

# *APOE*- $\epsilon$ 4 Carrier Status and Donepezil Response in Patients with Alzheimer's Disease

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## Abstract.

**Background:** Previous studies have investigated associations between apolipoprotein E (*APOE*)- $\epsilon$ 4 allele status and acetylcholinesterase inhibitor treatment response in patients with Alzheimer's disease. The ability to draw definitive conclusions regarding the effect of *APOE*- $\epsilon$ 4 genotype on treatment response has been hindered by inconsistent results among studies and methodological limitations that restrict interpretation of study findings.

**Objective:** To determine whether *APOE*- $\epsilon$ 4 carrier status influences the magnitude of change in 13-item Alzheimer's Disease Assessment Scale–Cognitive Subscale (ADAS-cog) score associated with acetylcholinesterase inhibitor treatment (i.e., donepezil).

**Methods:** Analyses were performed using pooled data from the donepezil and placebo treatment arms of three consecutive, similarly designed, 12-week, multi-national, randomized clinical studies that enrolled patients with mild-to-moderate Alzheimer's disease. Correlations between *APOE*- $\epsilon$ 4 carrier status and ADAS-cog scores were evaluated using analysis of covariance.

**Results:** No appreciable interaction between donepezil response and *APOE*- $\epsilon$ 4 carrier status or copy number was detected. Both carriers and non-carriers of *APOE*- $\epsilon$ 4 who received donepezil experienced significant improvements from baseline in ADAS-cog score versus placebo ( $p < 0.05$ ). Change from baseline to final observation in the donepezil treatment group was  $-2.95$  for *APOE*- $\epsilon$ 4 carriers and  $-4.09$  for non-carriers ( $p = 0.23$ ). In contrast, non-carriers of *APOE*- $\epsilon$ 4 in the placebo treatment group exhibited a greater improvement from baseline versus carriers ( $-2.38$  versus  $-0.60$ ,  $p = 0.05$ ).

**Conclusion:** Within this population, *APOE* genotype had no statistically significant effect on cognitive response to donepezil treatment; however, *APOE*- $\epsilon$ 4 allele status was associated with a difference in the magnitude of the change in ADAS-cog of placebo-treated patients.

Keywords: Acetylcholinesterase inhibitors, apolipoprotein E, genotype, placebo effect, treatment efficacy

## INTRODUCTION

The association between the  $\epsilon$ 4 allele of the gene encoding apolipoprotein E (*APOE*) and risk for late-onset familial and sporadic forms of Alzheimer's disease (AD) was first established more than 20 years

ago [1–4]. Accumulated clinical data and epidemiologic evidence have since implicated *APOE*- $\epsilon$ 4 as playing an etiologic role in as many as 50% of AD cases in the United States [5]. There is an apparent dosage effect related to the *APOE*- $\epsilon$ 4 allele such that homozygosity confers a substantially greater risk for AD than does carrying a single copy of the  $\epsilon$ 4 allele [6]. Moreover, the number of *APOE*- $\epsilon$ 4 alleles is inversely correlated with age of AD presentation in patients with

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late-onset disease [7]. The *APOE-ε4* allele is present in an estimated 13% of the general population worldwide, with regional frequencies varying from a low of 7% in India to a high of 22% in Oceania [8]. As would be expected given the disease linkage, the *APOE-ε4* allele is more pervasive among patients with AD compared with the general population [3, 9].

Although clearly influential in the pathogenesis of AD, the effect of *APOE* genotype on an individual's response to treatment is less well understood. Clinical trial data suggest that the presence or absence of *APOE-ε4* contributes to therapeutic response for a range of AD drugs, both approved (e.g., acetylcholinesterase inhibitors) and investigational (e.g., thiazolidinediones, immunotherapy agents) [10–12]. As the most commonly prescribed class of medication used to treat AD, acetylcholinesterase inhibitors have been of particular interest in this respect. Multiple studies have examined the effect of *APOE-ε4* carrier status on treatment response to donepezil, an acetylcholinesterase inhibitor that is frequently used in clinical practice. The results of these analyses have been mixed, with several studies suggesting that *APOE-ε4* carrier status does not affect treatment response [13–15], yet other analyses indicating that the presence of the *APOE-ε4* allele is associated with greater treatment-related improvements [16, 17] (Table 1). A similar inconsistent pattern across studies has been observed with other acetyl-

cholinesterase inhibitors. There is some evidence for a better response to treatment among carriers of the *APOE-ε4* allele versus non-carriers [18]; however, a few studies have suggested the opposite relationship [19–21], and the majority of assessments have shown no correlation between *APOE-ε4* carrier status and treatment response [22–26]. In many cases, the small sample size and the exploratory nature of the analyses make interpretation of the results difficult. In addition, disparate outcomes among these studies can be attributed to a multitude of factors, including differences in characteristics of the study population (e.g., baseline AD severity, duration of treatment, country), allele distribution, study drug properties, outcome measure, and other factors.

To better understand the possible link between treatment response and *APOE-ε4* carrier status, data were pooled from the donepezil and placebo treatment arms of three consecutive, comparable 12-week, multinational clinical trials conducted in eight countries that together included more than 300 patients with mild-to-moderate AD who were treated with donepezil or placebo. Assessment of treatment response according to *APOE-ε4* genotype was a pre-specified subgroup analysis in each of the included studies. The resulting dataset has yielded the largest and most geographically diverse evaluation of a possible correlation between *APOE-ε4* carrier status and donepezil treatment response available to date.

Table 1  
Previous studies of *APOE-ε4* allele status and clinical response to acetylcholinesterase inhibitor treatment

Study	Sample Size/ No. <i>APOE-ε4</i> +	Drug; duration	Efficacy assessment	Results
Winblad et al. [13]	142 / 98	Donepezil; 52 weeks	GBS scale, MMSE	–
Rigaud et al. [14]	117 / 56	Donepezil; 36 weeks	ADAS-cog, MMSE, IADL, CGIC	–
Choi et al. [16]	51 / 18	Donepezil; 48 weeks	ADAS-cog, IADL	>
Bizzarro et al. [17]	41 / 29	Donepezil; 12 to 16 months	Neuropsychiatric battery	>(in certain tests)
Kanaya et al. [15]	40 / NP	Donepezil; 3 years	ADAS-cog, MMSE	–
Aerssens et al. [22]	569 / NP	Galantamine; 3 to 12 months	ADAS-cog, DAD	–
MacGowan et al. [23]	68 / 49 36 / 20	Tacrine, Galantamine; 3 months	MMSE	–
Farlow et al. [24]	585 / 349	Metrifonate; 26 weeks	ADAS-cog, CIBIC-Plus	–
Farlow et al. [25]	367 / 246 <sup>1</sup>	Rivastigmine; 26 weeks	ADAS-cog	–
Sjögren et al. [19]	145 / 84	Tacrine; 1 year	CIBIC, MMSE	<(for CIBIC)
Farlow et al. [20]	374 / 245	Tacrine; 30 weeks	ADAS-cog	< <sup>2</sup>
Rigaud et al. [26]	76 / 33	Tacrine; variable	ADAS-cog <sup>3</sup>	–
Poirier et al. [21]	40 / 22	Tacrine; 30 weeks	ADAS-cog	< <sup>4</sup>
Almkvist et al. [18]	24 / 14	Tacrine; single dose	Measures of attention	>

<sup>1</sup>Sample size includes both rivastigmine- and placebo-treated patients; <sup>2</sup>greater effect size in *APOE-ε2,3+* versus *APOE-ε4+* women; <sup>3</sup>responsiveness to treatment was defined as a 4-point decrease in ADAS-cog score; <sup>4</sup>greater response among *APOE-ε2,3+* versus *APOE-ε4+* patients. >, greater response in *APOE-ε4* carriers; <, Less response in *APOE-ε4* carriers; —, no significant difference between *APOE-ε4* carriers and non-carriers; *APOE*, apolipoprotein E; ADAS-cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; CGIC, Clinical Global Impression of Change; CIBIC, Clinician's Interview Based Impression of Change; CIBIC-Plus, Clinician's Interview Based Impression of Change with Caregiver Input; DAD, Disability Assessment for Dementia; GBS, Gottfries-Bråne-Steen Rating Scale; IADL, Instrumental Activities of Daily Living; MMSE, Mini-Mental State Examination.

## MATERIALS AND METHODS

### Patients

Adult men and women aged 55 to 90 years who were diagnosed with mild-to-moderate AD, as defined by the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria for probable AD [27], were eligible for inclusion in the studies. Study participants must have scored between 10 and 24 (inclusive) on the Mini-Mental State Examination (MMSE), 10 or below on the Cornell Scale for Depression in Dementia, and 4 or below on the Modified Hachinski Ischemic Scale at the time of the first screening visit. Computed tomography or magnetic resonance imaging must have been performed for each subject within 36 months prior to randomization to exclude alternative causes for dementia. With the exception of AD, patients were to be in generally good health as indicated by medical history, findings at physical examination, vital sign measurements, results from laboratory profile, and results from 12-lead electrocardiography. Patients who had a history of donepezil treatment failure; who were receiving or had received medication for the treatment of AD within 60 days prior to the screening visit; who were currently taking or

had taken a vitamin K antagonist within 30 days of the screening visit; or who had clinically significant disease, including a history of neurologic disorders (e.g., Parkinson's disease, multi-infarct or vascular dementia, Huntington's disease, etc.), were not eligible to participate in the study. All study participants provided written informed consent.

### Study design

Data from three phase 2, 12-week, multi-national, randomized, double-blind, parallel-group clinical trials designed to evaluate the safety and efficacy of investigational agents for the treatment of mild-to-moderate AD conducted from 2009 to 2011 were pooled for this analysis (Table 2) [28–30]. Eight countries enrolled patients in 1 or more of the studies. Participating countries included Bulgaria, the Czech Republic, Russia, Slovakia, South Africa, Ukraine, the United Kingdom, and the United States. The trials had the same study design, with a screening period of up to 28 days followed by randomization to treatment with a low or high dose of an investigational drug (ABT-126, ABT-288, or ABT-384), placebo, or active control (donepezil) for 12 weeks, and a subsequent 30-day follow-up period (Fig. 1). Primary efficacy results from all three studies revealed a trend toward

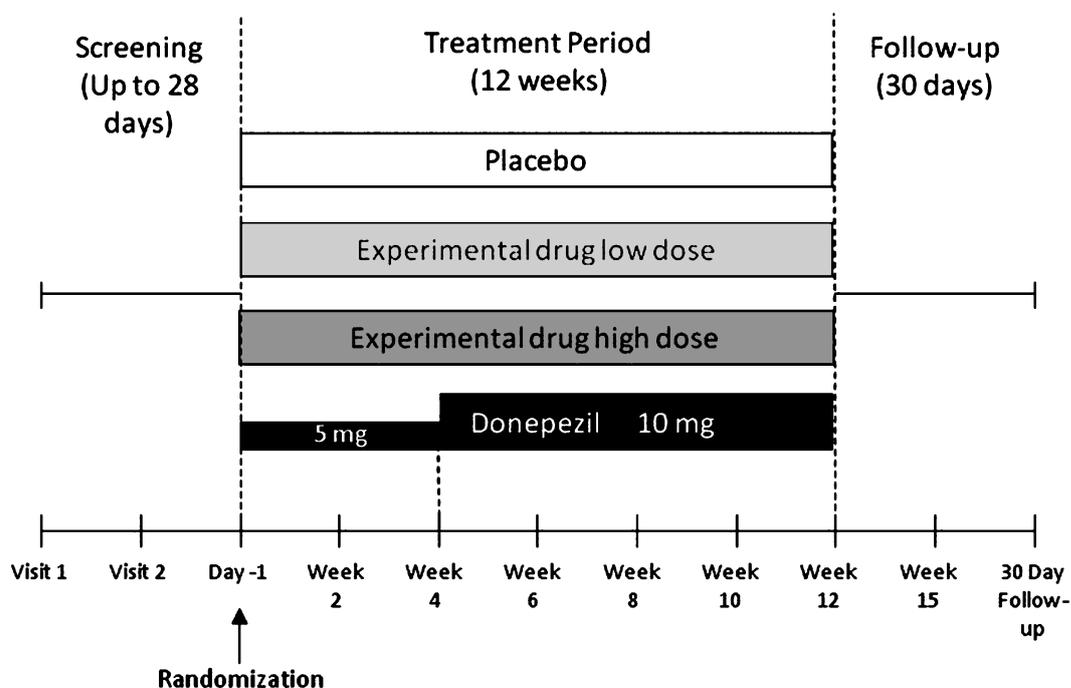


Fig. 1. Study schematic showing design shared among the three studies that contributed to the dataset. Only patients treated with placebo and donepezil were included in the analyses.

Table 2  
Descriptions of consecutive clinical trials by a single sponsor included in the analysis

Study Characteristic	Trial 1 [28] (NCT00948909)	Trial 2 [29] (NCT01018875)	Trial 3 [30] (NCT01137526)
Study drug	ABT-126	ABT-288	ABT-384
No. of study sites	27	21	30
Countries	Bulgaria, Czech Republic, Slovakia, South Africa, UK, US	Russia, Ukraine	Great Britain, Russia, South Africa, Ukraine
Study period	November 2009 to November 2010	December 2009 to February 2011	May 2010 to July 2011
Early termination due to futility	No	Yes	Yes
Patients randomized to all treatment groups, <i>n</i>	274	242	267
Patients randomized to placebo, <i>n</i> (% of total)	68 (24.8)	63 (26.0)	66 (24.7)
Patients randomized to donepezil, <i>n</i> (% of total)	68 (24.8)	60 (24.8)	66 (24.7)
Completed in all treatment groups, <i>n</i> (% of randomized)	257 (93.8)	153 (63.2) <sup>1</sup>	162 (60.7) <sup>2</sup>
Completed in placebo group, <i>n</i> (% of randomized)	65 (95.6)	38 (60.3)	44 (66.7)
Completed in donepezil group, <i>n</i> (% of randomized)	62 (91.2)	41 (68.3)	37 (56.1)

<sup>1</sup>68 (28.1%) patients discontinued early due to the sponsor decision to terminate the study for futility; <sup>2</sup>67 (25.1%) patients discontinued early due to the sponsor decision to terminate the study for futility; UK, United Kingdom; US, United States.

or a statistically significant improvement in the 13-item Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) among patients in the donepezil treatment arm [28–30]. Patients assigned to donepezil treatment were given a 5-mg dose once daily for the first 4 weeks of the treatment phase and a 10-mg dose once daily for the remaining 8 weeks, consistent with the dosing instructions in the product label. Study protocols received Institutional Review Board approval and study procedures were conducted in compliance with the principles of the Declaration of Helsinki and guidelines for Good Clinical Practice.

All three studies included protocol-specified, interim futility analyses to determine whether the experimental drug treatment arms were likely to demonstrate clinical benefit according to criteria specified in the Data Monitoring Committee charter. For two of the studies (NCT01018875 and NCT01137526), the criteria for futility were met and the studies were prematurely terminated; treatment was discontinued for all treatment arms and patients reported to their respective study sites for final study visits. Close to two-thirds of patients had completed the studies at the time of termination (Table 2) [29, 30].

#### Efficacy measures

Efficacy assessments were performed at 4, 8, and 12 weeks after treatment initiation. The ADAS-cog total score was generated by summing the component 13 items, which included word recall, commands, constructional praxis, delayed-word recall, naming objects and fingers, ideational praxis, orientation, word recognition, remembering test instructions, number cancellation, comprehension of spoken language,

spoken-language ability, and word-finding difficulty. Total scores for the 13-item ADAS-cog scale range from 0 to 85, with a higher score indicating greater impairment.

#### Sample collection and APOE-ε4 genotyping

Patients had the option of providing a blood sample for pharmacogenetic analysis; written informed consent was obtained for those who participated. One 4-mL whole blood sample for DNA isolation was collected into an ethylenediaminetetraacetic acid-containing tube from each consenting patient during the screening period and stored at –20°C until DNA extraction. DNA was isolated from whole blood using Qiagen reagent kits (Qiagen Inc, Valencia, CA) and applied to an AutoGenprep 3000 (AutoGen Inc, Holliston, MA) automated DNA extraction instrument.

Genotypes were determined for the following nucleotide APOE polymorphisms: 3937T>C (rs429358) and 4075C>T (rs7412). All genotypes were determined using the Pyrosequencing detection method (Pyrosequencing Inc, Westborough, MA) following standard operating procedures. Primer sequences for pyrosequencing were as follows: 5'-GGACATGGAGGACGTG-3' (APOE 3937T>C) and 5'-CCGATGACCTGCAGAAG-3' (APOE 4075C>T). Primer sequences used for allele-specific PCR included 5'-AAGGAGCTGCAGCGGCGCAGG-3' (forward) and 5'-GGATGGCGCTGAGGCCGCGC-3' (reverse).

#### Statistical analyses

Data for the intent-to-treat (ITT) and completer populations of the donepezil and placebo-treatment groups

were pooled from the three studies. The ITT population included all randomized patients who received at least 1 dose of study drug ( $n = 391$ ). For those individuals who did not complete the study, efficacy evaluations were performed using the last-observation-carried-forward method. The completer population consisted of patients in the ITT population who completed the full treatment period and had data available for the 12-week efficacy assessment ( $n = 287$ ). The primary analyses were performed using the ITT dataset. Results from the completer analysis were reported if the conclusions differed from those of the ITT analysis.

Evaluation of treatment response according to APOE-ε4 genotype was pre-specified in the study protocols. For the assessment of APOE-ε4 carrier status on treatment response, least square (LS) mean change from baseline to final evaluation in the ADAS-cog total score was analyzed using analysis of covariance with

effects of age, treatment, study site, APOE-ε4 allele status, APOE-ε4-by-treatment interaction and covariate of baseline ADAS-cog total score. To examine the effect of APOE-ε4 copy number on treatment response, the same model was applied with APOE-ε4 allele status replaced by APOE-ε4 copy number.

## RESULTS

### Patient characteristics

In the ITT population, demographics and baseline characteristics were comparable between the pooled placebo and pooled donepezil groups (Table 3). Approximately 57% of the patients were female and nearly two-thirds had not previously received an agent approved for the treatment of AD. Mean baseline MMSE scores were approximately 19, and MMSE

Table 3  
Subject demographics and baseline characteristics (ITT population)

Parameter	Placebo ( $n = 170$ )	Donepezil ( $n = 165$ )	Total ( $n = 335$ )
Gender, $n$ (%)			
Female	104 (61.2)	87 (52.7)	191 (57.0)
Male	66 (38.8)	78 (47.3)	144 (43.0)
Age, years <sup>1</sup>	72.2 (8.5)	71.6 (8.4)	71.9 (8.5)
Non-carriers <sup>2</sup>	71.1 (9.4)	71.1 (9.2)	
Carriers of 1 APOE-ε4 copy <sup>2</sup>	73.8 (7.9)	72.9 (7.5)	
Carriers of 2 APOE-ε4 copies <sup>2</sup>	70.8 (6.4)	66.1 (4.8)	
Baseline MMSE score <sup>1</sup>	19.2 (4.0)	19.1 (3.8)	19.2 (3.9)
Baseline 13-item ADAS-cog total score <sup>1</sup>	38.4 (12.9)	39.9 (13.4)	39.2 (13.2)
ITT population			
Non-carriers	38.6 (13.4)	38.8 (13.6)	
Carriers of 1 APOE-ε4 copy	37.9 (12.1)	41.6 (13.4)	
Carriers of 2 APOE-ε4 copies	39.6 (14.3)	37.0 (11.2)	
Completer population			
Non-carriers	37.9 (13.0)	38.7 (13.9)	
Carriers of 1 APOE-ε4 copy	36.4 (11.7)	42.0 (13.5)	
Carriers of 2 APOE-ε4 copies	37.3 (12.4)	36.8 (11.7)	
History of AD medication use, $n$ (%)			
Yes	62 (36.5)	59 (35.8)	121 (36.1)
No	108 (63.5)	106 (64.2)	214 (63.9)
Years of formal education <sup>1</sup>			
Non-carriers	12.4 (3.3)	11.3 (3.2)	
Carriers	11.6 (3.1)	12.0 (3.2)	
APOE-ε4 carrier status, $n$ (%) <sup>3,4</sup>			
Non-carriers	76 (44.7)	84 (50.9)	160 (47.8)
Carriers	94 (55.3)	81 (49.1)	175 (52.2)
APOE-ε4 copy number, $n$ (%) <sup>3,4</sup>			
0	76 (44.7)	84 (50.9)	160 (47.8)
1	72 (42.4)	72 (43.6)	144 (43.0)
2	22 (12.9)	9 (5.5)	31 (9.2)

Data are for the intent-to-treat (ITT) population unless otherwise noted. <sup>1</sup>Data are means (standard deviation); <sup>2</sup>Mean values are comparable in the completer population; <sup>3</sup>In the completer population, 61, 53, and 20 placebo-treated patients carried 0, 1, and 2 copies of the APOE-ε4 allele, respectively, for a total of 61 non-carriers and 73 carriers; <sup>4</sup>In the completer population, 67, 56, and 7 donepezil-treated patients carried 0, 1, and 2 copies of the APOE-ε4 allele, respectively, for a total of 67 non-carriers and 63 carriers; AD, Alzheimer's disease; ADAS-cog, Alzheimer's Disease Assessment Scale-Cognitive Subscale; APOE, apolipoprotein E; MMSE, Mini-Mental State Examination.

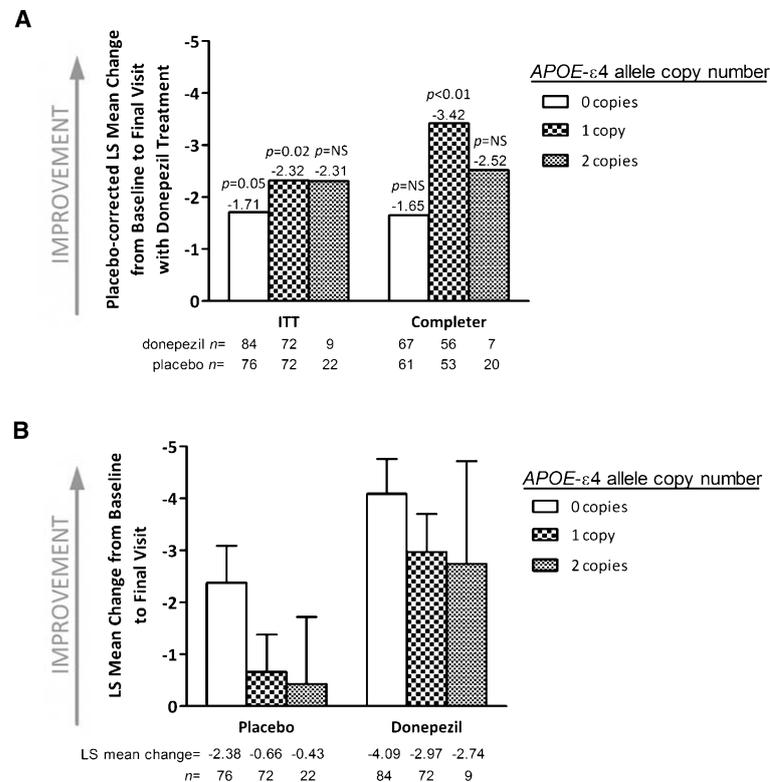


Fig. 2. Total score on the 13-item Alzheimer's Disease Assessment Scale-Cognitive Subscale by apolipoprotein E (*APOE*)- $\epsilon$ 4 carrier status. A) Placebo-corrected change from baseline to the final visit with donepezil treatment in the intent-to-treat (ITT) and completer populations. *p*-values are provided for the comparison of donepezil treatment effect versus placebo. B) Least square (LS) mean change from baseline values for the donepezil and placebo treatment groups (ITT population). Error bars in panel B represent standard error. NS, not significant.

and ADAS-cog scores were similar between patients assigned to placebo or donepezil. *APOE-ε4* genotype data were available for 170 out of 197 patients who received placebo and 165 out of 194 patients who received donepezil. Homozygosity for the *APOE-ε4* allele was more common among placebo-treated patients versus donepezil-treated patients (12.9% and 5.5%, respectively).

#### *Influence of APOE genotype on change from baseline to final observation in ADAS-cog score for placebo- and donepezil-treated patients*

Both *APOE-ε4* carriers and non-carriers showed a statistically significant improvement (indicated by a decrease in ADAS-cog total score) with donepezil treatment. The LS mean difference between donepezil- and placebo-treated patients in ADAS-cog at the final evaluation was  $-2.34$  for carriers and  $-1.71$  for non-carriers ( $p=0.01$  and  $p=0.05$ , respectively) in the ITT population (Fig. 2A). In the smaller completer population ( $n=264$ ), donepezil showed a statistically

significant treatment effect in the *APOE-ε4* carriers ( $n=136$ ,  $-3.53$ ;  $p<0.001$ ), but not among non-carriers ( $n=128$ ,  $-1.62$ ;  $p=0.113$ ). Overall, however, the interaction between *APOE-ε4* carrier status and treatment effect on the ADAS-cog was not significant in either the ITT ( $p=0.61$ ) or completer ( $p=0.20$ ) populations.

Improvement from baseline in mean ADAS-cog score with donepezil treatment was comparable in carriers and non-carriers of the *APOE-ε4* allele (Fig. 2B). In the ITT population, change from baseline to final observation in ADAS-cog was  $-2.95$  for *APOE-ε4* carriers and  $-4.09$  for non-carriers ( $p=0.23$ ). Similar results were observed within the completer population. Interestingly, *APOE-ε4* carrier status was associated with a difference in the magnitude of response on the ADAS-cog in placebo group. The LS mean change from baseline to final visit in ADAS-cog was significantly greater among *APOE-ε4* non-carriers versus carriers ( $-2.38$  and  $-0.60$ , respectively;  $p=0.05$ ). This divergence increased over time (Fig. 3) and was more apparent within the completer population ( $-3.34$  and  $0.13$ , respectively;  $p=0.002$ ). Together, these results

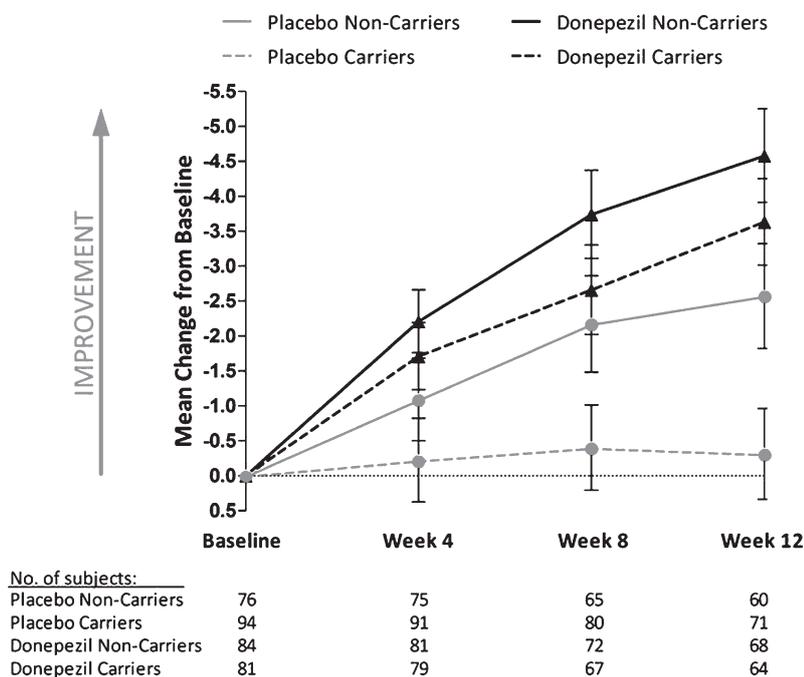


Fig. 3. Changes over time in mean Alzheimer's Disease Assessment Scale-Cognitive Subscale score stratified by treatment group and apolipoprotein E (*APOE*-ε4) carrier status (intent-to-treat population). Error bars represent standard error.

suggest that *APOE*-ε4 carrier status does not affect the magnitude of the improvement observed with donepezil treatment, but may be associated with a difference in the extent of the placebo response.

To rule out a possible influence of enrollment country on change in ADAS-cog score among *APOE*-ε4 non-carriers in the placebo treatment group, an analysis was performed using a statistical model that included the effect of study site within country, country, *APOE*-ε4 status, and covariates of subject age and baseline ADAS-cog score. The influence of country was determined to be insignificant, indicating that the results in the placebo group were not driven by outcomes from any single country.

#### *APOE*-ε4 copy number and ADAS-cog response

The influence of *APOE*-ε4 carrier status on the donepezil-placebo treatment difference and the change from baseline to final observation in donepezil- and placebo-treated patients was further investigated by subdividing the samples into specific genotype groups (i.e., non-carriers [ $n = 160$ ], heterozygous [ $n = 144$ ], and homozygous [ $n = 31$ ] *APOE*-ε4 patients). Within the ITT population, a significant donepezil treatment effect was observed relative to placebo among patients with 0 or 1 copy of the *APOE*-ε4 allele (Fig. 4A). The

difference in mean ADAS-cog score between patients treated with donepezil versus placebo was  $-1.71$  for non-carriers ( $p = 0.05$ ) and  $-2.32$  for patients with 1 *APOE*-ε4 allele ( $p = 0.02$ ). The treatment difference in patients homozygous for the *APOE*-ε4 allele was similar to that of *APOE*-ε4 heterozygotes ( $-2.31$  and  $-2.32$ , respectively), but did not achieve statistical significance, likely due to the comparatively small number of patients in the *APOE*-ε4 homozygote group. In the completer analysis, the only significant donepezil treatment effect was detected among carriers of 1 *APOE*-ε4 allele ( $-3.42$ ;  $p = 0.004$ ). Nonetheless, the interaction between *APOE*-ε4 copy number and treatment was not significant in either the ITT ( $p = 0.89$ ) or completer ( $p = 0.54$ ) populations.

Similar to the results described above that examined response to donepezil treatment in carriers and non-carriers, change from baseline to final observation in ADAS-cog scores of donepezil-treated patients was not influenced by the presence of 0, 1, or 2 *APOE*-ε4 alleles. Results from the ITT population are presented in Fig. 4B and are similar to those of the completer analysis. In the placebo group, however, LS mean change from baseline in ADAS-cog demonstrated less improvement among patients with 1 or 2 *APOE*-ε4 alleles versus non-carriers (Fig. 4B). This difference approached statistical significance for the comparison

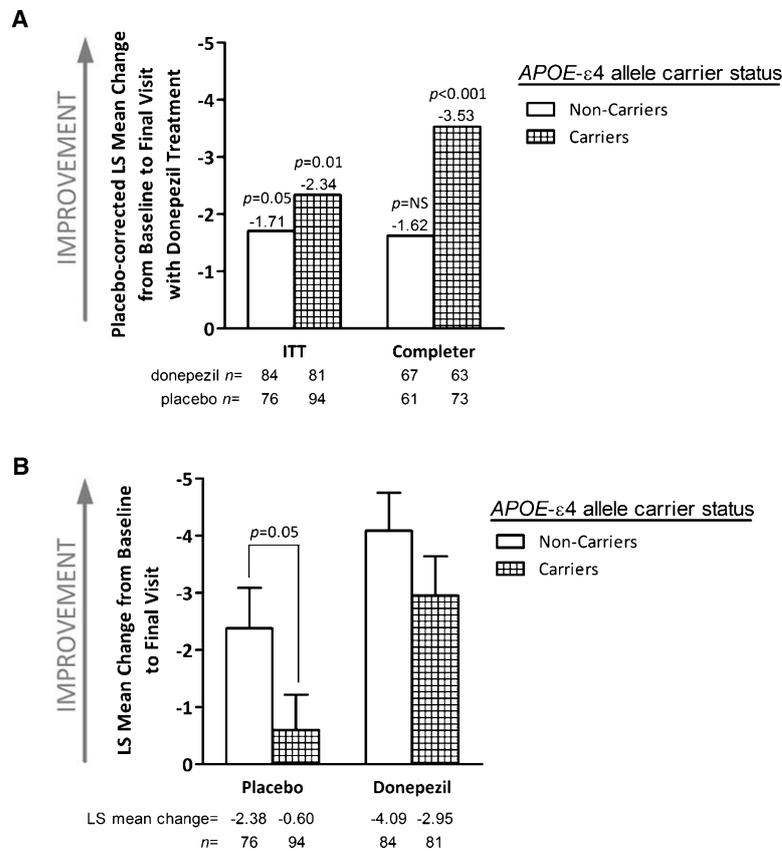


Fig. 4. Change from baseline to final assessment in Alzheimer's Disease Assessment Scale-Cognitive Subscale total score by apolipoprotein E (*APOE*)- $\epsilon$ 4 allele copy number. A) Placebo-corrected change from baseline to the final visit with donepezil treatment in the intent-to-treat (ITT) and completer populations. *p*-values are for the comparison of donepezil treatment effect versus placebo. B) Least square (LS) mean change from baseline values for the donepezil and placebo treatment groups (ITT population). Error bars in panel B represent standard error. NS, not significant.

of 1 copy versus no copies of *APOE*- $\epsilon$ 4 in the ITT population (between-group difference, 1.72; *p* = 0.08) and was statistically significant within the completer population (between-group difference, 3.15; *p* = 0.008). A significant difference in ADAS-cog score change from baseline was also observed in carriers of 2 *APOE*- $\epsilon$ 4 alleles versus non-carriers in the completer analysis (between-group difference, 4.36; *p* = 0.008).

## DISCUSSION

Pooled data from three consecutive, randomized, placebo-controlled, multi-national clinical trials conducted from 2009 to 2011 demonstrated that *APOE*- $\epsilon$ 4 allele status has no statistically significant effect on the difference in treatment response between donepezil and placebo or the change from baseline to final observation in donepezil-treated patients as measured by ADAS-cog. Tests of interaction revealed no significant

effect of either *APOE*- $\epsilon$ 4 presence or copy number on donepezil efficacy, and comparable findings were observed in both the ITT and completer populations. The pooled dataset utilized for these analyses is the largest and most geographically diverse dataset in which the relationship between *APOE*- $\epsilon$ 4 carrier status and donepezil treatment response has been examined.

The analyses performed in this assessment were prompted by the mixed results from previous studies that evaluated the effect of *APOE* genotype on response to donepezil or other acetylcholinesterase inhibitors. If donepezil studies are considered independently of other acetylcholinesterase inhibitor clinical trials (Table 1), a pattern emerges such that the larger studies (i.e., those that enrolled 100 or more patients) reported no difference in response to treatment in *APOE*- $\epsilon$ 4 carriers versus non-carriers [13, 14], whereas smaller studies (i.e., those that enrolled approximately 50 patients or fewer) tended to indicate

a correlation between outcomes and *APOE-ε4* carrier status [15–17]. Our analysis, which included 165 patients who were treated with donepezil, is consistent with the findings of the larger studies, which were also multi-national and enrolled patients with mild-to-moderate AD [13, 14]. An important caveat in the comparison of our data with previous evaluations of *APOE-ε4* carrier status and treatment response is that the prior analyses were derived from trials of longer duration than the 12-week period covered by our dataset.

Our results are also consistent with larger evaluations of *APOE* genotype and treatment response that involved other acetylcholinesterase inhibitors, including clinical trials of metrifonate, galantamine, and rivastigmine [22, 24, 25]. In the largest assessment to date, which included 585 patients treated with metrifonate, no interaction was detected between *APOE-ε4* allele status and response to treatment [24]. Similarly, Aerssens and colleagues reported no effect of *APOE-ε4* presence or copy number on the rate of cognitive and functional decline in a population of 569 patients treated with galantamine [22]. In a slightly smaller assessment that included 367 patients with mild-to-moderate AD, Farlow and colleagues observed greater cognitive decline among *APOE-ε4* non-carriers during treatment with either rivastigmine or placebo, yet because the deterioration was observed with both active treatment and placebo, the net result was a comparable treatment response in the *APOE-ε4* carrier and non-carrier subgroups [25]. Datasets for all of these analyses were derived from pooling of multi-center, double-blind, placebo-controlled studies that enrolled patients with mild-to-moderate AD and evaluated treatment response using ADAS-cog, a global measure of cognition [22, 24, 25].

An intriguing finding in these analyses was the influence of *APOE-ε4* carrier status on change from baseline to final measurement in ADAS-cog score in the placebo-treated group. Individuals heterozygous or homozygous for the *APOE-ε4* allele demonstrated an attenuated placebo improvement compared with patients without an *APOE-ε4* allele. These results are consistent with observations from many previous studies that have suggested that *APOE-ε4* carriers exhibit a lower placebo response or a greater degree of decline over time in AD clinical trials [31–33] or in observational cohorts [34–36]. There is evidence to suggest that brain structure and function in patients with AD are influenced by *APOE* genotype, raising the possibility that the lack of improvement and increased rate of decline observed in *APOE-ε4* carriers

in clinical trials is related to underlying differences in disease progression. Investigators have found that carriers of the *APOE-ε4* allele have more pronounced hippocampal atrophy compared with non-carriers [37, 38]. Moreover, functional magnetic resonance imaging has revealed significant differences in the activity of various brain regions in carriers compared with non-carriers [39].

Another possible explanation for the differences in response to placebo among *APOE-ε4* carrier groups is a differential impact of repeated ADAS-cog testing. Improvement in scores after repeated administration of cognitive measurement instruments (i.e., practice effects) have been reported among patients with AD [40, 41]. In their recent assessment of amyloid levels and *APOE-ε4* carrier status in clinically normal older individuals, Mormino and colleagues [42] observed the highest rate of short-term cognitive decline among patients who were *APOE-ε4+* and had high amyloid-β levels. Our results, together with that of Mormino et al., indicate that if *APOE* genotype influences practice effects, *APOE-ε4* non-carriers benefit to a greater extent than do *APOE-ε4* carriers.

If the findings for the relationship between *APOE-ε4* carrier status and placebo response are replicated in other datasets, this relationship has the potential to influence clinical trial results. *APOE-ε4* carriers represent a considerable proportion of the study population (58% to 67%) in AD clinical trials [43]. Differences in distribution of *APOE-ε4* carriers among treatment groups could have an important impact on the ability to generate a consistent placebo response and to quantify the efficacy of study drug treatment. To minimize this potential source of bias, stratification of patients by *APOE-ε4* carrier status could be considered for future AD pharmacotherapy trials. In addition, enriching study populations with *APOE-ε4*-positive patients may augment the power to detect treatment differences by minimizing placebo group improvement; however, the potential that the increased burden of screening requirements for such a design would outweigh the benefit must be taken into account [32].

There are several limitations inherent in this analysis of pooled data that have relevance for the interpretation of the results. First, the early termination of two of the trials at a point when fewer than two-thirds of patients had completed the 12-week treatment may have led to an underestimation of the final donepezil and placebo responses. Second, the short duration of treatment precluded an assessment of the long-term effects of genotype on treatment response. MacGowan and colleagues [23]

noted that, although their data revealed no short-term effect of *APOE-ε4* allele status on response to acetylcholinesterase inhibitor therapy, there was the suggestion of an effect over the long-term. It is worth noting, however, that in larger 36- and 52-week studies of donepezil, no such trend was observed [13, 14]. Third, whereas evaluation of treatment response by *APOE-ε4* genotype was prespecified as a secondary analysis in the protocols of the contributing studies, the trials themselves were powered to evaluate differences in ADAS-cog improvement between the investigational agents and placebo. Lastly, other factors that were not evaluated in this analysis could potentially affect the influence of *APOE* genotype on treatment response. For example, Morgen and colleagues recently reported an interaction between the *APOE-ε4* allele and a single-nucleotide polymorphism in the phosphatidylinositol-binding clathrin assembly protein (*PICALM*) gene in patients with very mild or mild AD dementia [44]. Modifier interactions such as this may underlie the inconsistencies that have been observed from studies evaluating the impact of *APOE* genotype on acetylcholinesterase inhibitor treatment response.

The current study adds to the growing body of literature indicating that *APOE-ε4* carrier status does not appreciably affect the magnitude of treatment response to donepezil in patients with mild-to-moderate AD. The detection of a significant variation in response to placebo based on *APOE-ε4* allele status, however, does merit consideration for future clinical study design.

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