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Treatment outcome of tacrine therapy depends on apolipoprotein genotype and gender of the subjects with Alzheimer's disease

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Article abstract—We studied the effects of apolipoprotein E (*APOE*) genotype and gender on clinical response to tacrine in patients with mild to moderate Alzheimer's disease (AD). We analyzed data from a previously reported 30-week, double-blind, placebo-controlled trial of tacrine, in which *APOE* genotypes were determined from previously collected plasma samples. Patients were assigned to placebo or tacrine with daily dosages of 80, 120, or 160 mg/day. The outcome measures were Alzheimer's Disease Assessment Scale-Cognitive Component, Clinician Interview Based Impression, Mini-Mental State Examination, and the Caregiver-rated Clinical Global Impression of Change. An intent-to-treat (ITT) analysis of patients with available genotypes (n = 528) did not reveal response differences by genotype, although the effect size was twice as large in the ε2-3 as the ε4 group (−2.62 versus −1.25). The association of treatment effect with *APOE* genotype varied significantly according to gender ($p < 0.002$ for ITT; $p < 0.05$ for evaluables). The treatment effect was larger in the ε2-3 compared with ε4 women (ITT, 4.24 points, $p = 0.03$; evaluable, 7.20 points, $p = 0.01$). In contrast, treatment effect size was not different between ε2-3 and ε4 of men with AD. *APOE* genotype and gender may predict response to tacrine in patients with AD.

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Alzheimer's disease (AD) is characterized by a severe loss of presynaptic cholinergic neurons in nucleus basalis and their projections to the cerebral cortices and decreased levels of choline acetyl transferase in the cortex.[1,2] This loss of cholinergic activity in the central nervous system (CNS) in patients with AD correlates with worsening of scores on dementia rating scales.[3] Currently, cholinesterase inhibition is the most widely studied and developed approach for treating symptoms of AD.[4]

Tacrine, a centrally active potent cholinesterase inhibitor, has been the most commonly used drug for the treatment of AD. It has consistently produced, in some patients, mild to moderate improvements in memory and cognitive abilities.[5,6] However, only 25 to 50% of treated patients have had a significant clinical response in previously reported clinical trials, with the percentage of responders influenced by study design, length of treatment, instruments for measuring efficacy, and methods of analysis.[4] Results from previous studies have also suggested that achieving adequate dosage, higher plasma levels of tacrine, and higher levels of cholinesterase inhibition in peripheral blood are all important factors associated with greater probability of clinical response.[4] However, many patients cannot tolerate high dosage of tacrine because this medication has significant dose-related side effects that can include hepatotoxicity and systemic cholinergic symptoms such as dyspepsia, nausea, vomiting, and diarrhea. These limitations in treatment response and the significant side effects have led us to search for other predictive markers for patients' clinical response.

The apolipoprotein E (*APOE*) gene constitutes a major susceptibility factor for the development of the familial and sporadic forms of late-onset AD.[7,8] The 34-kDa protein, apolipoprotein E (apoE), is also believed to play a key role in membrane remodeling associated with synaptic plasticity.[9] The gene for apoE is located on the long arm of the human chromosome 19 (19q13). Three common alleles (ε2, ε3, and ε4) encode the three major apoE isoforms. The risk for AD is higher, and the age of onset lower, for ε4 heterozygotes and even more so for ε4 homozy-

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gotes compared with the risk and onset age in patients with other genotypes.[7] Furthermore, *APOE*-ε4 has been associated with decreased numbers of cholinergic markers in temporal cortex and the hippocampus in neuropathologic studies of subjects with AD.[10] Because previous studies have consistently shown that only 25 to 50% of patients are responders to the cholinesterase inhibitor tacrine and neuropathologic studies have indicated a greater deterioration in the cholinergic system, it seems reasonable to propose that *APOE*-ε4 patients with AD will fail to improve with this drug.[5,6,10]

Gender was not associated with any differences in clinical response to tacrine in one previous analysis.[11] However, a subanalysis of data from another recent trial suggested that women receiving estrogen replacement therapy were likely to have a large magnitude of improvement with tacrine than those who were not.[12] Also, another study suggested that women heterozygous for ε4 are more likely to develop AD at an earlier age than men heterozygous for ε4.[13] It seems reasonable to propose an interaction between gender and *APOE* type on treatment effect. The aim of this study is to determine the effects of *APOE* genotype and gender on clinical response to tacrine in patients with AD.

Methods. In the present study, all eligible patients are men and women diagnosed by NINCDS-ADRDA criteria with probable AD.[14] They were all at least 50 years of age and mildly to moderately impaired in their cognitive functions at baseline, as estimated by Mini-Mental State Examination (MMSE) scores of 10 to 26 inclusive.[15] Patients were otherwise healthy and met inclusion criteria as described in the original report of this trial.[6] Patients were only included if a caregiver was available to ensure compliance with the study regimen and to complete patient assessments. Patients and their legal representatives or caregivers provided written informed consent.

Study design. This was a 30-week, double-blind, placebo-controlled trial that incorporated forced escalation of dosage in the tacrine-treated groups at 6-week intervals.[6] Medication was given in four daily doses. There were four treatment groups. Patients in Group 1 received placebo for the entire 30 weeks. Patients in Group 2 received 40 mg/day of tacrine for 6 weeks and then 80 mg/day for 24 weeks. Patients in Group 3 received 40 mg/day for 6 weeks, 80 mg/day for 6 weeks, and 120 mg/day for 18 weeks. Group 4 received 40 mg/day for 6 weeks, 80 mg/day for 6 weeks, 120 mg/day for 6 weeks, and 160 mg/day for the last 12 weeks. Patients were randomly assigned to groups, but the numbers were unequal (Group 1, n = 3; Group 2, n 1; Group 3, n = 3, Group 4, n = 4) with more patients assigned to Group 4 in anticipation of a higher dropout rate from cholinergic side effects. Patients who completed 30 weeks were eligible to receive open-label tacrine, as were patients who dropped out of the double-blind trial because of side effects. Patients were discontinued from the study if serum alanine aminotransferase (ALT) levels exceeded three times the upper limits of normal but were eligible for open-label tacrine 30 days after ALT levels returned to normal limits. Efficacy assessments were performed at baseline and at 6-week intervals during the study. Plasma samples were collected at 6-week intervals during the study with an aliquot saved at −70 °C in a freezer. *APOE* types were determined from frozen samples as previously described.[16]

Outcome measures. The outcome measures used in this analysis were the Alzheimer's Disease Assessment Scale (ADAS), the ADAS-Cognitive component (ADAS-Cog), the Clinician Interview-Based Impression (CIBI), the MMSE, and the caregiver-rated Clinical Global Impression of Change (CGIC).[17-20] The ADAS measures cognition and behavioral dysfunction found in AD subjects on a 0- to 115-point scale. The cognitive component of this scale (ADAS-Cog) is the primary measure on which our statistical analyses were focused. It objectively measures memory, language, and praxis on a 70-point scale. Decreasing scores in this scale indicate improvement. The CIBI is a physician's interview-based global rating of a patient's functions relative to a baseline established at the beginning of the study. No input is allowed from family, clinic staff, or the results of neuropsychological testing. A seven-point rating scale is used of 1 (very much better) to 7 (very much worse) with 4 being "no change." The MMSE is a brief, standardized evaluation of cognitive function with scores ranging from 0 to 30 points, with a score of 24 or above considered normal. The caregiver also provided a global evaluation of the patients' overall status relative to baseline (caregiver-rated CGIC).

Statistical analyses. The primary outcome was the change in ADAS-Cog score from baseline to week 30. Most major analyses were also performed for changes in MMSE and ADAS and for CIBI and CGIC at week 30. The evaluable population consisted of all patients who completed the 30-week trial in double-blind on study medication. The intent-to-treat (ITT) population comprised all patients who completed 30 weeks on study medication, retrieved dropouts who were evaluated at 30 weeks, and if an evaluation at 30 weeks was not available, the last observation carried forward. These analyses were also performed in both the ITT and evaluable patient groups to establish consistency within the study. In preliminary analyses, *t*-tests were used to compare the outcomes between the two genotype groups (defined by presence or absence of ε4) and between men and women. Baseline characteristics were similarly compared between the ε2-3 and ε4 groups within each of the placebo and combined tacrine groups, using *t*-tests and chi-square tests. To investigate where the treatment effect of tacrine was modified by *APOE* genotype, we used two-way ANOVA, which included the effects of treatment, *APOE* type, and their interaction on the outcome variables. The interaction of gender and treatment was similarly investigated. To examine the combined effects of *APOE* type and gender on the treatment effect, we used a three-way ANOVA model that included three-way interaction between *APOE*, gender, and treatment. When a significant three-way interaction was detected, we used two-way ANOVA to investigate the interaction between *APOE* type and treatment within each gender. Multiple regression analyses were also performed to adjust for other covariates such as age and baseline disease stage.

Secondary analyses included the estimation of changes in ADAS-Cog within each *APOE*-by-gender-by-treatment

Table 1 Baseline characteristics of 528 ITT patients by APOE-ε4 *and treatment*

	Placebo		Tacrine	
	ε2-3	ε4	ε2-3	ε4
n	62	92	129	245
Gender: male (%)	47	49	52	45
Race: white (%)	98	95	98	92
Education (%)				
Elementary	11	11	15	10
High school	50	48	39	42
College	39	41	46	48
Age, y (mean ± SD)	72.5 ± 9.8	72.3 ± 7.0	72.8 ± 9.4	72.7 ± 7.0
ADAS-Cog (mean ± SD)	31.6 ± 11.7	29.2 ± 11.9	28.8 ± 11.5	28.6 ± 11.7
Completed 30 weeks	45 (73%)	60 (65%)	42 (33%)	86 (35%)

ADAS-Cog = Alzheimer's Disease Assessment Scale-Cognitive component.

subgroup. Specifically, the changes over the course of 30 weeks were plotted for the placebo groups to reflect the natural course of disease for the four *APOE*-by-gender subgroups. To display the interaction of *APOE* genotype and gender with treatment in more clinically meaningful ways, we categorized the 30-week change in ADAS-Cog by whether the score had increased or decreased by more than four points, which is generally regarded as a clinically important change.[21] The distribution of response categories was plotted for the two genotype groups within each gender. Finally, the mean change in ADAS-Cog was tabulated by dose of tacrine for each of the gender-by-*APOE* subgroups.

Results. In the period between October 1991 and June 1992, 663 patients (316 men and 347 women) were randomized to study medication with a large subset of patients also having *APOE* type available for both evaluable and ITT patient analysis. Of these patients, *APOE* genotypes were obtained from retrieved plasma samples in 528 subjects (277 women and 251 men). There were 20 patients (4%) homozygous for ε4 and 317 (60%) heterozygous for this polymorphism. There were 191 patients (36%) heterozygous or homozygous with combination of ε2 and ε3. Because the number of ε4 homozygotes was small, these patients were added together with the ε4 heterozygotes into a combined ε4 group. All patients with only ε2 and/or ε3 alleles were put together in a combined ε2-3 group.

Baseline characteristics. The baseline characteristics of the ITT population with *APOE* types are presented for four subgroups cross-classified by treatment (tacrine versus placebo) and *APOE* type (ε4 versus ε2-3) in table 1. In all groups, there was an approximately equal proportion of men and women and a somewhat lower percentage of minority patients participating than are represented in the general population. The distribution of gender, race, and education and mean age did not differ significantly across the four groups ($p > 0.1$). The mean ADAS-Cog scores were also similar across all four groups. None of these characteristics differed between the patients who did or did not have *APOE* types available (data not shown). Of the 528 ITT patients, 233 went on to complete 30 weeks, with the proportion of evaluable patients substantially higher (>65%) in the placebo groups than in the tacrine groups (<35%).

Interaction of APOE-ε4 *and gender with tacrine on ADAS-Cog scores.* In these analyses, the outcome variable was the change in ADAS-Cog score from baseline to 30 weeks. *APOE* type was coded as ε4 versus ε2-3 and the treatment effect was coded as tacrine (any dose) versus placebo. In our analysis, there was no significant interaction between *APOE* type and treatment in both the ITT and the evaluable populations ($p > 0.2$), although the estimated mean treatment effect in the ε2-3 group was about twice as large as that in the ε4 group (−2.62 versus −1.25 for ITT, −4.2 versus −2.0 for evaluables). Gender, by itself, also had no significant interaction with treatment on the outcome. However, when the combined effects of *APOE* type and gender on treatment effects were considered, there was a significant interaction among *APOE*, gender, and treatment on the outcome. Table 2 summarizes the mean change in ADAS-Cog scores for each of the eight subgroups defined by gender, *APOE*, and treatment. These results are graphically displayed in figure 1 for the evaluable patients.

A significant three-way interaction among *APOE*, gender, and treatment meant that the treatment effect depended on the gender-*APOE* combination of the patients. Therefore, we investigated the treatment effect in the various subgroups to examine how treatment effect varies with *APOE* and gender. Because the response of patients on placebo could affect the estimate of treatment effects, we first examined the mean change in ADAS-Cog from baseline to 30 weeks in the four gender-by-*APOE* subgroups. From table 2, we can see that both male groups on placebo had a significant deterioration, whereas women deteriorated only in the ε2-3 group but not in the ε4 group. The trend was similar between the overall ITT group and the subgroup of evaluable patients. The change in ADAS-Cog from baseline is plotted every 6 weeks through week 30 for the four subgroups of evaluable patients in figure 2; this reflects the natural course of cognitive decline in AD patients by gender and *APOE* type. When we examined the patients on tacrine, neither the ITT or evaluable patients had a significant change in mean ADAS-Cog from

Table 2 *Change in ADAS-Cog scores* from baseline to 30 weeks by gender,* APOE *genotype, and treatment*

			ITT		Evaluables	
Gender	*APOE*	Treatment	n	Change in ADAS-Cog from baseline	n	Change in ADAS-Cog from baseline
Men	ε2-3	Tacrine	66	1.30 ± 0.74	30	1.10 ± 1.12
		Placebo	29	3.21 ± 1.56†	22	4.09 ± 1.88†
		Difference		−1.91		−2.99
Men	ε-4	Tacrine	108	−0.14 ± 0.69	46	−1.58 ± 1.05
		Placebo	43	3.30 ± 0.91	28	2.00 ± 1.14
		Difference		−3.44‡		−3.58‡
Women	ε2-3	Tacrine	62	0.66 ± 0.96	12	4.25 ± 2.35
		Placebo	31	4.0 ± 1.05	21	3.43 ± 1.25†
		Difference		−3.34‡		7.68‡
Women	ε-4	Tacrine	134	1.22 ± 0.64	37	−0.84 ± 1.03
		Placebo	46	0.30 ± 0.96	31	−0.35 ± 1.24
		Difference		0.92		−0.49

* Negative change indicates improvement.
† Significantly different from zero ($p < 0.05$) indicating significant change from baseline to 30 weeks.
‡ Significant difference between tacrine and placebo groups ($p < 0.05$).

ADAS-Cog = Alzheimer's Disease Assessment Scale-Cognitive component; ITT = intent-to-treat.

baseline to 30 weeks in any of the gender-by-*APOE* subgroups.

When tacrine was compared with placebo in each of the four gender-by-*APOE* subgroups, a significant treatment effect (tacrine versus placebo) was found in the ε4 subgroup of men and in the ε2-3 subgroup of women. This finding was consistent in both the ITT and evaluable analyses (table 2).

When ANOVA was performed separately for men and women, we observed that the treatment effect (tacrine versus placebo) differed significantly between ε2-3 and ε4 groups of women for both the ITT ($p = 0.03$) and for the evaluable ($p = 0.01$) analyses. In both cases, the treatment effect was significantly larger in the ε2-3 group than in the ε4 group by a mean difference of 4.24 points in the ITT and 7.20 points in the evaluable analyses. In contrast, the treatment effect did not differ between the two *APOE* types in men in either the ITT ($p > 0.4$) or the evaluable ($p > 0.8$) analyses.

Analyses adjusting for the covariates age, baseline score, and disease stage at entry did not change the direction, magnitude, or significance of the observed differences seen between the *APOE* and gender-defined groups.

To display the differences in clinically meaningful responses to treatment, we divided the above patient groups into those whose ADAS-Cog from baseline to 30 weeks worsened by greater than 4 points, deteriorated by 0 to 4 points, improved by 0 to 4 points, and improved by greater than 4 points. In the ε2-3 and ε4 male groups, the percentages of subjects that improved or worsened was not markedly influenced by *APOE* genotype (data not shown). However, the pattern of the women's differential response to placebo or tacrine therapy varied substantially between the two *APOE* types (figure 3 for evaluable women). The pattern of response for *APOE*-ε4 women treated with tacrine was virtually the same as for those treated with placebo. In contrast, among ε2-3 women, 75% of placebo patients had worsened by the end of 30 weeks, whereas only 17% of those on tacrine had deteriorated from baseline.

Dose-response. In the evaluable population, we examined the effects of tacrine dose on each genotype and gender-determined subgroup. In both ε2-3 and ε4 men, a modest treatment response was seen in those taking 160 mg/day tacrine (table 3). In contrast, ε2-3 women exhibited a much larger treatment response in both the 120- and 160-mg/day subgroups. However, ε4 women showed little response to treatment regardless of the dose.

Other outcome measures. The MMSE, CIBI, and the CGIC all showed similar patterns as those seen using the ADAS-Cog for groups defined by *APOE* type and gender (table 4). For all outcomes, the estimated treatment effect (tacrine versus placebo) in men is similar between the genotype groups or even slightly stronger in the *APOE*-ε4 group, whereas the treatment effect in women is stronger in the *APOE*-ε2-3 group than in the *APOE*-ε4 group. However, only ADAS showed a significant three-way interaction between gender, genotype, and treatment. Overall, the results are consistent across various outcome measures.

Discussion. The aim of the present study was to determine the effects of *APOE* genotype and gender

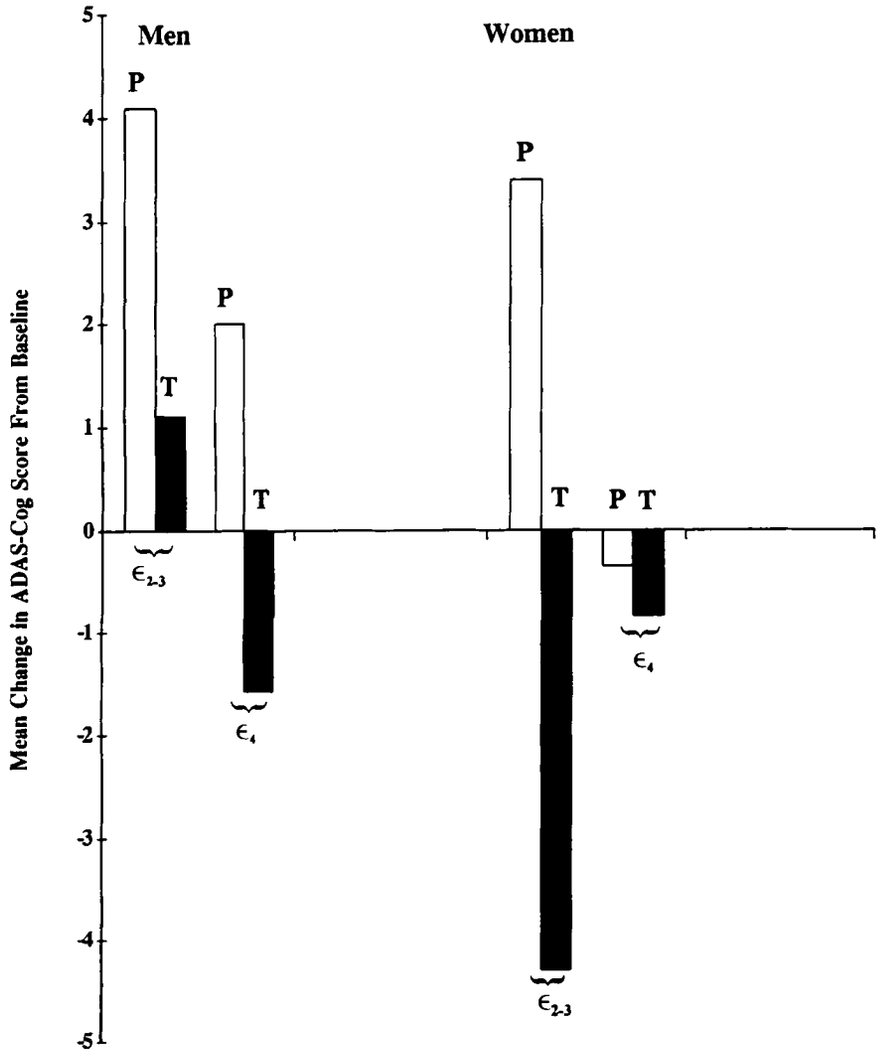

Figure 1. Effect of placebo and tacrine on the cognitive ability of men and women with different APOE *genotypes. The mean changes in ADAS-Cog score from baseline were shown with different subjects divided by either treatment, gender, or genotype. The treatment group includes all patients on tacrine at three different doses: 80, 120, and 160 mg. Results were presented for men (left) and women (right) with either ϵ2-3 or ϵ4 genotype. Both placebo and tacrine-treated subjects were separately indicated for each genotype shown here. Open bars indicate placebo; solid bars indicate tacrine.*

on clinical response to treatment with placebo and tacrine in patients with AD. This study was based on previous reports that showed mild improvement in memory and cognitive function of approximately 25 to 50% of treated patients and from AD autopsy studies suggesting that *APOE*-ϵ4 is associated with decreased numbers of cholinergic markers in temporal cortex and the hippocampus.[6,7,22]

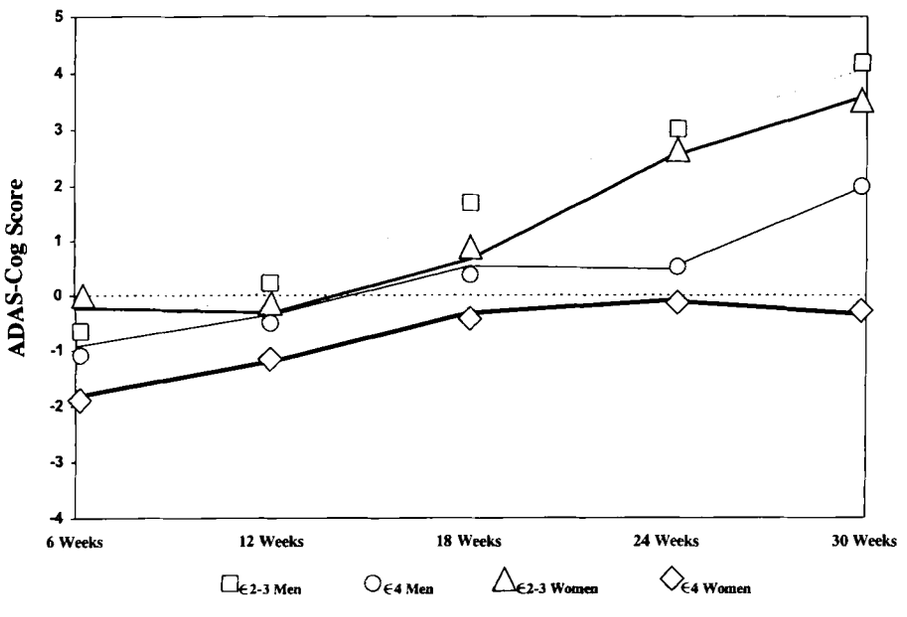

Figure 2. Effect of placebo and APOE *genotype on the progression of disease as measured by ADAS-Cog at different times. The mean changes in ADAS-Cog score from baseline were shown for subjects belonging to ϵ2-3 men (□), ϵ4 men (○), ϵ2-3 women (△), or ϵ4 women (◇). Improvement or deterioration of the subjects on the ADAS-Cog scale was determined at intervals of 6, 12, 18, 24, and 30 weeks.*

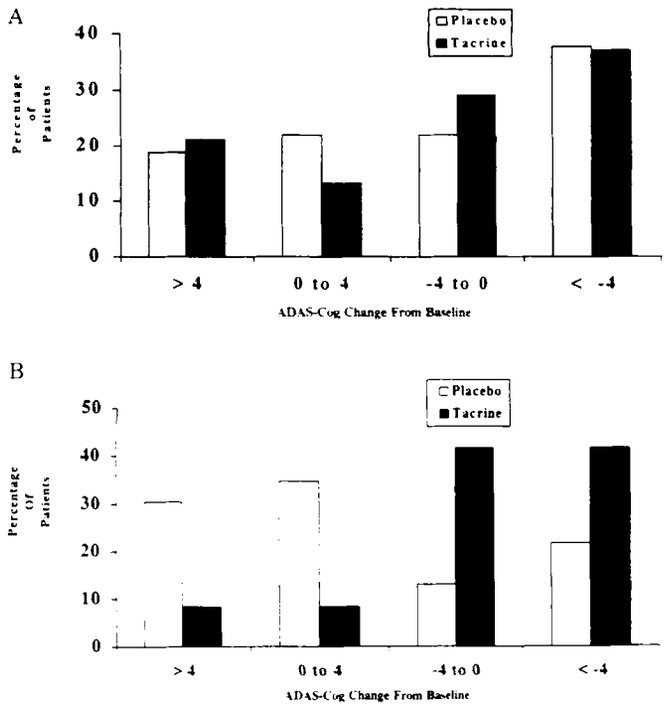

Figure 3. Effect of placebo and tacrine on patients in four subgroups based on ADAS-Cog change. Percentage of patients that exhibited different ADAS-Cog change from baseline was shown for either APOE-*ε4 women (A) or* APOE-*ε2-ε3 women (B). The analysis was performed by dividing patient groups into those who from baseline after 30 weeks on ADAS-Cog worsened by greater than 4 points (>4), deteriorated by 0 to 4 points (0 to 4), improved by 0 to 4 points (−4 to 0), and improved by greater than 4 points (< −4). In each category, the results were presented for both placebo and tacrine-treated subjects.*

In this study of 528 patients, a significant difference was observed with *APOE*-ε4 genotype influencing the rate of change in placebo-treated patients with AD. Surprisingly, placebo ε4 patients over the course of 30 weeks appeared to change at a much slower rate than ε2-3 patients. Slower rate of change or progression in ε4 patients was previously reported in a longitudinal study examining disease progression in patients with AD who were not participating in a drug trial.[23] Two other smaller studies suggested no significant relationship between *APOE* type and disease course.[24,25] It has also been reported that ε4 patients with an isolated memory deficit (possible prodromal AD) followed in a prospective study were more likely to progress to AD than similar ε2-3 patients over the course of 3 years.[26] It is thus possible that genotype may influence rates of disease progression differently during various stages of the illness. Whether these results are generalization or somewhat specific to this class study population remains to be determined.

With regard to response to cholinesterase therapy, the original proposed hypothesis that AD patients with the ε4 genotype would be less responsive to treatment was supported.[22] There does clearly seem to be greater response with tacrine therapy in the ε2-3 group as compared with AD patients in the ε4 group; however, it is much less clear that the reason for greater response in the ε2-3 group relates to more intact cholinergic function in these patients. The differences observed between these groups largely relates to more rapid deterioration in measures of cognitive function in the ε2-3 placebo group compared with the ε4 placebo group. This phenomenon is not age related because the ε2-3 and ε4 placebo groups had similar mean ages. Treatment with tacrine appeared to at least temporarily delay this decline in cognitive function for ε2-3 AD patients. The lack of significant difference with treatment in the ε4 group versus the ε2-3 group would not be consistent with previous neuropathologic studies involving older patients, possibly with more aggressive illness. This may suggest greater deterioration in the cholin-

Table 3 *Change in ADAS-Cog* from baseline to 30 weeks by gender,* APOE *genotype, and dose group of tacrine*

		Placebo		80 mg		120 mg		160 mg	
		n	Mean (SE)	n	Mean (SE)	n	Mean (SE)	n	Mean (SE)
ITT									
Men	ε2-3	29	3.2 (1.6)	5	3.2 (2.3)	18	2.4 (1.1)	43	0.6 (1.0)
	ε4	43	3.3 (0.9)	19	2.5 (1.7)	42	−0.2 (1.1)	47	−1.2 (1.0)
Women	ε2-3	31	4.0 (1.0)	8	−1.0 (2.5)	24	0.5 (1.6)	30	1.3 (1.4)
	ε4	46	0.3 (1.0)	15	−0.3 (1.5)	57	1.3 (1.0)	62	1.5 (1.0)
Evaluables									
Men	ε2-3	22	4.1 (1.9)	3	1.3 (3.5)	7	2.6 (1.1)	19	0.5 (1.6)
	ε4	26	2.0 (1.1)	7	−0.7 (2.3)	23	0.1 (1.7)	16	−4.4 (1.3)
Women	ε2-3	21	3.4 (1.3)	2	−1.0 ( )	3	−6.3 (4.1)	7	−4.3 (3.8)
	ε4	31	−0.4 (1.2)	10	−0.7 (1.8)	17	−0.2 (1.6)	10	−2.1 (2.1)

* Negative change indicates improvement.

ADAS-Cog = Alzheimer's Disease Assessment Scale cognitive component; ITT = intent-to-treat.

Table 4 *Four measures of change* from baseline to 30 weeks by gender,* APOE *genotype, and treatment for ITT and evaluable patients*

			ADAS†		CIBI		CGIC		MMSE	
			n	Mean (SE)	n	Mean (SE)	n	Mean (SE)	n	Mean (SE)
ITT										
Men	ε2-3	Placebo	29	3.0 (1.6)	29	4.2 (0.2)	29	44.3 (4.8)	28	−0.4 (0.7)
		Tacrine	66	1.6 (0.9)	66	4.0 (0.1)	38	55.1 (4.0)	64	0.2 (0.4)
	ε4	Placebo	43	5.3 (1.3)	44	4.3 (0.1)	37	44.9 (4.2)	43	−1.3 (0.6)
		Tacrine	108	0.0 (0.9)‡	110	4.0 (0.1)	62	46.8 (3.1)	103	0.3 (0.4)‡
Women	ε2-3	Placebo	31	6.4 (1.7)	32	4.4 (0.2)	25	33.1 (3.6)	30	−1.2 (0.6)
		Tacrine	62	0.5 (1.3)‡	62	4.1 (0.1)	24	48.7 (3.9)‡	60	−0.7 (0.5)
	ε4	Placebo	46	0.7 (1.4)	47	4.1 (0.1)	39	50.4 (3.1)	45	−0.4 (0.5)
		Tacrine	133	2.6 (0.9)	135	4.2 (1.0)	60	46.1 (2.7)	123	−0.2 (0.3)
Evaluables										
Men	ε2-3	Placebo	22	3.9 (2.0)	22	4.3 (0.2)	21	45.2 (5.2)	21	−0.9 (0.9)
		Tacrine	29	1.3 (1.4)	30	3.8 (0.2)	29	57.6 (4.9)	28	1.0 (0.7)
	ε4	Placebo	26	3.1 (1.4)	28	4.3 (0.2)	28	46.2 (4.9)	27	−0.9 (0.7)
		Tacrine	46	−1.1 (1.4)‡	48	3.9 (0.1)	47	48.3 (3.8)	48	0.6 (0.6)
Women	ε2-3	Placebo	21	4.3 (1.8)	23	4.3 (0.2)	22	33.7 (4.1)	23	−0.4 (0.7)
		Tacrine	12	−4.8 (3.2)‡	12	3.7 (0.3)	12	51.5 (6.1)‡	12	2.2 (0.9)‡
	ε4	Placebo	31	0.0 (1.7)	32	4.0 (0.2)	31	54.7 (3.3)	31	−0.1 (0.6)
		Tacrine	37	−0.2 (1.7)	38	4.0 (0.2)	38	50.2 (3.5)	37	0.9 (0.6)

* Lower values of ADAS and CIBI and higher values of CGIC and MMSE reflect improvement.
† Significant gender × *APOE*-ε4 × treatment interaction ($p < 0.002$ for ITT, $p < 0.05$ for evaluables).
‡ Significantly different from placebo within the same gender × *APOE*-ε4 subgroup ($p < 0.05$).

ITT = intent-to-treat; ADAS = Alzheimer's Disease Assessment Scale; CIBI = Clinician Interview Based Impression; CGIC = Clinical Global Impression of Change; MMSE = Mini-Mental State Examination.

ergic systems of the CNS of the ε4 patients.[22] Proposing a proper hypothesis to explain the following results is complicated by having to also account for the influence of gender; in men, the effects of *APOE* genotype on treatment response are modest and do not seem to change the magnitude of improvement achieved with drug therapy versus baseline or versus placebo. ε4 men seem to respond about as well to tacrine as ε2-3 men with AD. However, ε4 women treated with tacrine had very little change from baseline or in comparison at 30 weeks with their corresponding placebo groups. Cholinesterase inhibition appears to have little effect on cognitive function in this group. In contrast, ε2-3 women respond significantly better than all other groups versus baseline and versus their corresponding placebo group. The ε2-3 women on placebo deteriorated more than any other group.

The mechanism for a gender-specific *APOE* genotype interaction with response to cholinesterase therapy is not fully understood. One can speculate that higher levels of estrogen in women amplify the effects of apoE, possibly by changing circulating levels of the protein or its ability to deliver lipids in the CNS. It has recently been reported that women heterozygous for ε4 may have greater probability for developing AD at an earlier age than heterozygous ε4 men.[13] Data from other reports show similar statistical trends but do not quite achieve clinical significance.[27,28] It has also been reported that the lifetime risk of AD for relatives of identified male probands with probable AD and the ε3-4 genotype is similar to that seen in male probands with the ε3-3 genotype.[29] In this study, most men had the ε3-4 genotype. We postulate that patients in this group may have similar levels of cholinergic dysfunction and probabilities of responding to cholinesterase therapy as men who carry the ε3-3 genotype. In women, however, the ε3-4 genotype confers almost the same greater lifetime risk of AD at earlier age of onset as the ε4-4 genotype.[29] Because increased risk and lower age of onset of AD are associated with the ε3-4 genotype in women, it seems reasonable to propose more deterioration in cholinergic function similar to that seen at autopsy in homozygous ε4 patients. This hypothesis would explain decreased responsiveness to cholinesterase therapy for these women but would not be consistent with the lack of deterioration seen in the corresponding placebo group, which showed the slowest rate of disease progression. The mechanism underlying these observations remains to be elucidated. To understand whether other factors besides *APOE* genotypes played a role in treatment outcomes, we analyzed the effects of the level of educa-

tion, race, and age on the treatment effects. We did not observe any significant differences because of the limited range in the selection criteria.

In this clinical trial, patients were highly selected on the basis of good health and relatively early onset with the result that both gender- and *APOE* genotype-defined groups had a mean age of 72. Therefore, older AD patients were not well represented, and the sample has a selection bias that may influence the rate of change of subjects after randomized intervention. Although not representative of the general population, this is an appropriate sample to study the interaction of tacrine with gender and *APOE* status.

The mechanisms by which gender and *APOE* status influence the age of onset of AD, and now possibly response to cholinesterase therapy, remain relatively mysterious. Future studies examining estrogen and apoE levels in women may be helpful and may develop methodology to more accurately quantitate CNS cholinergic dysfunction in living patients. *APOE* genotype may also influence treatment response of other drugs that are being investigated for their ability to delay disease progression. For example, in vitro studies have suggested that apoE4 protein may be less effective in neutralizing free radicals that are potentially toxic to cortical neurons.[30] If free radical-mediated toxicity does play a role in AD, it might be predicted that potentially protective medications such as vitamin E and selegeline would be more effective in patients carrying one or more copies of the $\epsilon 4$ allele.

Newly discovered genes such as presenilin-1 and presenilin-2 may interact in ways that are not yet known.[31] It is known that *APOE* genotype modulates the age of onset in families with mutations in the beta amyloid precursor protein (APP) but not in families with chromosome 14-linked early-onset AD.[32] Whether there are *APOE* genotype-associated difference in damage to the cholinergic system that are present in APP but not chromosome 14-linked patients with AD remains to be determined. There is evidence from the cell culture studies demonstrating that apoE3 and apoE4 have differential effects on neuronal growth.[33] Moreover, apoE4 interacts more readily than apoE3 protein with the amyloidogenic fragment of APP.[34] APP has also been shown to be regulated by the drug, tacrine, in neuronal cultures.[35] The mechanisms, through which *APOE* type and processing of APP by tacrine and estrogen influence cholinomimetic therapy, remain to be fully explored.

As with all retrospective analyses, the present study suggests, but does not prove, a significant relationship between gender, *APOE* genotype, and response to cholinesterase therapy in patients with AD. The described differences in this retrospective analysis must be demonstrated prospectively in a treatment trial. The differences seen in placebo group progression are consistent with previously reported longitudinal data after progression in AD and suggest that it would be helpful to stratify patients in future drug trials by *APOE* genotype. In the future, *APOE* genotype may prove to be a useful predictor of response to therapy in these patients, either alone or in conjunction with other biological markers.

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Second impact syndrome

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Article abstract—Diffuse cerebral swelling with delayed catastrophic deterioration, a known complication of brain trauma, has been postulated to occur after repeated concussive brain injury in sports—the "second impact syndrome" (SIS). Certain current concussion management guidelines are contingent upon this assumption. We established criteria for definite, probable, and possible SIS and analyzed all published cases. A total of 17 cases were identified in which the reports described the cases as being consistent with SIS. Of these, only five probable cases of SIS were found based on our diagnostic criteria. We also studied the accuracy of recalled episodes of minor concussion in football players by their teammates because the diagnosis of SIS is usually based on such accounts. We found overreporting of recalled episodes of concussion in teammates when compared with self reports and videotape analysis. Based on case reports, the claim that SIS is a risk factor for diffuse cerebral swelling is not established. Prevention strategies for sports-related cerebral swelling are difficult to implement in the absence of established risk factors.

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Diffuse cerebral swelling is a rare but well recognized cause of delayed catastrophic deterioration resulting in death or persistent vegetative state after an apparently minor head injury. Risk factors for this posttraumatic complication have not been clearly established, although most cases have occurred in children and adolescents.[1-8] This phenomenon has also been observed in collision sports. It has been postulated that a specific form of cerebral swelling may be the consequence of a repeated minor head injury. Specifically, the "second impact syndrome" (SIS) has been defined as occurring when "an athlete who has sustained an initial head injury, most often a concussion, sustains a second head injury before symptoms associated with the first have fully cleared" (p. 27).[9] It is postulated that this second impact sets in motion the rapid development of cerebral vascular congestion which in turn causes increased intracranial pressure, usually resulting in brainstem herniation and death.[9]

Documentation of a witnessed initial impact is crucial to the central issue as to whether repeated

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