffected children of patients with AD have been shown to fall into two distributions on memory tasks[37], and at-risk, nondemented relatives of early-onset FAD were more likely to show

decline in cognitive test-retest scores when followed up longitudinally.[38] If our results can be confirmed, they should be investigated further in the National Heart, Lung, and Blood Institute twins, as well as in female cohorts. When seen at the third examination, 6 to 7 years ago, the twins averaged 63 years in age. At a fourth examination, the decline in cognitive function of individuals with and without the ε4 allele could be studied. Magnetic resonance imaging of the brain would also permit the detection of subjects with early subclinical brain atrophy or ischemic changes that may ultimately lead to dementia. If our hypothesis is correct, those with the ε4 allele may be more likely to show greater cognitive decline or abnormalities in brain morphology than their cohorts without the ε4 allele.

From the current study, it is already possible to identify subjects with ε4 who consistently performed poorly on all tests or displayed deficits in one test relative to their cotwin without the ε4 allele. These subjects will be of particular interest for follow-up studies. In two populations of elderly adults, a decline in the frequency of individuals with the ε4 allele has been reported compared with younger cohorts.[34,39] The loss in individuals with the ε4 allele in these older populations suggest that this gene may mark those who are at risk for accelerated aging in part through increased risk for dementia or earlier ischemic heart disease. Our results may have wider implications for the ability to use methods to predict which of these individuals in the general population are at greater risk to develop dementia, given their ε4 genotype and cognitive test scores. Identified individuals may ultimately be treated to delay the clinical onset of functional dementia.

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