Published in final edited form as:
J Alzheimers Dis. 2008 April ; 13(3): 255–266.

Multiple SNPs Within and Surrounding the Apolipoprotein E Gene Influence Cerebrospinal Fluid Apolipoprotein E Protein Levels

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Abstract

The ε4 allele of the apolipoprotein E gene (*APOE*) is associated with increased risk and earlier age at onset in late onset Alzheimer's disease (AD). Other factors, such as expression level of apolipoprotein E protein (apoE), have been postulated to modify the *APOE* related risk of developing AD. Multiple loci in and outside of *APOE* are associated with a high risk of AD. The aim of this exploratory hypothesis generating investigation was to determine if some of these loci predict cerebrospinal fluid (CSF) apoE levels in healthy non-demented subjects. CSF apoE levels were measured from healthy non-demented subjects 21–87 years of age (n = 134). Backward regression models were used to evaluate the influence of 21 SNPs, within and surrounding *APOE*, on CSF apoE levels while taking into account age, gender, *APOE ε4* and correlation between

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Communicated by Thomas Montine

SNPs (linkage disequilibrium). *APOE ε*4 genotype does not predict CSF apoE levels. Three SNPs within the *TOMM40* gene, one *APOE* promoter SNP and two SNPs within distal *APOE* enhancer elements (ME1 and BCR) predict CSF apoE levels. Further investigation of the genetic influence of these loci on apoE expression levels in the central nervous system is likely to provide new insight into apoE regulation as well as AD pathogenesis.

Keywords

Apolipoprotein E gene; apolipoprotein E protein; cerebroshinal fluid; enhancer; promoter; SNP

INTRODUCTION

Apolipoprotein E (apoE) is involved in lipid transport and binds to cell surface receptors to mediate lipoprotein uptake. It is the major apolipoprotein synthesized in the brain [23]. The apoE protein exists in three extensively studied isoforms, E2, E3, and E4 that are the result of two non-synonymous SNPs, rs429358 and rs7412, located in exon 4 of the *APOE* gene. Structural consequences of the exon 4 *APOE* haplotype appear to be that the apoE E4 protein binds preferentially to plasma very low density lipids (VLDLs) whereas apoE E2 and E3 bind preferentially to plasma high density lipoproteins (HDLs) [13]. In addition, apoE isoforms appear to influence plasma cholesterol levels [5,11], neuronal growth [15,28,41] and amyloid deposition [9,27,31].

The *APOE ε4* allele, defined by the rs429358 SNP, and age are currently the only risk factors strongly associated with late onset Alzheimer's disease (AD) [36]. However, a family history of dementia is associated with an increased risk of AD in the elderly only among *APOE ε4* carriers and a large proportion of *APOE ε4* carriers who survive into old age remain cognitively normal suggesting other genetic factors besides *APOE ε4* play a role in AD [12,14].

Cerebrospinal fluid apoE levels

There is no clear consensus on whether cerebrospinal fluid (CSF) apoE levels are associated with AD. Studies thus far indicate CSF apoE is either lower in AD [4,17,21] or has no association with AD [20,26]. CSF apoE levels in healthy populations appear to be associated with age, but not with gender [16,20,45] although one study did not find an association with age [20]. In addition, *APOE ε4* appears not to be associated with CSF apoE levels in healthy populations or AD [4,17,20,21,26,45], but is associated with lower apoE plasma levels in healthy subjects [21,37]. Interestingly, Fukumoto et al. report that apoE plasma levels are lower in AD compared to the mildly cognitively impaired [10] and *APOE e3/e4* heterozygotes have a higher proportion of the apoE E3 isoform than the E4 in plasma [10]. In contrast, the opposite proportion is present in CSF, suggesting a differential metabolism or regulation of apoE isoforms depending on the biological compartment measured; plasma or CSF [10]. Because plasma apoE is produced by the liver and CSF apoE is secreted by brain glial cells [22,33], it appears that apoE and possibly apoE isoforms may be metabolized or regulated differently in the two compartments.

APOE regulatory elements and apoE expression

AD risk has been reported to be associated with *APOE* promoter polymorphisms directly upstream of the transcription start site. Characterized *APOE* promoter polymorphisms include −491, −427, −219 (Th1/E47cs), and +113. Establishing AD risk associated with *APOE* promoter polymorphisms independent of *APOE ε4* has been difficult, with

contrasting reports likely due to strong linkage disequilibrium (LD) in the region [3,6,30,34,42].

Regulatory sites distal to *APOE* have been characterized. Multienhancer 1 and 2 (ME1 and ME2) are homologous regulatory regions are located 3.3 and 15.9 kb distal to *APOE* and influence *APOE* regulation in macrophages and adipocytes [24,38]. Two homologous hepatic control regions (HCR1 and HCR2) play a role in *APOE* regulation in the liver and are located approximately 15 kb and 27 kb distal to *APOE* [1,39]. Recently, a brain control region (BCR) has been described and is located 41.9 kb distal to *APOE* between *APOC2* and *CLPTM1* [47].

The influence of *APOE* promoter SNPs on apoE levels in plasma and brain has been characterized [18] but, to our knowledge, the influence of *APOE* promoter SNPs and *APOE* distal regulatory regions on CSF apoE levels has not been investigated.

APOE region and linkage disequilibrium

Several SNPs within the *TOMM40* gene, in a region 15 kb proximal to the *APOE* promoter, are associated with AD risk, but are also in strong LD with *APOE ε4* [25,46]. When these SNPs are entered into a logistic regression model that includes *APOE ε4*, only the effect of *APOE ε4* remains significantly associated with AD [46]. These observations diminish the possibility that loci in the *TOMM40* gene, proximal to *APOE*, have a major effect on AD risk independent of *APOE ε4*. LD with *APOE ε4* exists distal to the *APOE* gene in a region that contains the regulatory element ME1, but is weak in the region of the HCR2 and does not exist in the BCR, suggesting that the ME1 regulatory element is dependent on the presence or absence of *APOE ε4*, but HCR2 and BCR may be independent of *APOE ε4* [46].

Aim of the investigation

Because SNPs upstream of *APOE* (proximal), as well as SNPs downstream of APOE (distal) within ME1, HCR2 and BCR, are either within a LD block surrounding the *APOE* gene or are associated with *APOE* regulation, we hypothesized that genetic elements in the region proximate to *APOE* along with the *APOE* promoter and distal regulatory elements (ME1 and BCR), predict apoE expression in CSF, in a manner that can be dependent or independent of *APOE ε4*. We further hypothesized that HCR2, a hepatic control region of *APOE*, would not have an effect on the central nervous system and, thus, would not predict CSF apoE levels.

Thus, the aim of this exploratory investigation was to evaluate the potential influence of multiple genetic loci, within and surrounding the *APOE* gene, on CSF apoE levels in a cognitively normal population. The first aim was to characterize CSF apoE levels according to age, gender and *APOE ε4* status in a sample of cognitively normal subjects. The second aim was to explore the influence of the *APOE* proximal, promoter and distal region SNPs on CSF apoE levels.

METHODS

Subjects

All procedures were approved by the institutional review boards of the participating institutions. Subjects were 148 healthy carefully evaluated non-demented adults age 21–87 years. All subjects provided written consent. Mini-Mental State Examination (MMSE) scores were between 26–30 and Clinical Dementia Rating Scale (CDR) scores were 0 as previously described [32]. All 148 subjects were genotyped for 22 SNPs within a 70 kb region within and surrounding the *APOE* gene. Fourteen subjects lacked complete data for

all 22 SNPs leaving 134 subjects to be utilized in the analysis. The mean age, gender ratio, *APOE ε4* status, and mean CSF apoE levels are described in Table 1.

Cerebrospinal fluid

CSF was collected in the morning after an overnight fast using Sprotte 24-g atraumatic spinal needles as previously described [32]. Samples were frozen immediately on dry ice and stored at −80°C until assay at which time apoE concentrations (mg/dl) were measured using a nephelometer (Dade Behring). Nephelometry is an antibody-based automated method that uses the BN II Nephelometer and a kit from Dade Behring for apoE measurement. Briefly, 10 μls of the CSF sample is used undiluted in a reaction between antigen (apoE) and antibody (human anti-apoE). Antibody positive and negative controls are provided with the kit. Nepholometry is a highly standardized method with an average CSF apoE inter-assay variability of less than 1%. No values with an inter-assay variability of greater than 5% are accepted.

Genotyping, DNA sequencing, SNP selection

Genomic DNAs, 5ng each, were dispensed into individual wells of a 384 plate and air dried overnight. Plates were then covered with adhesive lid tape and stored at 4°C. For TaqMan allelic discrimination detection on the 384 well plates, using a final volume of 3 μl per well. For each reaction; 0.075 μl of 20× SNP TaqMan Assay (Applied Biosystems, CA), 1.5 μl of TaqMan Universal PCR Master Mix (Applied Biosystems, CA) and 1.425 μl of dH_2O were pipetted into each well. PCR reactions were carried out using a 9700 Gene Amp PCR System (Applied Biosystems, CA). The PCR profile was 95°C for 10 min and then 50 cycles at 92°C for 15 sec and 60°C for 90 sec. Plates were then subjected to end-point read in a 7900 Real-Time PCR System (Applied Biosystems, CA). Twenty one SNPs were genotyped including *APOE* proximal SNPs, *APOE* promoter SNPs, SNPs within the *APOE* gene and *APOE* distal SNPs (Table 3). The results were first evaluated by cluster variations, the allele calls were then assigned automatically before transferred and integrated into the genotype database, except for the ME1 and HCR2 SNPs.

The ME1 and HCR2 SNPs are located within a duplicated region, which has extremely high homology with ME2 and HCR1 elements, and thus were first PCR amplified (95°C for 15 min, 30 cycles of 92°C for 20 sec, 55°C for 30 sec, 72°C for 15 min) using primer sets specific to the one region surrounding the SNPs (not the duplication) and then subsequently DNA sequenced to determine the nucleotide content at each SNP site. BigDye terminator cycle sequencing kit (Applied Biosystems, CA) in a final volume of 10 μl, and the sequence information were collected from an 377 DNA Sequencer (AB). Primer sequences for region-specific PCR and sequencing is listed in Supplementary Table 1.

SNPs were selected in the region surrounding the *APOE* gene. SNPs included SNPs proximate to the *APOE* gene that have been previously described [46], SNPs distal to *APOE*, and SNPs within ME1, HCR2 and BCR. All SNPs are described in Table 2.

Statistical analysis

We examined the relationship between CSF apoE levels and *APOE* region SNPs, gender, and age using backward stepwise linear regression models. One SNP (rs157582) was omitted from all analyses because it was perfectly correlated with another SNP (rs157581) leaving 21 SNPs for the analyses. Except for rs429358, SNP genotypes were coded as 1, 2, or 3 and treated as continuous variables. The contribution of the rs429358 SNP was modeled using *APOE ε4* status (coded as 1 or 2, where 2 denotes no ε4 alleles are present).

Backward elimination was performed using a variable selection procedure in which all variables are entered into the equation and then sequentially removed until the stopping criterion is reached [8]. The variable with the smallest partial correlation with the dependent variable was considered first for removal. Variables were removed listwise from the model depending on the significance of the partial F-value (p-value > 0.10). After the first variable was removed, the variable remaining in the equation with the smallest partial correlation was considered next. The procedure stopped when there were no variables in the equation that satisfied the removal criteria (p-value > 0.10). R^2 values were determined to assess the proportion of variability in CSF apoE levels that is explained by the predictor variables. The backward regression p-values do not take into account multiple comparisons. Bonferroni multiple comparison corrections were performed (Table 3,Table 5). Backwards regression based on the Akaike Information Criterion was also performed with the same results as the backward regression shown in Table 3 (data not shown) [43].

RESULTS

APOE ε4 genotype, gender, age and CSF apoE levels

To determine if CSF apoE levels in our study sample (n = 134) are consistent with previous studies in which CSF apoE levels were not found to be associated with *APOE ε4* [4,17,20,21,26]or gender but were associated with age [16,20,45], CSF apoE levels were stratified by *APOE* genotype, the presence or absence *APOE ε4*, gender and age (Fig. 1). No significant difference in CSF apoE levels between *APOE* genotypes (p = 0.59 in ε2/ε3/ε4 genotype and p = 0.23 in ε4 allele, Fig. 1, panel A and panel B) and gender (p = 0.18, Fig. 1, panel C) was found. In contrast, a significant increase in CSF apoE levels with increasing age was observed (p = 0.002, Fig. 1, panel D).

APOE regional SNPs and correlations with APOE ε4

SNPs (Table 2) were selected either on the basis of our previous study that found most of these SNPs to be in LD with *APOE ε4* as well as associated with AD risk [46], or on the basis of their position within ME1, HCR2 or BCR. These SNPs (n = 21) span approximately 70 kb and range from a proximal SNP (rs6857) located 5′ of the *TOMM40* gene, to a distal SNP (rs7247551) within the *APOE* BCR.

All SNPs (n = 21) are in Hardy Weinberg equilibrium (data not shown). In addition, we determined the LD structure, (utilizing D′ and R^2), within this study sample and found it to be similar to our previous findings (Table 4) [46]. These results suggest the presence of LD across the region, which is consistent with our previous observation [46].

APOE region SNPs predict CSF apoE levels

We applied backward regression models to assess the effects of the 21 SNPs on CSF apoE levels (Table 3) while taking into account *APOE* ε4 status, gender and age. The models show that six SNPs may predict CSF apoE levels, including three SNPs in the proximal region (rs11556506, n17664883, rs157584), one in the promoter (rs449647; −491), and two in the distal region (n17684509; ME1 and rs7247551; BCR) of *APOE*. The proportion of variance explained by the model, as calculated by R^2, is 19%. Backward regression models based on the Akaike Information Criterion gave similar results where the same six SNPs predicted CSF apoE levels (data not shown).

In models in which only age and each of the six SNPs are entered alone, without other SNPs, into a linear regression model, either with or without *APOE* ε4 present in the model, only rs449647, independent of *APOE* ε4, and n17664883, in the presence of *APOE* ε4, predict CSF apoE levels (data not shown).

To determine if carriers of the more rare alleles (minor-allele-homozygoteplus heterozygote) have low CSF apoE levels, each SNP was collapsed into “non-variant” group (with major-allele-homozygote only) and “variant” group (with minor-allele-homozygote plus heterozygote) and linear regression was performed while taking into account age but not *APOE ε*4. In this model n17664883 (p-value, 0.002) and n17684509 (p-value, 0.021) predict CSF apoE levels while rs449647 marginally predicts CSF apoE levels (p-value, 0.088) (Fig. 2). However, after Bonferroni correction for multiple comparisons, only n17664883 remains significant (p-value, 0.030). Mean CSF apoE levels for SNP variant carriers compared to non-variant carriers while taking into account age are shown in Fig. 2.

To further address the question of whether a combination of SNPs may contribute to CSF apoE levels the six positive SNPs identified in the backward regression models were analyzed for their ability to predict CSF apoE levels. All possible combinations of these six SNPs were analyzed. Five haplotypes were found to predict CSF apoE levels while taking into account age and while taking into account all the haplotype combinations within the haplotype tested (Table 5: see haplotype p-values). After correcting for multiple comparisons only two of these haplotypes predict CSF apoE levels (see Bonferroni p-values for haplotype: n17684509, rs7247551 and haplotype: n17664883, rs449647, n17684509, rs7247551) (Table 5).

DISCUSSION

Because *APOE ε4* and age are important AD risk factors, the characterization of regulatory elements surrounding the *APOE* gene that may predict the level of apoE E4 protein, is vital in understanding AD pathogenesis. The influence of *APOE* promoter SNPs on apoE levels in plasma and brain has been studied [18,19] but, to our knowledge, the influence of *APOE* promoter SNPs or *APOE* distal regulatory regions on CSF apoE levels has not been characterized. In a previous investigation, SNPs proximal to *APOE* were found to be associated with AD risk [46] leading us to wonder if SNPs in the region proximal to *APOE* lay within an un-characterized *APOE* regulatory element. Thus, in this investigation we evaluated the differences in healthy subjects CSF apoE levels while taking into account age, gender and *APOE ε4* status, as well as associations with 21 SNPs in a large 70 kb region surrounding the *APOE* gene. This study was an exploratory investigation to test the hypothesis that multiple genetic loci surrounding *APOE* can predict CSF apoE levels. The results indicate that in addition to the *APOE* promoter, SNPs in the *TOMM40* gene, the ME1 and the BCR regions may also predict CSF apoE levels.

Consistent with the previous reports, CSF apoE levels increased with increasing age (Fig. 1, Panel D); and the levels were not associated with *APOE ε4* genotype (Fig. 1, Panel A) or allele (Fig. 1, Panel B) or gender (Fig. 1, Panel C) [4,16,17,20,21,26,45].

Backward linear regression models indicate that out of the 21 SNPs entered into the models, six SNPs may predict CSF apoE levels (three SNPs within *TOMM40*; rs11556505, n17664883, rs157584, one *APOE* promoter SNP, rs449647, one SNP within ME1; n17684509, and one SNP within the BCR; rs7247551; Table 3). Of these six SNPs only two SNPs predict CSF apoE levels either with *APOE ε*4 (n17664883) or without *APOE ε*4 (rs449647) present in the model (data not shown).

Interestingly, previous reports indicate that the *APOE* −491 promoter polymorphism (rs449647) is associated with AD risk, although it is unclear whether LD is obscuring these results [6,30,42]. In our investigation the non-variant (AA genotype) carriers of −491 polymorphism appeared to have higher CSF apoE levels than the variant (AT and TT genotypes) carriers both alone and as part of a haplotype (Fig. 2;Table 5) which is consistent

with previous reports by Laws et al. where higher brain apoE levels [18] and plasma apoE levels [19] are associated with the −491 AA genotype. To our knowledge this is the first investigation of the association between CSF apoE levels and *APOE* promoter SNPs. These results reported here, along with previous reports, implicate the −491 promoter polymorphism as an important regulator of apoE levels.

The association with the novel proximal SNP, n17664883, suggests that an *APOE* regulatory element exists in the region distantly proximal to *APOE* that may predict CSF apoE levels. This region within intron 4 of the *TOMM40* gene, may contain a regulatory element that contributes to high CSF apoE levels when the major homozygote genotype (non-variant) is present (Fig. 2, Table 5). Whereas the minor homozygote or the heterozygotes (variant) may contribute to lower CSF apoE levels (Fig. 2, Table 5). Alternatively, CSF apoE levels may be consequences of *TOMM40* gene action. The *TOMM40* gene encodes the TOM40 protein which is the pore subunit of the mitochondrial outer membrane protein translocator [35]. Currently, there is no evidence of protein-protein interaction between TOM40 and apoE. However, recent reports suggest that AβPP may be targeted to the mitochondria and translocated across the mitochondrial membrane via the TOM40 protein [2]; additionally, the translocation arrest of AβPP in the TOM40 pore may lead to mitochondria dysfunction and neuronal loss in AD [7]. Therefore, it is possible that variants of TOM40 may be more susceptible to the translocation arrest of AβPP and may lead to more profound decline of mitochondrial function and neuronal damage. The biological feedback mechanism would then be activated and produce more apoE for the neuronal repair or regeneration, a well-known function of apoE.

We also assessed whether the *APOE* distal regulatory elements (HCR, ME and BCR) can predict CSF apoE levels. Because evidence suggests that the HCR is primarily active in the liver [1,39], we hypothesized that only the SNPs within the ME1 (rs483082, n17684509, rs584007) and BCR (rs7247551) but not HCR2 (rs35136575), would predict apoE expression in CSF. Indeed, in our analyses the HCR2 SNP did not have an effect on CSF apoE levels, but SNPs from both ME1 and BCR did (Table 3, Fig. 2, Table 5). Interestingly, only one of the three SNPs within ME1, a small region which spans 620 bp, is predicted to be associated with CSF apoE levels (Table 3). This result may be attributed to high correlation of other SNPs in the model with SNPs in the ME1 region, thus, leading to the elimination of other ME1 SNPs from the regression model. This result does not necessarily reflect a lack of a biological influence on CSF apoE levels by the other ME1 SNPs.

The hypothesis generated by these results is that there may be additional regulators of *APOE* in the proximal region as far upstream as 15 kb. The proximal region may act in combination with regulatory elements in the distal region, such as the ME1 and the BCR to increase the activity of the *APOE* promoter. An example of distant proximal enhancers can be demonstrated by the *APOB* gene, which has a *cis*-element enhancer located 54 to 62 kilobases 5′ to the structural gene [29]. Further support of our hypothesis is demonstrated by our finding whereby multiple loci together have effects on CSF apoE levels (Table 3,Fig. 2,Table 5) implicating an influence on CSF apoE levels by a large haplotype. Such a concept is in line with studies suggesting that promoter haplotypes of *APOE* can influence plasma apoE levels [40,44]. But, our study goes beyond previous studies by investigating contributions by distal regulatory regions, such as ME1, HCR2 and BCR, on CSF apoE levels.

There were limitations of this exploratory investigation. First, our study sample size (n = 134) may be too small to detect small effects contributed by a few of the SNPs tested that have low minor allele frequencies. Even though we required all minor allele frequencies to be equal to or greater than 2%, and collapsed genotypes into variant and non-variant groups,

some of the haplotype numbers were low (Table 5). Second, the statistical results should be approached with caution because stepwise linear regression models do not take into account multiple comparisons so that p-values do not represent true significance until corrected for multiple comparisons (Table 3). Thus, it is important to note that this is an exploratory investigation intended to generate further hypotheses.

In summary, linear regression models were used to search for *APOE* regulatory SNPs that predict CSF apoE levels. These SNPs are located within a large region surrounding the *APOE* gene. Six SNPs were found to predict CSF apoE levels; three *TOMM40* SNPs (rs11556505, n17664883, rs157584), one *APOE* promoter SNP (rs449647; −491) and two *APOE* distal SNPs (ME1; n17684509, BCR; rs7247551). For two of these SNPs, the novel SNP within the *TOMM40* gene (n17664883) and the *APOE* promoter SNP (rs449647; −491), there is a significant difference in CSF apoE levels between genotypes and these two SNPs also appear to contribute to haplotype CSF apoE levels. These results support the hypothesis that modestly penetrant SNPs within *APOE* regulatory elements may explain part of the variation in CSF apoE protein levels. Our data indicate that a multigenetic approach may be more powerful in explaining the variation in apoE levels than a monogenetic approach. However, the total contribution of several SNPs together was modest, with a large proportion of the variation remaining unaccounted for (R^2 value, 0.19; Table 3), which may suggest that future evaluation of molecular haplotypes in the *APOE* gene region in a larger study population is required to explain more specifically the variation in apoE levels. In addition, given that *APOE* proximal SNPs within the *TOMM40* gene predict CSF apoE levels, a possible independent influence on CSF apoE levels by the TOM40 protein may exist.

The mechanism whereby *APOE ε4* increases the risk of AD is uncertain. *APOE ε4* carriers show substantial variance for age at onset of AD. Some individuals who are ε4 homozygotes may be spared from AD even if they live into their 9^{th} or 10^{th} decade [12,14]. Factors that alter apoE protein expression, such as an *APOE ε4* haplotype that includes proximal and distal regulatory elements, may help to explain this variance. In addition to aging, we have now shown that certain SNPs appear to be associated with levels of apoE protein in CSF in cognitively normal individuals. If these SNPs are also associated with younger age of onset in AD, this would implicate levels of apoE as a factor in AD pathogenesis. An extension of this study would be an investigation of familial AD cases to evaluate the influence of family history of AD on AD age-at-onset and associations between SNPs within and around APOE, *APOE ε4* status, CSF apoE levels as well as other AD biomarkers such as CSF Aβ_{40}, Aβ_{42}, and tau.

In conclusion, this exploratory investigation has generated further hypotheses regarding the possible influence on CSF apoE levels by multiple genetic loci within and surrounding the *APOE* gene suggesting that the actual effect is likely to be determined by these loci's haplotype structure.

Acknowledgments

Supported by grants from the National Alzheimer's Association (IIRG-03-4750), NIA (R21 AG24486-01), Veterans Affairs Biomedical Laboratory Research Development Merit Review (ID1127558), Ruth L Kirschstein National Research Service Award (NR-SA) (5T32AG000258-07), NINDS (R01 NS48595), VISN20 MIRECC, NIA (AG05136), NIH P01HL30086, Alzheimer's Center Grant (AG08017), National Alzheimer's Coordinating Center, Friends of Alzheimer's Research, Alzheimer's Association of Western and Central Washington, an anonymous foundation, and the Department of Veterans Affairs. Dr. Kaye is on the advisory board of Myriad Pharmaceuticals and receives grant support from the Intel Corporation. Dr. Farlow is a consultant for Abbott Labs, Accera Inc., Adamas Pharm, Cephalon Inc., Comentis, Eisai, GlaxoSmithKline, Medivation, Inc., Memory Pharm, Merck and Co., Neurochem, Novartis, OctaPharma, Sanofi-Aventis Groupe, Shering Plough, Takada Pharmaceuticals, Talecris Biotherapeutics, and Worldwide Clinical Trials. Additionally, Dr. Farlow receives grant

support from Ono Pharmanet, Eli Lilly and Co., Wyeth, and Elan Pharmaceuticals. Dr. Quinn is a member of the speaker's bureau of Pfizer and Forest.

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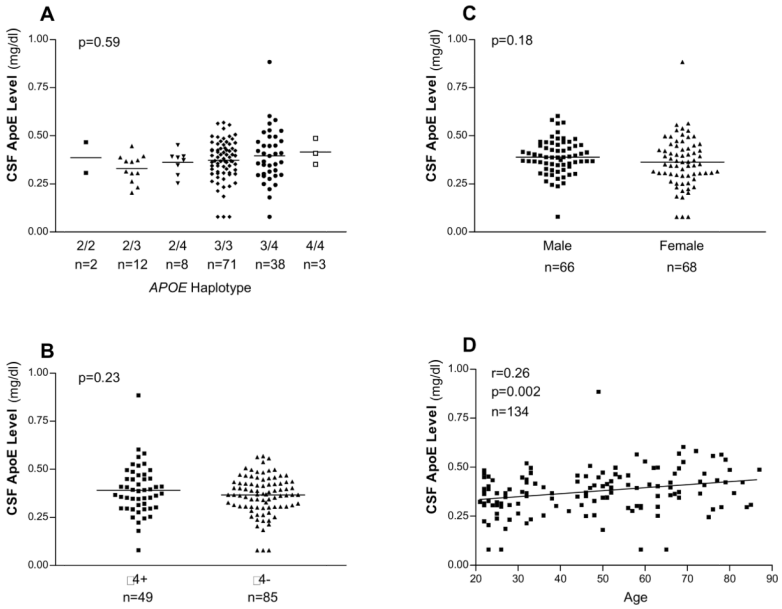

Fig. 1. APOE ε4, gender, age and CSF apoE levels

There is no significant difference in CSF apoE levels between *APOE* genotypes (Panel A) or between the presence or absence of *APOE ε4* (Panel B). There is not a significant difference in CSF apoE levels between genders (Panel C). There is a significant increase in CSF apoE with increasing age (Panel D).

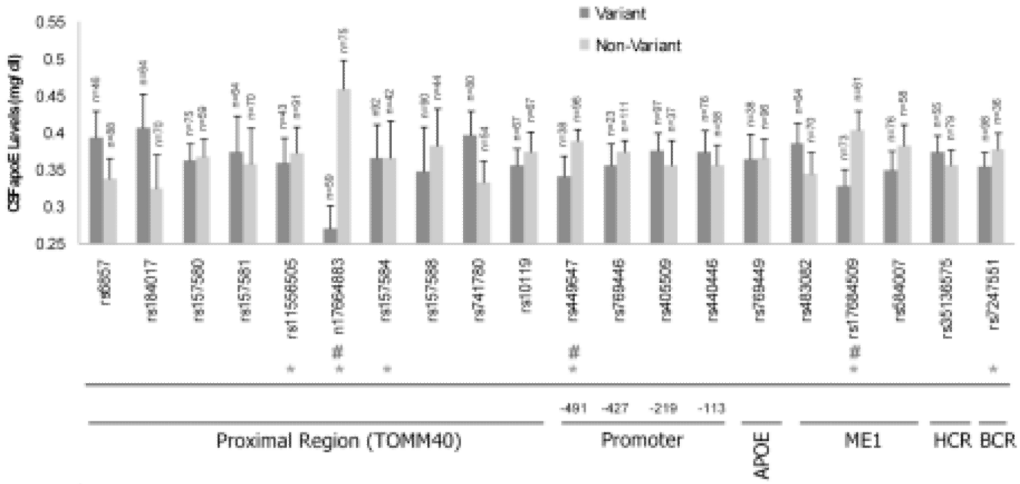

Fig. 2. CSF apoE mean levels

Bars represent CSF apoE means for variant or non-variant carrier groups for each SNP after taking into account age. Error bars represent standard error and n equals the number of individuals within the group analyzed. SNPs found to predict CSF apoE levels in backward regression models shown in Table 3 are designated with a *. SNPs found to predict CSF apoE levels after taking into account age and other SNPs (n17664883 p-value, 0.002: rs449647 p-value, 0.088, n17684509 p-value, 0.021) are designated with a #.

Table 1

Study Subject Characteristics. Age, APOE ε4 status, and mean CSF apoE levels are described for the entire cognitively normal study sample and for males and females

	All	Males	Females
Category	*n* = 134	66	68
Age (mean + SD)	47 ± 19	46 ± 20	49 ± 18
APOE ε4 Status (% ε4+)	37	38	35
Mean CSF ApoE (mg/dl)	0.376 ± 0.112	0.389 ± 0.09	0.363 ± 0.129

Table 2

SNP description. The 21 SNPs investigated are described including; SNP ID, Gene or gene element, gene region, SNP position, allele, and genotype frequency for the study sample (*n* = 134)

No	SNP ID	Gene/Element	Region	Position in NT 011109.15	Allele	Genotype Frequency in CSF Sample All (*n* = 134)
1	rs6857	5′ TOMM40		17660472	G/a	0.657_0.321_0.022
2	rs184017	TOMM40	Intron 2	17663187	A/c	0.522_0.410_0.067
3	rs157580	TOMM40	Intron 2	17663484	A/g	0.440_0.440_0.119
4	rs157581	TOMM40	Exon 3	17663932	T/c	0.522_0.403_0.075
5	rs11556505	TOMM40	Exon 4	17664362	C/t	0.679_0.291_0.030
6	n17664883*	TOMM40	Intron 4	17664883	C/a	0.560_0.396_0.045
7	rs157584	TOMM40	Intron 4	17665117	A/g	0.313_0.440_0.246
8	rs157588	TOMM40	Intron 6	17666482	C/t	0.328_0.433_0.239
9	rs741780	TOMM40	Intron 8	17672649	A/g	0.403_0.410_0.187
10	rs10119	TOMM40	Exon 10	17674891	G/a	0.500_0.381_0.119
11	rs449647	APOE −491	5′UTR	17676782	A/t	0.716_0.254_0.030
12	rs769446	APOE −427	5′UTR	17676846	T/c	0.828_0.172_0.000
13	rs405509	APOE −219	5′UTR	17677054	T/g	0.276_0.485_0.239
14	rs440446	APOE +113	Intron 1	17677385	G/c	0.433_0.418_0.149
15	rs769449	APOE	Intron 2	17678220	G/a	0.716_0.261_0.022
16	rs429358	APOE ε4	Exon 4	17680159	T/c	0.634_0.343_0.022
17	rs483082	ME1		17684396	G/t	0.522_0.373_0.105
18	n17684509*	ME1		17684509	C/t	0.455_0.425_0.119
19	rs584007	ME1		17684696	G/a	0.433_0.418_0.149
20	rs35136575	HCR2		17707381	C/g	0.590_0.336_0.075
21	rs7247551	BCR		17722984	A/g	0.269_0.508_0.224

* novel SNPs.

Table 3

Backward Linear Regression Models. Two backward linear regression models were utilized to evaluate the influence of *APOE* region SNPs on CSF apoE levels. CSF apoE level for each cognitively normal individual (n = 134) was entered into a backward linear regression model as the dependent variable and SNPs (n = 21); including the SNP representing *APOE ε 4* status, age, and gender were entered as the independent variables. SNPs were entered either as genotype (coded as 1, 2 or 3)

Condition R^2	0.19 beta (std error)	p-value	Bonferroni Corrected p-value*
SNPs			
rs11556505	0.072 (0.031)	0.020	0.142
n17664883	−0.075 (0.027)	0.007	0.049
rs157584	0.055 (0.019)	0.004	0.028
rs449647	−0.044 (0.018)	0.016	0.113
n17684509	−0.053 (0.019)	0.007	0.050
rs7247551	−0.028 (0.013)	0.035	0.242
Covariates			
Age	0.002 (0.000)	0.000	0.002
Gender	–	–	–
APOE ε 4	–	–	–
(Intercept)	0.400 (0.064)	0.000	0.000

* The backward regression model p-value does not correct for multiple comparisons. The Bonferroni corrected p-value shows the p-value after.

Table 4

Linkage Disequilibrium Surrounding the *APOE* gene. A 70 kb region from the 5′ proximal region at rs6857 to the 3′ distal region at rs7247551. Measures of linkage disequilibrium including D′ and R^2

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
		rs6857	**rs184017**	**rs157580**	**rs157581**	**rsl1556505**	**n17664883**	**rs157584**	**rs157588**	**rs741780**	**rs10119**	**rs449647**	**rs769446**	**rs405509**	**rs440446**	**rs769449**	**rs429358**	**rs483082**	**n17684509**	**rs584007**	**rs35136575**	**rs7247551**	**R^2**
1	**rs6857**	—	0.53	0.12	0.50	0.71	0.58	0.15	0.09	0.14	0.38	0.00	0.00	0.11	0.00	0.46	0.46	0.22	0.10	0.00	0.00	0.00	1
2	**rs184017**	1.00	—	0.23	0.95	0.38	0.87	0.00	0.01	0.00	0.26	0.02	0.04	0.00	0.13	0.28	0.44	0.47	0.20	0.06	0.03	0.00	2
3	**rs157580**	1.00	1.00	—	0.24	0.05	0.16	0.53	0.54	0.34	0.01	0.00	0.01	0.35	0.61	0.08	0.14	0.07	0.13	0.40	0.06	0.01	3
4	**rs157581**	1.00	1.00	1.00	—	0.38	0.78	0.00	0.00	0.00	0.26	0.04	0.04	0.00	0.13	0.24	0.44	0.52	0.20	0.07	0.03	0.01	4
5	**rs11556505**	0.92	0.94	0.71	0.95	—	0.45	0.21	0.16	0.12	0.27	0.00	0.00	0.17	0.00	0.57	0.29	0.14	0.09	0.00	0.00	0.01	5
6	**n17664883**	1.00	1.00	1.00	1.00	0.93	—	0.02	0.02	0.02	0.22	0.04	0.07	0.01	0.04	0.30	0.33	0.33	0.19	0.00	0.02	0.00	6
7	**rs157584**	0.80	0.06	1.00	0.05	1.00	0.22	—	0.94	0.67	0.17	0.00	0.00	0.65	0.40	0.15	0.04	0.01	0.40	0.29	0.02	0.00	7
8	**rs157588**	0.65	0.11	1.00	0.10	0.91	0.20	1.00	—	0.66	0.14	0.00	0.00	0.67	0.40	0.12	0.02	0.00	0.32	0.21	0.01	0.00	8
9	**rs741780**	1.00	0.06	1.00	0.10	1.00	0.25	1.00	1.00	—	0.27	0.01	0.00	0.61	0.33	0.09	0.16	0.02	0.50	0.20	0.03	0.00	9
10	**rs10119**	0.90	0.55	0.13	0.54	0.83	0.56	0.53	0.53	0.94	—	0.11	0.02	0.07	0.03	0.26	0.44	0.37	0.22	0.02	0.03	0.00	10
11	**rs449647**	0.01	0.22	0.01	0.30	0.04	0.24	0.00	0.04	0.25	0.50	—	0.02	0.02	0.01	0.03	0.00	0.02	0.05	0.00	0.01	0.00	11
12	**rs769446**	0.35	0.38	0.17	0.41	0.02	0.53	0.11	0.11	0.01	0.57	1.00	—	0.00	0.00	0.00	0.01	0.06	0.05	0.02	0.03	0.00	12
13	**rs405509**	0.72	0.03	0.79	0.01	1.00	0.18	0.82	0.85	1.00	0.39	0.31	0.00	—	0.49	0.15	0.09	0.00	0.30	0.27	0.08	0.00	13
14	**rs440446**	0.17	0.76	0.80	0.76	0.08	0.54	0.94	0.91	1.00	0.33	0.28	0.03	0.97	—	0.08	0.14	0.25	0.13	0.69	0.18	0.01	14
15	**rs769449**	0.84	0.87	1.00	0.85	0.82	0.83	0.88	0.89	1.00	0.93	1.00	0.08	1.00	1.00	—	0.60	0.32	0.07	0.08	0.01	0.01	15
16	**rs429358**	0.72	0.84	1.00	0.87	0.66	0.68	0.34	0.27	1.00	0.91	0.23	0.69	0.60	1.00	1.00	—	0.49	0.12	0.13	0.03	0.00	16
17	**rs483082**	0.69	0.72	0.52	0.74	0.59	0.67	0.16	0.08	0.25	0.61	0.23	0.50	0.09	1.00	1.00	0.96	—	0.22	0.25	0.06	0.00	17
18	**n17684509**	1.00	1.00	0.70	1.00	1.00	1.00	0.89	0.82	0.82	1.00	0.69	1.00	0.80	0.75	1.00	1.00	1.00	—	0.24	0.02	0.00	18
19	**rs584007**	0.19	0.51	0.65	0.54	0.16	0.04	0.81	0.65	0.79	0.27	0.10	0.65	0.73	0.83	1.00	1.00	1.00	1.00	—	0.18	0.00	19
20	**rs35136575**	0.04	0.54	0.35	0.52	0.08	0.49	0.30	0.18	0.44	0.44	0.39	1.00	0.54	0.60	0.50	0.64	0.67	0.35	0.58	—	0.03	20
21	**rs7247551**	0.12	0.08	0.16	0.13	0.21	0.05	0.03	0.04	0.02	0.02	0.07	0.03	0.00	0.16	0.28	0.10	0.08	0.04	0.01	0.30	—	21
	D′	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	

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Table 5

Haplotype mean CSF apoE levels. Mean CSF apoE levels for haploytype combinations of all six SNPs in Table 3 were evaluated taking into account age. Only those haplotypes that predict (p-value ~ 0.05) CSF apoE levels are shown. Only the variant carrier and non-variant carrier haplotype mean values are shown as they have the lowest and highest CSF apoE levels, respectively. The p-value represents the overall significance of all haplotype combinations within the haplotype group shown

									95% CI			
Region	**Allele**	**Haplotype**				**n=**	**ApoE Mean**	**Std. Error**	**Lower**	**Upper**	**Haplotype p-value** *	**Bonferroni p-value** *
TOMM40-APOE		n17664883	rs449647									
	Variant	CA, AA	AT, TT			20	0.34	0.02	0.29	0.39		
	Non-Variant	CC	AA			57	0.40	0.01	0.38	0.43	0.042	0.170
APOE-ME1		rs449647	n17684509									
	Variant	AT, TT	CT, TT			12	0.32	0.03	0.26	0.38		
	Non-Variant	AA	CC			35	0.41	0.02	0.37	0.44	0.057	0.228
ME1-BCR		n17684509	rs7247551									
	Variant	CT, TT	AG, GG			55	0.37	0.01	0.35	0.40		
	Non-Variant	CC	AA			18	0.43	0.03	0.38	0.48	0.000	0.000
APOE-ME1-BCR		rs449647	n17684509	rs7247551								
	Variant	AT, TT	CT, TT	AG, GG		7	0.30	0.04	0.22	0.38		
	Non-Variant	AA	CC	AA		12	0.47	0.03	0.41	0.53	0.023	0.185
TOMM40-ME1-BCR		n17664883	n17684509	rs7247551								
	Variant	CA, AA	CT, TT	AG, GG		16	0.34	0.03	0.29	0.40		
	Non-Variant	CC	CC	AA		6	0.52	0.04	0.44	0.61	0.054	0.430
TOMM40-APOE-ME1-BCR		n17664883	rs449647	n17684509	rs7247551							
	Variant	CA, AA	AT, TT	CT, TT	AG, GG	3	0.34	0.06	0.22	0.46		
	Non-Variant	CC	AA	CC	AA	5	0.53	0.05	0.44	0.62	0.000	0.000

* p-values are for within haplotype comparisons.