Sex, Apolipoprotein E ε4 Status, and Hippocampal Volume in Mild Cognitive Impairment

Adam Fleisher, MD; Michael Grundman, MD, MPH; Clifford R. Jack, Jr, MD; Ronald C. Petersen, PhD, MD; Curtis Taylor, PhD; Hyun T. Kim, MS; Denise H. B. Schiller, BA; Victor Bagwell, MSIT, MBA; Drahomira Sencakova, MD; Myron F. Weiner, MD; Charles DeCarli, MD; Steven T. DeKosky, MD; Christopher H. van Dyck, MD; Leon J. Thal, MD; for the Alzheimer’s Disease Cooperative Study

Background: Subjects with mild cognitive impairment (MCI) have been shown to have reduced hippocampal volumes relative to normal elderly control subjects. The presence of the apolipoprotein E ε4 (APOE*Ε4) allele has been associated with greater hippocampal atrophy in women than in men with Alzheimer disease. This relationship has not been demonstrated in MCI.

Objective: To examine the relationship between APOE genotype and hippocampal volume in men and women with MCI.

Design: This study evaluated MCI in 193 subjects (86 women and 107 men) participating in a multicenter clinical trial, all of whom underwent magnetic resonance imaging at their baseline visit. We evaluated the association among the number of APOE*Ε4 alleles, memory performance, and hippocampal volume in men and women with tests of means and multiple linear regressions.

Results: Compared with MCI subjects with no APOE*Ε4 alleles, women with 1 or 2 APOE*Ε4 alleles were found to have significantly reduced hippocampal volume, whereas men only showed a significant reduction in hippocampal volume when carrying 2 APOE*Ε4 alleles. Worsening of performance on a delayed word recall task (Alzheimer’s Disease Assessment Scale cognitive sub-scale) showed an identical pattern in association with APOE*Ε4 allele dose and sex. Furthermore, when controlling for memory performance on delayed word recall, the APOE*Ε4 effect on hippocampal volumes was attenuated in men, but remained significant in women.

Conclusion: The APOE*Ε4 genotype status appears to have a greater deleterious effect on gross hippocampal pathology and memory performance in women than in men.

Arch Neurol. 2005;62:953-957
have not been demonstrated in MCI subjects. The central focus of this study is to determine if APOE status is related to hippocampal volume among men and women participating in an MCI trial.

METHODS

SUBJECTS

One hundred ninety-three MCI subjects underwent structural brain MRI. These individuals were recruited from 24 sites participating in an MCI trial to evaluate the efficacy of donepezil hydrochloride (Aricept; Eisai Inc, Research Triangle Park, NC), vitamin E, and placebo on slowing the rate of cognitive decline and the progression from MCI to AD. The operational criteria for MCI in this study are described in full detail in a previous publication from our group. All subjects provided blood samples for APOE genotyping.

IMAGING PROTOCOL

Volume measurements of the hippocampi were derived from a T1-weighted 3-dimensional volumetric spoiled-gradient recalled-echo sequence, with 124 contiguous partitions, 1.6-mm section thickness, a 22 × 16.5-cm field of view, 192 sections, and 25° flip angle. Intrarater test-retest coefficient of variation of hippocampal volumetric measurements is 1.9% with the methods used.21 The borders of the hippocampi were manually traced by operators blinded to clinical data. Typically, 25 imaging sections were measured for each hippocampus. In-plane hippocampal anatomic boundaries were defined to include the CA1 to CA4 sectors of the hippocampus proper, the dentate gyrus, and the subiculum.22 The posterior boundary of the hippocampus is determined by the oblique coronal anatomic section in which the crura of the fornices were defined in full profile. Normalized hippocampal volume was calculated according to the following formula: (raw hippocampal volume/total intracranial volume) × 1000.

STATISTICAL ANALYSES

Because this study hypothesized that APOE would have differential effects on hippocampal volume, we analyzed the data for men and women separately. Mean normalized hippocampal volume ratios (NHVRs) of subjects who were heterozygous and homozygous for APOE*E4 alleles were compared with the mean volume ratios of subjects with no APOE*E4 alleles (APOE*E4-negative subjects) using independent t tests. In further analyses, a linear regression model was used to predict the hippocampal volume of men and women based on their APOE status, while controlling for age and memory performance (determined using a 10-word delayed recall list derived from the Alzheimer's Disease Assessment Scale cognitive subscale23). Unless otherwise indicated, data are expressed as mean±SD.

RESULTS

Of the 193 subjects, 86 (44.6%) were women and 107 (55.4%) were men. Demographic characteristics of this sample are presented in Table 1. Magnetic resonance imaging data are presented in Table 2; these data include total intracranial and raw hippocampal volumes and NHVR. Men had significantly larger raw hippocampal (t=4.01;P<.001) and total intracranial volumes (t=10.45; P<.001); however, there was no significant sex difference with respect to NHVR (t=−1.13; P=.26). With respect to APOE*E4, 68 subjects (35.2%) had no APOE*E4 alleles and 125 subjects (64.8%) had the allele. Among the APOE*E4-positive group, 102 (81.6%) had 1 APOE*E4 allele and 23 (18.4%) had 2 alleles. The distribution of APOE*E4 status by sex was not significant (χ²=0.87; P=.65).

Planned t tests performed to compare the effects of having 1 or 2 APOE*E4 alleles on NHVRs are shown in Figure 1. Men with no APOE*E4 alleles had a mean NHVR of 3.28±0.08. This was not significantly different from the mean NHVR of 3.35±0.06 in men with a
single APOE*E4 allele \( (P = .52) \). Men with 2 APOE*E4 alleles had a mean NHVR of 3.00±0.10, which was significantly smaller than that of the men with no APOE*E4 alleles \( (P = .04) \). The profile of hippocampal volume in women was different. The APOE*E4-negative women had a mean NHVR of 3.65±0.09. Unlike the men, women with a single APOE*E4 allele had a significantly smaller NHVR compared with their APOE*E4-negative counterparts \( (P < .001) \), with a mean NHVR of 3.23±0.06. Women with 2 APOE*E4 alleles had a mean NHVR of 3.00±0.15, which was significantly lower than that of the APOE*E4-negative women \( (P = .02) \). In summary, men with a single APOE*E4 allele were not significantly different from men with no APOE*E4 allele, whereas women with a single APOE*E4 allele had a significantly smaller NHVR than those without an APOE*E4 allele (Figure 2).

To further examine the relation among APOE*E4, sex, and hippocampal volume, linear regression was used to observe the relative contribution of 1 or 2 APOE*E4 alleles in men and women while simultaneously controlling for age and memory performance (Table 3). For men, after controlling for age and memory performance, the presence of a single APOE*E4 allele was not found to be a significant predictor of normalized hippocampal volume \( (P = .32) \), and neither was having 2 such alleles \( (P = .13) \). For women, after controlling for these same variables, having a single APOE*E4 allele was a significant predictor of hippocampal atrophy \( (P = .002) \), as was having 2 APOE*E4 alleles \( (P = .007) \).

We performed planned \( t \) tests to compare the effect of APOE*E4 status on memory performance, as measured with the delayed recall list derived from the Alzheimer’s Disease Assessment Scale cognitive subscale. \(^{23} \) Men and women were not significantly different in delayed recall performance when controlling for age and education \( (P = .33) \). Men who carried 2 APOE*E4 alleles showed significantly worse memory performance compared with men without an APOE*E4 allele \( (P = .006) \). Likewise, women who carried 2 APOE*E4 alleles showed significantly worse memory performance compared with women without an APOE*E4 allele \( (P = .002) \). However, sex differences were seen in individuals carrying a single APOE*E4 allele. The men with a single APOE*E4 allele were not significantly different from those who did not carry the allele \( (P = .19) \), whereas women with only 1 allele performed significantly worse than women who did not carry an allele \( (P < .001) \). These results parallel those found with respect to normalized hippocampal volume. Specifically, men with a single APOE*E4 allele were not significantly different from men with no APOE*E4 allele, whereas women with a single APOE*E4 allele had a significantly worse memory than those without the allele.

**COMMENT**

Men and women with MCI who carried 2 APOE*E4 alleles showed a significant reduction in hippocampal volume when compared with their same-sex APOE*E4-negative peers. When compared with APOE*E4-negative individuals, women with a single APOE*E4 allele showed a marked reduction in hippocampal volume, whereas men with a single allele did not. When controlling for memory performance, women continued to show a significant negative relationship between the presence of an APOE*E4 allele and hippocampal volume, whereas men did not. This attenuation of significance between the APOE*E4 allele and hippocampal volume reduction in men infers a more coherent link between these factors in

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**Figure 1.** Mean normalized hippocampal volume and apolipoprotein E (APOE) status. APOE*E4 indicates the allele producing the e4 type of APOE; asterisk, \( P = .52 \) for men with 1 APOE*E4 allele and \( P < .001 \) for women with 1 APOE*E4 allele compared with APOE*E4-negative subjects; and dagger, \( P = .04 \) for men with 2 APOE*E4 alleles and \( P = .02 \) for women with 2 APOE*E4 alleles compared with APOE*E4-negative subjects.

**Figure 2.** Reduction in hippocampal volume and apolipoprotein E (APOE) status relative to that in subjects with no allele producing the e4 type of APOE (APOE*E4).

**Table 3. Predictors of Hippocampal Volume in Men and Women**

<table>
<thead>
<tr>
<th>Factor</th>
<th>( \beta ) Coefficient</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>4.327</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.013</td>
<td>.12</td>
</tr>
<tr>
<td>Memory performance</td>
<td>-0.041</td>
<td>.06</td>
</tr>
<tr>
<td>Presence of 1 APOE*E4 allele</td>
<td>-0.095</td>
<td>.32</td>
</tr>
<tr>
<td>Presence of 2 APOE*E4 alleles</td>
<td>-0.241</td>
<td>.13</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>4.774</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.013</td>
<td>.052</td>
</tr>
<tr>
<td>Memory performance</td>
<td>-0.040</td>
<td>.06</td>
</tr>
<tr>
<td>Presence of 1 APOE*E4 allele</td>
<td>-0.343</td>
<td>.002</td>
</tr>
<tr>
<td>Presence of 2 APOE*E4 alleles</td>
<td>-0.425</td>
<td>.007</td>
</tr>
</tbody>
</table>

Abbreviation: APOE*E4, the allele producing the e4 type of apolipoprotein E.
women, and perhaps emphasizes the greater effect of APOE*E4 alleles on disease neuropathology in women. This also suggests that with respect to MCI and AD, hippocampal volume loss may be an independent indicator of disease progress after controlling for impairment in delayed recall.

Further analysis of memory performance showed associations of sex with the APOE*E4 allele that paralleled our MRI findings. There were no group differences between men and women on delayed recall testing results overall. However, men showed significant performance reductions on results of delayed recall testing only when 2 copies of the APOE*E4 allele were present. Women had significant performance reductions with 1 or 2 alleles when compared with APOE*E4-negative individuals. These collective results suggest that presence of the APOE*E4 allele confers a greater risk of hippocampal pathology and decline in memory performance for women than it does for men. In this study, men with MCI require homozygosity to show significant hippocampal volume loss and worsened memory performance; women with MCI are vulnerable to the negative effects of even a single APOE*E4 allele.

Our finding that women with no APOE*E4 allele have a larger NHVR than men with no APOE*E4 allele (3.65 vs 3.28) was unexpected (Figure 2). To date, studies of normalized hippocampal volumes in men and women have typically involved younger individuals without disease. Even so, men are reported to have more rapid hippocampal atrophy in normal aging when compared with women. Our study is unique in that it focuses on an older population with MCI. With no literature precedence of hippocampal volume and sex differences in this population, our findings must be considered preliminary. We are inclined to believe that the normalized volume differences seen herein represent true sex differences in neuropathology.

The most evident difference between men and women older than 55 years is the presence of menopause and its associated neuroendocrine changes. The sex differences in AD neuropathology might be explained, at least in part, by the influence of serum or brain levels of estrogen, which may act in concert with certain APOE genotypes. Per-haps, at some point after menopause, estrogen levels drop below a critical threshold that protects against senile plaque formation. Estrogen promotes a cleavage pathway that protects against the conversion of amyloid precursor protein into β-amyloid. For women at greater risk of development of AD (ie, those with the APOE*E4 allele), available estrogen may help reduce the buildup of amyloid plaques and the subsequent neural damage. Another potential protective effect of estrogen may be reducing the toxic effects of amyloid that has already built up.

Beyond risks associated with amyloid deposits, estrogen may help offset other dementia risk factors related to APOE*E4 alleles. Relative to the APOE*E3 allele, the APOE*E4 allele has been shown to decrease neurite outgrowth in vitro. This may equate to less neuronal remodeling in the aging brain. Estrogen helps to increase dendritic spine density of hippocampal neurons. Estrogen has also been shown to cause an increase of synaptic sprouting via an APOE-dependent mechanism, suggesting that the combined influence of estrogen loss and the presence of the APOE*E4 genotype is a possible reason why risk of AD appears to be greater in women carrying an APOE*E4 allele than in men.

Although men will not have the same protection from estrogen as women, testosterone may provide protective influence in the hippocampus for men with 1 or 2 APOE*E4 alleles. Testosterone can be locally converted to estrogen via aromatization, potentially providing some of the benefits discussed previously. Moreover, testosterone has been shown to reduce β-amyloid secretions in neuronal cultures and reduce the hyperphosphorylation of tau protein, which is a major contributor to the neurofibrillary tangles associated with AD. Lower levels of available testosterone have been associated with an increased risk for AD. Overall these data support that the risk of hippocampal degeneration and short-term memory loss related to MCI is affected by complex interactions among sex and neurophysiologic, neuroendocrine, and genetic factors that warrant further exploration. At present, we are analyzing longitudinal data in a recently completed randomized, placebo-controlled clinical trial of donepezil and vitamin E in 769 MCI subjects. Given that this study...
represents a cross-sectional cohort, further analysis will be required to infer any longitudinal outcome variation due to sex and APOE status or an association between these hippocampal volume differences and clinical progression. It is clear, however, that the results of this analysis point to the necessity of evaluating differences by sex in future clinical trials.

Accepted for Publication: October 14, 2004.

Correspondence: Adam Fleisher, MD, Alzheimer’s Disease Cooperative Study, 8950 Villa La Jolla Dr, Suite C227, La Jolla, CA 92037 (afleisher@ucsd.edu).

Author Contributions: Study concept and design: Grundman, Taylor, Kim, and Schiller. Acquisition of data: Jack, Petersen, Kim, Sencakova, Weinir, DeKosky, van Dyck, and Thal. Analysis and interpretation of data: Fleisher, Grundman, Jack, Taylor, Kim, Schiller, Bagwell, DeCarli, DeKosky, and van Dyck. Drafting of the manuscript: Fleisher, Grundman, Taylor, Kim, Schiller, Weinir, and van Dyck. Critical revision of the manuscript for important intellectual content: Fleisher, Jack, Petersen, Taylor, Schiller, Bagwell, Sencakova, DeCarli, DeKosky, van Dyck, and Thal. Statistical analysis: Taylor and Kim. Obtained funding: Peterson and Thal. Administrative, technical, and material support: Grundman, Schiller, Bagwell and van Dyck. Study supervision: Fleisher, Grundman, and DeCarli.

Funding/Support: This study was supported by grant U01 AG010483 from the National Institute on Aging, Gaithersburg, Md; by Pfizer Inc, New York, NY; and Eisai Inc, Research Triangle Park, NC. Study medication was provided by Pfizer Inc, Eisai Inc, and Roche Vitamins Inc, Parsippany, NJ. Additional funding for the magnetic resonance imaging component of the trial was provided from the Institute for the Study of Aging, New York.


