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*E4*) 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*E4* 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*E4* alleles, women with 1 or 2 *APOE*E4* alleles were found to have significantly reduced hippocampal volume, whereas men only showed a significant reduction in hippocampal volume when carrying 2 *APOE*E4* alleles. Worsening of performance on a delayed word recall task (Alzheimer's Disease Assessment Scale cognitive subscale) showed an identical pattern in association with *APOE*E4* allele dose and sex. Furthermore, when controlling for memory performance on delayed word recall, the *APOE*E4* effect on hippocampal volumes was attenuated in men, but remained significant in women.

Conclusion: The *APOE*E4* 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

Author Affiliations: Department of Neurosciences, University of California–San Diego, La Jolla (Drs Fleisher, Taylor, and Thal, Messrs Kim and Bagwell, and Ms Schiller); Elan Corporation, San Diego, Calif (Dr Grundman); Departments of Diagnostic Radiology (Drs Jack and Sencakova) and Neurology (Dr Petersen), Mayo Clinic and Foundation, Rochester, Minn; Department of Psychiatry, The University of Texas Southwestern Medical Center, Dallas (Dr Weiner); Department of Neurology, University of California–Davis, Sacramento (Dr DeCarli); Department of Neurology, University of Pittsburgh, Pittsburgh, Pa (Dr DeKosky); and Department of Psychiatry, Yale University School of Medicine, New Haven, Conn (Dr van Dyck).

Group Information: A complete list of participants from the Alzheimer's Disease Cooperative Study appears in a box at the end of the text.

THE AMNESTIC FORM OF MILD cognitive impairment (MCI) is characterized by progressive memory deficits without equivalent declines in other areas of cognition and behavior. It is of particular clinical interest because MCI is perceived by many as a prodromal state for Alzheimer disease (AD). The presence of 1 or 2 copies of the apolipoprotein E ε4 (*APOE*E4*) allele has been associated with memory impairment in nondemented individuals and an increased risk of development of AD.[1,2] In patients with MCI (hereafter referred to as MCI patients or subjects) and in nondemented individuals, studies have shown a relation between the *APOE*E4* allele and hippocampal or entorhinal atrophy.[3-6] Furthermore, atrophy of the hippocampus has been associated with impaired memory performance in individuals with AD[7] and MCI[8] and is a predictor of AD conversion among MCI patients.[9,10] The *APOE*E4* allele has also been linked to variability in medial temporal lobe pathophysiology by neurochemistry findings in MCI subjects[11] and by positron emission tomography[12,13] and functional magnetic resonance imaging (MRI)[14] findings in preclinical disease. In addition, neuroimaging studies have indicated a negative correlation between the number of *APOE*E4* allele copies and the volume of medial temporal brain structures, including the hippocampus, in subjects with AD.[15,16]

Previous studies have shown an association between atrophy of the hippocampus and entorhinal cortex with the *APOE*E4* allele in MCI and preclinical disease.[17] Women of a given *APOE* genotype (eg, *APOE*E3/APOE*E4* or *APOE*E4/APOE*E4*) appear to have a higher odds ratio of development of AD than men with the identical genotype.[18,19] In 1 study of individuals with AD, those who carried the *APOE*E4* allele showed a 45% reduction in hippocampal volume relative to matched control subjects. When this group underwent analysis by sex, the women with the *APOE*E4* allele showed a reduction of 55%, whereas the men only showed a 45% reduction.[16] At present, differences between men and women and *APOE* status

Table 1. Demographic Characteristics*

	All Subjects (N = 193)	Men (n = 107)	Women (n = 86)
Age, mean (SD), y	72.89 (6.70)	72.95 (6.22)	72.81 (7.30)
Education, mean (SD), y†	15.01 (3.04)	15.74 (3.30)	14.09 (2.41)
Marital status, %†			
Married	81.9	90.7	70.9
Widowed	9.3	2.8	17.4
Divorced	7.3	4.7	10.5
Never married	1.6	1.9	1.2
Apolipoprotein genotype status, %			
*APOE**E3/APOE**E3*	35.2	37.4	32.6
*APOE**E3/APOE**E4*	52.8	52.3	53.5
*APOE**E4/APOE**E4*	11.9	10.3	14.0
Ethnicity, %			
Native American	0.5	0.0	1.2
Asian American	1.0	0.9	1.2
African American	2.1	1.9	2.3
Hispanic	3.1	4.7	1.2
White	93.3	92.5	94.2
ADAS-Cog total score, mean (SD)	10.93 (4.20)	10.70 (3.82)	11.20 (4.63)
Mini-Mental Status Examination score, mean (SD)	27.48 (1.80)	27.51 (1.82)	27.44 (1.80)

Abbreviations: ADAS-Cog, Alzheimer's Disease Assessment Scale cognitive subscale; *APOE**E3*, the allele producing the ε3 type of apolipoprotein E; *APOE**E4*, the allele producing the ε4 type of apolipoprotein E.
*Unless otherwise indicated, data are expressed as percentage of subjects. Percentages have been rounded and may not total 100.
†Indicates significant difference by sex ($P<.01$).

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 publications from our group.[20] 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)×10^3.

Table 2. Hippocampal Baseline Data in Subjects Undergoing MRI*

	All Subjects (N = 193)	Men (n = 107)	Women (n = 86)
Total intracranial volume, $mm^3 \times 10^6$†	1.49	1.58	1.39
Raw hippocampal volume, $mm^3 \times 10^3$†	4.94	5.17	4.67
Normalized hippocampal volume, (raw hippocampal/total intracranial volume) × 1000	3.32	3.29	3.36

Abbreviation: MRI, magnetic resonance imaging.
*Data are expressed as means.
†Indicates significant difference by sex ($P<.001$).

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 subscale[23]). 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 ($\chi^2=0.87$; $P=.65$).

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

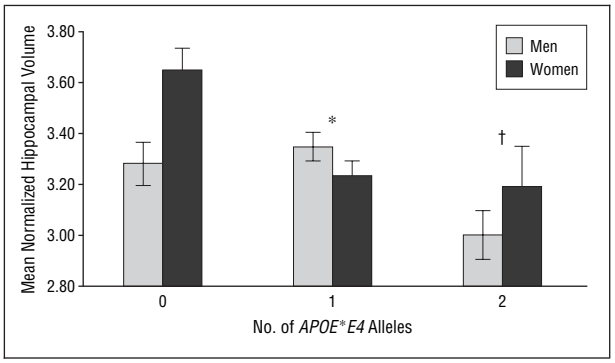

Figure 1. Mean normalized hippocampal volume and apolipoprotein E (APOE) status. *APOE*E4* indicates the allele producing the ε4 type of APOE; asterisk, $P=.52$ for men with 1 *APOE*E4* allele and $P<.001$ for women with 1 *APOE*E4* allele compared with *APOE*E4*-negative subjects; and dagger, $P=.04$ for men with 2 *APOE*E4* alleles and $P=.02$ for women with 2 *APOE*E4* alleles compared with *APOE*E4*-negative subjects.

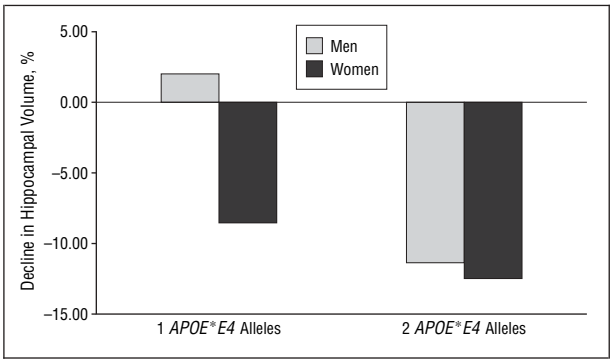

Figure 2. Reduction in hippocampal volume and apolipoprotein E (APOE) status relative to that in subjects with no allele producing the ε4 type of APOE (*APOE*E4*).

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.

Table 3. Predictors of Hippocampal Volume in Men and Women

Factor	β Coefficient	*P* Value
Men		
Constant	4.327	<.001
Age	−0.013	.12
Memory performance	−0.041	.06
Presence of 1 *APOE*E4* allele	−0.095	.32
Presence of 2 *APOE*E4* alleles	−0.241	.13
Women		
Constant	4.774	<.001
Age	−0.013	.052
Memory performance	−0.040	.06
Presence of 1 *APOE*E4* allele	−0.343	.002
Presence of 2 *APOE*E4* alleles	−0.425	.007

Abbreviation: *APOE*E4*, the allele producing the ε4 type of apolipoprotein E.

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

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.[24] 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.[25] Perhaps, at some point after menopause, estrogen levels drop below a critical threshold that protects against senile plaque formation.[26] Estrogen promotes a cleavage pathway that protects against the conversion of amyloid precursor protein into β-amyloid.[27] 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.[28,29]

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.[30] This may equate to less neuronal remodeling in the aging brain.[31] Estrogen helps to increase dendritic spine density of hippocampal neurons.[32] 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.[33]

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.[34] Moreover, testosterone has been shown to reduce β-amyloid secretions in neuronal cultures[35] and reduce the hyperphosphorylation of tau protein, which is a major contributer to the neurofibrillary tangles associated with AD.[36,37] Lower levels of available testosterone have been associated with an increased risk for AD.[38,39]

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.[20] 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, Weiner, 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, Weiner, 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.

REFERENCES

1. Petersen RC, Waring SC, Smith GE, Tangalos EG, Thibodeau SN. Predictive value of APOE genotyping in incipient Alzheimer's disease. *Ann N Y Acad Sci*. 1996; 802:58-69.
2. Bondi MW, Salmon DP, Monsch AU, et al. Episodic memory changes are associated with the APOE-epsilon 4 allele in nondemented older adults. *Neurology*. 1995;45:2203-2206.
3. Moffat SD, Szekely CA, Zonderman AB, Kabani NJ, Resnick SM. Longitudinal change in hippocampal volume as a function of apolipoprotein E genotype. *Neurology*. 2000;55:134-136.
4. den Heijer T, Oudkerk M, Launer LJ, et al. Hippocampal, amygdalar, and global brain atrophy in different apolipoprotein E genotypes. *Neurology*. 2002;59: 746-748.
5. Jack CR, Petersen RC, Xu Y, et al. Rates of hippocampal atrophy correlate with change in clinical status in aging and AD. *Neurology*. 2000;55:484-489.
6. Killiany RJ, Hyman BT, Gomez-Isla T, et al. MRI measures of entorhinal cortex vs hippocampus in preclinical AD. *Neurology*. 2002;58:1188-1196.
7. Petersen RC, Jack CR Jr, Xu Y-C, et al. Memory and MRI-based hippocampal volumes in aging and AD. *Neurology*. 2000;54:581-587.
8. Grundman M, Jack C, Petersen R, et al. Hippocampal volume is associated with memory but not nonmemory cognitive performance in patients with mild cognitive impairment. *J Mol Neurosci*. 2003;20:241-248.
9. Jack CR Jr, Petersen RC, Xu YC, et al. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology*. 1999;52:1397-1403.
10. Grundman M, Sencakova D, Jack CR Jr, et al. Use of brain MRI volumetric analysis in a mild cognitive impairment trial to delay the diagnosis of Alzheimer's disease. In: Fillit H, O'Connell A, eds. *Drug Discovery and Development for Alzheimer's Disease 2000*. New York, NY: Springer Publishing Co Inc; 2001:24-32.
11. DeKosky ST, Ikonomovic MD, Styren SD, et al. Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Ann Neurol*. 2002;51:145-155.
12. Small GW, Mazziotta JC, Collins MT, et al. Apolipoprotein E type 4 allele and cerebral glucose metabolism in relatives at risk for familial Alzheimer disease. *JAMA*. 1995;273:942-947.
13. Reiman EM, Caselli RJ, Chen K, Alexander GE, Bandy D, Frost J. Declining brain activity in cognitively normal apolipoprotein E epsilon 4 heterozygotes: a foundation for using positron emission tomography to efficiently test treatments to prevent Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2001;98:3334-3339.
14. Bookheimer SY, Strojwas MH, Cohen MS, et al. Patterns of brain activation in people at risk for Alzheimer's disease. *N Engl J Med*. 2000;343:450-456.
15. Hashimoto M, Yasuda M, Tanimukai S, et al. Apolipoprotein E epsilon 4 and the pattern of regional brain atrophy in Alzheimer's disease. *Neurology*. 2001;57: 1461-1466.
16. Juottonen K, Lehtovirta M, Helisalmi S, Riekkinen PJ Sr, Soininen H. Major decrease in the volume of the entorhinal cortex in patients with Alzheimer's disease carrying the apolipoprotein E epsilon4 allele. *J Neurol Neurosurg Psychiatry*. 1998;65:322-327.
17. Poirier J, Davignon J, Bouthillier D, et al. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet*. 1993;342:697-699.
18. Farrer LA, Cupples LA, Haines JL, et al; APOE and Alzheimer Disease Meta Analysis Consortium. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. *JAMA*. 1997; 278:1349-1356.
19. Bretsky PM, Buckwalter JG, Seeman TE, et al. Evidence for an interaction between apolipoprotein E genotype, gender, and Alzheimer disease. *Alzheimer Dis Assoc Disord*. 1999;13:216-221.
20. Grundman M, Petersen RC, Ferris SH, et al. Mild cognitive impairment can be distinguished from Alzheimer's disease and normal aging for clinical trials. *Arch Neurol*. 2004;61:59-66.
21. Jack CR Jr, Bentley M, Twomey CK, Zinsmeister AR. MR-based volume measurements of the hippocampal formation and anterior temporal lobe: validation studies. *Radiology*. 1990;176:205-209.
22. Jack CR Jr, Petersen RC, Xu YC, et al. Medial temporal atrophy on MRI in normal aging and very mild Alzheimer's disease. *Neurology*. 1997;49:786-794.
23. Mohs RC, Knopman D, Petersen RC, et al. Development of cognitive instruments for use in clinical trials of antidementia drugs: additions to the Alzheimer's Disease Assessment Scale (ADAS) that broaden its scope: The Alzheimer's Disease Cooperative Study. *Alzheimer Dis Assoc Disord*. 1997;11(suppl 2):S13-S21.
24. Pruessner JC, Collins DL, Pruessner M, Evans AC. Age and gender predict volume decline in the anterior and posterior hippocampus in early adulthood. *J Neurosci*. 2001;21:194-200.
25. Yaffe K, Haan M, Byers A, Tangen C, Kuller L. Estrogen use, APOE, and cognitive decline: evidence of gene-environment interaction. *Neurology*. 2000;54:1949-1954.
26. Ghebremedhin E, Schultz C, Thal DR, et al. Gender and age modify the association between APOE and AD-related neuropathology. *Neurology*. 2001;56:1696-1701.
27. Xu H, Gouras GK, Greenfield JP, et al. Estrogen reduces neuronal generation of Alzheimer beta-amyloid peptides. *Nat Med*. 1998;4:447-451.
28. Blennow K, Cowburn RF. The neurochemistry of Alzheimer's disease. *Acta Neurol Scand Suppl*. 1996;168:77-86.
29. Green PS, Gridley KE, Simpkins JW. Estradiol protects against beta-amyloid (25-35)–induced toxicity in SK-N-SH human neuroblastoma cells. *Neurosci Lett*. 1996; 218:165-168.
30. Nathan BP, Bellosta S, Sanan DA, et al. Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro. *Science*. 1994;264:850-852.
31. Nathan BP, Chang KC, Bellosta S, et al. The inhibitory effect of apolipoprotein E4 on neurite outgrowth is associated with microtubule depolymerization. *J Biol Chem*. 1995;270:19791-19799.
32. Poirier J, Delisle MC, Quirion R, et al. Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. *Proc Natl Acad Sci U S A*. 1995;92:12260-12264.
33. Stone DJ, Rozovsky I, Morgan TE, Anderson CP, Finch CE. Increased synaptic sprouting in response to estrogen via an apolipoprotein E–dependent mechanism: implications for Alzheimer's disease. *J Neurosci*. 1998;18:3180-3185.
34. Sherwin BB. Steroid hormones and cognitive functioning in aging men: a mini-review. *J Mol Neurosci*. 2003;20:385-393.
35. Gouras GK, Xu H, Gross RS, et al. Testosterone reduces neuronal secretion of Alzheimer's beta-amyloid peptides. *Proc Natl Acad Sci U S A*. 2000;97:1202-1205.
36. Spillantini MG, Goedert M. Tau protein pathology in neurodegenerative diseases. *Trends Neurosci*. 1998;21:428-433.
37. Mitchell TW, Mufson EJ, Schneider JA, et al. Parahippocampal tau pathology in healthy aging, mild cognitive impairment, and early Alzheimer's disease. *Ann Neurol*. 2002;51:182-189.
38. Moffat SD, Zonderman AB, Metter EJ, et al. Free testosterone and risk for Alzheimer disease in older men. *Neurology*. 2004;62:188-193.
39. Okun MS, DeLong MR, Hanfelt J, Gearing M, Levey A. Plasma testosterone levels in Alzheimer and Parkinson diseases. *Neurology*. 2004;62:411-413.