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Association of cerebrospinal fluid $A\beta_{42}$ with *A2M* gene in cognitively normal subjects

Steven P. Millard[a,*], **Franziska Lutz**[b,c], **Ge Li**[d], **Douglas R. Galasko**[e], **Martin R. Farlow**[f], **Joseph F. Quinn**[g], **Jeffrey A. Kaye**[g], **James B. Leverenz**[d,h], **Debby Tsuang**[b,d], **Chang-En Yu**[b,c], **Elaine R. Peskind**[a,d], and **Lynn M. Bekris**[b,c]

[a]Northwest Network VISN-20 Mental Illness Research, Education, and Clinical Center, Seattle, WA, 98108, USA

[b]Geriatric Research, Education, and Clinical Center, Seattle, WA, 98108, USA

[c]Department of Medicine, University of Washington, Seattle, WA, 98195, USA

[d]Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA, 98195, USA

[e]Department of Neurosciences, University of California at San Diego, San Diego, CA, 92093, USA, and VA Medical Center San Diego, San Diego, CA, 92161, USA

[f]Department of Neurology, Indiana University School of Medicine, Indianapolis, IN, 46202, USA

[g]Department of Neurology, Oregon Health and Science University, Portland, OR, 97239, USA, and Portland VA Medical Center, Portland, OR, 97239, USA

[h]Department of Neurology, University of Washington, Seattle, WA, 98195, USA, and Northwest Network VISN-20 Parkinson's Disease Research, Education, and Clinical Center, VA Puget Sound Health Care System, Seattle, WA, 98108, USA

Abstract

Low cerebrospinal fluid (CSF) $A\beta_{42}$ levels correlate with increased brain Aβ deposition in Alzheimer's disease (AD), which suggests a disruption in the degradation and clearance of Aβ from the brain. In addition, *APOE* ε4 carriers have lower CSF $A\beta_{42}$ levels than non-carriers. The hypothesis of this investigation was that CSF $A\beta_{42}$ levels correlate with regulatory region variation in genes that are biologically associated with degradation or clearance of Aβ from the brain. CSF $A\beta_{42}$ levels were tested for associations with Aβ degradation and clearance genes and *APOE* ε4. Twenty-four SNPs located within the 5′ and 3′ regions of 12 genes were analyzed. The study sample consisted of 99 AD patients and 168 cognitively normal control subjects. CSF $A\beta_{42}$ levels were associated with *APOE* ε4 status in controls but not in AD patients; *A2M* regulatory region SNPs were also associated with CSF $A\beta_{42}$ levels in controls, but not in AD patients, even after adjusting for *APOE* ε4. These results suggest that genetic variation within the *A2M* gene influences CSF $A\beta_{42}$ levels.

Address correspondence to Steven P. Millard, PhD, VA Puget Sound Health Care System, 1660 S. Columbian Way, S-116-MIRECC, Seattle, WA 98108, USA; Phone: 1-206-277-5056; Fax: 1-206-768-5364; SteveMillard@comcast.net.

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Keywords

Alzheimer's disease; cerebrospinal fluid; $A\beta_{42}$; alpha-2-macroglobulin; A2M; APOE

1 Introduction

Amyloid precursor protein (APP) is an integral membrane protein that is expressed in many cell types, including neurons (Brunholz, et al., 2012, Chasseigneaux and Allinquant, 2012). APP has been implicated in synapse formation and neuronal plasticity (Hoe, et al., 2012, Marcello, et al., 2012), but it is best known as the protein whose cleavage generates amyloid beta (Aβ), a peptide that is the primary component of the amyloid plaques found in Alzheimer's disease (AD) (Hyman, 2011).

Many proteins are involved in the posttranslational cleavage of APP into Aβ, but there are two main pathways through which APP appears to undergo cleavage. In a pathway that negates the production of Aβ, an APPα fragment is produced through cleavage by the enzyme α-secretase. A number of proteins have been implicated as having α-secretase activity, including ADAM10 (Deuss, et al., 2008, Postina, 2008). In an alternative cleavage pathway, a soluble APPβ fragment and a C terminal fragment (βCTF) are produced through APP cleavage by β-secretase, perhaps by two enzymes, BACE1 and BACE2, that are known to have β-secretase activity (Ewers, et al., 2008, Stockley and O'Neill, 2007, Sun, et al., 2005, Wu, et al., 2008, Zetterberg, et al., 2008). The βCTF produced by this β-secretase activity is bound to the membrane and can subsequently undergo γ-secretase cleavage to produce an Aβ peptide ranging in size from 35–42 amino acids. Several protein subunits form the γ-secretase protein complex, including presenilin (either presenilin 1 or presenilin 2), nicastrin, APH1, and PEN2 (Baulac, et al., 2003, Francis, et al., 2002, Kimberly, et al., 2003).

Multiple APP cleavage products, including Aβ, are normally present in the brain and cerebrospinal fluid (CSF) (Seubert, et al., 1992), and the levels of these cleavage products may be associated with cognitive decline. For example, low CSF $A\beta_{42}$ levels are associated with age and the apolipoprotein E (*APOE*) ε4 genotype in cognitively normal adults (Peskind, et al., 2006), and CSF $A\beta_{42}$ levels are significantly lower in patients with AD and mild cognitive impairment compared to cognitively normal controls (Galasko, et al., 1998, Motter, et al., 1995, Sunderland, et al., 2003). In addition, low CSF $A\beta_{42}$ levels are associated with both AD and Aβ deposition in the brain (Fagan, et al., 2006, Tapiola, et al., 2009, Visser, et al., 2009).

Evidence suggests that the steady-state levels of CSF Aβ are maintained not only by its production but also by its degradation and clearance (Sagare, et al., 2012). Modulated production and clearance of the Aβ peptide in the brain is implicated in the complex pathological cascade of AD, which may be caused by overproduction of the Aβ peptide or by impaired clearance of Aβ from the brain. Clearance decreases with increased aggregation, decreased degradation, disturbed blood-brain barrier transport, or inefficient peripheral removal of the peptide (Liu, et al., 2012, Miners, et al., 2011, Sagare, et al., 2012). Previous studies have shown that ApoE interacts with and promotes the clearance of Aβ from the interstitial fluid of the brain (Castellano, et al., 2011). Many other proteins have been associated with Aβ degradation and clearance in AD, including neprilysin, endothelin-converting enzyme, angiotensin I converting enzyme, insulin-degrading enzyme, plasminogen, matrix metallopeptidase 9, receptor for advanced glycation end products, low-density lipoprotein receptor-related protein 1, low-density lipoprotein receptor-related

protein 8, ATP-binding cassette, sub-family B (P-glycoprotein), clusterin (apolipoprotein J), and α2-macroglobulin (Liu, et al., 2012, Miners, et al., 2011, Sagare, et al., 2012).

Given that multiple proteins have been associated with the degradation and clearance of Aβ, and that lower CSF $A\beta_{42}$ levels are associated with AD, older age, and the *APOE* ε4 allele, we hypothesized that SNPs within the noncoding regions of genes that encode proteins biologically implicated in the degradation and clearance of Aβ may correlate with CSF $A\beta_{42}$ levels. We therefore analyzed the relationship between CSF $A\beta_{42}$ levels and 24 SNPs located within the 5′ and 3′ regions of 12 Aβ degradation and clearance genes, as well as *APOE* SNPs that designate the *APOE* ε4 allele, while taking into account age, gender, race, and *APOE* ε4 status.

2 Methods

2.1 Participants

Following informed consent, 168 healthy, cognitively normal, older control subjects (age > 51) and 99 AD patients (Table 1) underwent extensive evaluation that consisted of medical history, family history, physical and neurologic examination, laboratory tests, and neuropsychological assessment; information was obtained from participants and from informants for all AD patients. All procedures were approved by the institutional review boards of the participating institutions.

All control subjects were found to have normal cognition after undergoing a thorough clinical and neuropsychological assessment including Logical memory (immediate and delayed), Category fluency for animals and Letter S, and Trail Making tests A and B. All controls had Mini-Mental State Exam (MMSE) scores > 26 and Clinical Dementia Rating (CDR) scale scores of 0 (Peskind, et al., 2006).

All AD patients were participants in research clinical cores at their respective institutions. Clinical diagnoses of AD were made according to well-established consensus criteria (McKhann, et al., 1984, Petersen, et al., 1999). No AD patients had a known AD-causing mutation or a family history of AD that would suggest autosomal-dominant AD.

2.2 Cerebrospinal fluid (CSF)

All CSF samples were collected in the morning after participants fasted overnight. CSF samples were collected using the Sprotte 24-g traumatic spinal needle while participants were in either a lateral decubitus or sitting position (Peskind, et al., 2006, Peskind, et al., 2005). The samples were aliquoted at the bedside, frozen immediately on dry ice, and then stored at −80°C until they were assayed. Results reported here are from assays run from comparable lumbar puncture fractions to limit variability from rostrocaudal concentration gradients. $A\beta_{42}$ was measured using a sensitive multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use–only reagents) as previously described (Olsson, et al., 2005, Shaw, et al., 2009). The intra-assay coefficient of variation was < 10% for all assays.

2.3 Genes and SNP selection

Twelve genes were chosen for their biologically characterized role in APP degradation and clearance. SNPs were chosen within these genes according to the following criteria: (1) A chosen SNP was located within a known or putative regulatory region of the gene, and when necessary, tagging SNPs were chosen to capture putative regulatory regions; (2) a chosen SNP had a minor allele frequency (MAF) of ≥ 0.1 in the HapMap Caucasian (CEU) population and a minor genotype frequency in our study sample of ≥ 0.01; and (3) a SNP

genotyping assay was commercially available for a chosen SNP. Based on these criteria, 24 SNPs were selected (Table 2). An additional SNP (rs429358) was also genotyped to determine *APOE* ε4 status.

2.4 SNP genotyping

Genomic DNA was genotyped using TaqMan allelic discrimination detection on 384 well plates as described previously (Bekris, et al., 2008). For each reaction, SNP TaqMan Assay (Applied Biosystems), TaqMan Universal PCR Master Mix (Applied Biosystems), and DNA were pipetted into each well. PCR was carried out using a 9700 Gene Amp PCR System (Applied Biosystems). Plates were then subjected to an end-point read on a 7900 Real-Time PCR System (Applied Biosystems). The results were first evaluated by cluster variations; the allele calls were then assigned automatically before being integrated into the genotype database.

2.5 Statistical analysis

SNP genotype frequencies (FF vs. EE vs. EF, where F denotes the major allele and E denotes the minor allele) were tested for Hardy-Weinberg equilibrium and also compared between groups (AD patients vs. control subjects) using the chi-squared test. To adjust for age, gender, race, and *APOE* ε4 status, we performed logistic regression using these covariates and the particular SNPs as predictor variables and the disease status as the response variable. We also compared the frequency of collapsed genotype groups (absence or presence of the minor allele; i.e., FF vs. combined EE and EF) between AD patients and control subjects after adjusting for covariates. All subsequent analyses involving SNPs were based on the collapsed genotype group.

CSF $A\beta_{42}$ levels were initially compared between AD patients and control subjects using the t-test. We then used a linear regression model to examine the relationship between *APOE* ε4 status and CSF $A\beta_{42}$ levels while taking into account gender, age, race, and disease status. This regression model included CSF $A\beta_{42}$ level as the dependent variable; gender, age, race, *APOE* ε4 status, and disease status as the predictor variables; and a disease status–by–*APOE* ε4-status interaction term to allow for the effect of *APOE* ε4 status to potentially vary between disease groups. Next, we used regression models to examine the relationship between the SNPs and CSF $A\beta_{42}$ levels. Each initial model (one for each SNP) included the CSF $A\beta_{42}$ level as the dependent variable and the same predictor variables as in the previously described *APOE* ε4 model, along with the SNP and a disease status–by–SNP interaction term to allow the effect of SNP to potentially vary between disease groups. In cases where a significant SNP effect was found, we then investigated the effect of that SNP separately within each disease status group, allowing for a SNP–by–*APOE* ε4-status interaction term. Diagnostic plots of model residuals were inspected to assess any major departures from normality or homoscedasticity.

As a sensitivity analysis, we constructed a random forest model (Breiman, 2001, Liaw, 2002) using CSF $A\beta_{42}$ level as the dependent variable and disease status, gender, age, race, *APOE* ε4 status, and all 24 SNPs as the predictor variables. The random forest algorithm uses multiple tree-based models to predict $A\beta_{42}$ levels, has built-in cross validation, and inherently adjusts for interactions between predictor variables. The importance of a predictor is quantified by the average increase in mean squared error across all trees that occurs when the values of the predictor are randomly permuted for the out-of-bag (OOB) cases (i.e., the set of observations that are not used for building the current tree). Following the recommendation of Nicodemus et al. (Nicodemus, et al., 2010), we used unscaled measures of importance in these analyses.

Statistical analyses were performed in SPSS (version 14) and R (version 2.12.2; R Development Core Team, 2009; http://www.R-project.org.). When correcting for multiple comparisons, the Holm method was used (Holm, 1979).

3 Results

3.1 SNP genotype frequency

Twenty-four SNPs from 12 APP degradation- and clearance-related genes were genotyped (Table 2). All 24 SNPs passed the test for Hardy-Weinberg equilibrium (i.e., $p > 0.05$ for all tests). A comparison of genotype frequency between AD patients and control subjects indicated that only the frequency of *NEP* rs12765 was significantly different between disease groups, and this difference only occurred when the genotypes were collapsed into two groups (i.e., major genotype FF vs. combined minor genotypes EE and EF), both before and after adjusting for age, gender, race, and *APOE* ε4 status (unadjusted $p = 0.020$, adjusted $p = 0.038$). However, after correcting for multiple comparisons, the significance of this frequency difference disappeared.

3.2 CSF $A\beta_{42}$ levels by disease group and *APOE* ε4 status

Figure 1 shows CSF $A\beta_{42}$ levels for cognitively normal control subjects and AD patients, including 95% confidence intervals for the means. We found that CSF $A\beta_{42}$ levels were significantly lower in AD patients (mean: 106 pg/ml, standard deviation (SD): 28 pg/ml) than in controls (mean: 152 pg/ml, SD: 37 pg/ml; 46 pg/ml reduction, $p < 0.001$, effect size: 1.4, 95% confidence interval (CI) for reduction: 37, 54pg/ml). Figure 2 shows CSF $A\beta_{42}$ levels by disease group and *APOE* ε4 status and includes unadjusted 95% confidence intervals for the means. After controlling for gender, age, and race, there was a significant disease group–by–*APOE* ε4-status interaction ($p = 0.002$). Control subject $A\beta_{42}$ levels were significantly lower for *APOE* ε4 carriers than non-carriers (31 pg/ml reduction, $p < 0.001$, effect size: 1, 95% CI for reduction: 21, 41 pg/ml), whereas among AD patients there was no significant difference in $A\beta_{42}$ levels between *APOE* ε4 carriers and non-carriers ($p = 0.46$).

3.3 SNP genotype effect on CSF $A\beta_{42}$ levels

Each SNP was tested individually for its effect on CSF $A\beta_{42}$ levels in AD patients and controls. Figure 3 shows the effect of SNP on $A\beta_{42}$ levels for each of the 24 APP degradation- and clearance-related SNPs based on the linear regression model described in the Methods section. For each disease group (i.e., control subjects or AD patients), the figure shows either an increase or decrease in $A\beta_{42}$ level for the minor SNP group (EE, EF) compared to the major SNP group (FF), including 95% confidence intervals unadjusted for multiple comparisons. For example, $A\beta_{42}$ levels were higher for minor allele carriers of the *A2M* rs226379 SNP within the control group.

Within the control group, CSF $A\beta_{42}$ levels were significantly higher for *A2M* rs1805667 minor allele carriers compared to major genotype carriers—and this significance remained after performing a Holm correction for multiple comparisons ($p = 0.002$, Holm corrected $p = 0.04$) (Figure 3). CSF $A\beta_{42}$ levels were also significantly higher for minor allele carriers compared to major genotype carriers for the *A2M* rs226376 SNP, and this finding almost achieved significance after correcting for multiple comparisons ($p = 0.003$, Holm corrected $p = 0.06$). None of the other SNPs reached significance after correction for multiple comparisons. For example, within the AD group, CSF $A\beta_{42}$ levels were significantly higher for minor allele carriers compared to major genotype carriers for the *LRP1* rs7956957 SNP, but this finding did not remain significant after correcting for multiple comparisons ($p = 0.03$, Holm corrected $p = 0.63$).

3.4 Effect of *APOE* ε4, A2M rs226376, and rs1805667 genotype on CSF $A\beta_{42}$ levels

The *A2M* gene was the only gene in this analysis that remained significant after performing a Holm correction for multiple comparisons (Figure 3). The nature of this difference is summarized in Figure 4 for both of the two relevant *A2M* SNPs: rs226376 and rs1805667. The effects of these SNPs were analyzed separately for control subjects and AD patients, and these analyses included gender, race, age, and *APOE* ε4 status as covariates, as well as a SNP-by–*APOE* ε4-status interaction term. The effects of SNP and *APOE* ε4 were additive, that is, neither of the SNPs displayed a significant SNP-by–*APOE* ε4 status interaction effect, either within controls ($p = 0.78$ for rs226379, $p = 0.46$ for rs1805667) or within AD patients ($p = 0.36$ for rs226379, $p = 0.11$ for rs1805667). Within control subjects, there was a significant difference between minor allele carriers and major genotype carriers for both SNPs, with the major genotype carriers showing decreased levels of $A\beta_{42}$ (15 pg/ml reduction, $p = 0.017$, effect size of 0.48, 95% CI for reduction: 3, 27 pg/ml for rs226379; 14 pg/ml reduction, $p = 0.026$, effect size of 0.45, 95% CI for reduction: 2, 26 pg/ml for rs1805667). Just as with *APOE* ε4 (Figure 2), within AD patients there was no significant difference in $A\beta_{42}$ levels between allele carrier groups for either SNP ($p = 0.78$ for rs226379; $p = 0.49$ for rs805667).

3.5 Random forest results

A random forest model with CSF $A\beta_{42}$ level as the dependent variable and disease status, gender, age, race, *APOE* ε4 status, and all 24 SNPs as the predictor variables explained 40% of the variance in $A\beta_{42}$ levels (Figure 5). The five most important variables, based on unscaled importance, were disease status (diagnosis), *APOE* ε4 status, age, and the two *A2M* SNPs: rs226376 and rs1805667.

4 Discussion

The aim of this investigation was to determine whether CSF $A\beta_{42}$ levels correlate with genetic variation in the regulatory regions of genes that are biologically associated with the degradation and clearance of Aβ in the brain. We analyzed twelve of these genes for correlations between the SNPs in their regulatory regions and CSF $A\beta_{42}$ levels, and we found that two SNPs in the *A2M* gene were significantly correlated with CSF $A\beta_{42}$ levels. Moreover, the 3′ region *A2M* SNP (rs1805667) remained significant after correction for multiple comparisons (Figure 2). These results are supported by a random forest analysis where the *A2M* SNPs were identified as the two most important variables for predicting CSF $A\beta_{42}$ levels after disease status, *APOE* ε4 status, and age (Figure 5).

In addition, we found that CSF $A\beta_{42}$ levels in cognitively normal subjects were significantly lower in *APOE* ε4 carriers than in non-carriers, whereas in AD patients the presence or lack of an ε4 allele showed no relationship to CSF $A\beta_{42}$ levels (Figure 2). These results are consistent with previous reports in cognitively normal controls of lower Aβ levels in *APOE* ε4 carriers than in ε4 non-carriers (Lim, et al., 2012, Peskind, et al., 2006). Given that among controls CSF $A\beta_{42}$ levels were lower in *APOE* ε4 carriers than in non-carriers, we further hypothesized that the effect of *APOE* ε4 on CSF $A\beta_{42}$ levels would be enhanced in the presence of certain *A2M* genotypes. Indeed, cognitively normal controls who carried *A2M* rare alleles (EF, EE) but not *APOE* ε4 had significantly higher CSF $A\beta_{42}$ levels than *A2M* common genotype (FF) carriers who also carried *APOE* ε4 (Figure 4). Given that low CSF $A\beta_{42}$ levels are associated with both AD and Aβ deposition in the brain (Fagan, et al., 2006, Tapiola, et al., 2009, Visser, et al., 2009), these results suggest that *A2M* rare allele carriers that are *APOE* ε4 non-carriers have the greatest level of protection against Aβ deposition, whereas *APOE* ε4 carriers with the *A2M* common genotype are the most susceptible to Aβ deposition.

Interestingly, in AD patients, we found no relationship between CSF $A\beta_{42}$ levels and *APOE* genotype (Figure 2), nor between CSF $A\beta_{42}$ levels and *A2M* genotype (Figure 4). This suggests that although genotype may influence CSF $A\beta_{42}$ levels in cognitively normal controls, genotype does not appear to play a role once subjects have progressed to AD. Furthermore, it is possible that other distant uncharacterized regulatory regions were not detected using the regulatory region tagging SNPs chosen for this study, and this may contribute to the lack of a CSF $A\beta_{42}$ association with other Aβ clearance or degradation genes in either AD or controls. Although an advantage of this investigation, in contrast to genome-wide association studies, is that our hypothesis tested a specific biological pathway, a limitation to this approach is that only a few SNPs were used to capture putative regulatory genetic variation within and surrounding the genes of interest. In addition, a positive SNP may represent a surrogate marker for a true functional SNP that was not analyzed in this study. Additional functional studies are needed to fine-map the specific contribution of the genetic variation within and surrounding the *A2M* gene to both protein activity and gene-expression levels.

The *A2M* gene encodes alpha-2-macroglobulin (alpha2M), a protein which has many diversified and complex functions, including the inhibition of a broad spectrum of proteases without the direct blockage of the protease active site (Rehman, et al., 2013). When activated by proteases, alpha2M functions as an extracellular chaperone that inhibits amorphous and fibrillar protein aggregation (Wyatt, et al., 2013). It is also implicated in the clearance of the extracellular Aβ constituent of amyloid plaques, where it acts as an extracellular chaperone that helps control amyloid formation and toxicity in vivo (Wilson, et al., 2008; Yerbury, et al., 2009, Yerbury and Wilson, 2010). A recent report on microglial Aβ binding has shown that alpha2M facilitates the clearance of Aβ in AD, whereas in AD with cerebral amyloid angiopathy, CD11b leads to Aβ accumulation (Zabel, et al., 2013).

An increase in alpha2M levels occurs in blood-brain barrier disruption and during inflammation (Cucullo, et al., 2003). In addition, dimeric and tetrameric alpha2M play a role in controlling amyloid fibril formation (Ozawa, et al., 2011), which suggests that the level of alpha2M expression is critical in amyloid deposition. An increase in serum or plasma alpha2M levels has also been reported in AD (Hye, et al., 2006, Zhang, et al., 2004). However, other studies have found no difference between AD patients and cognitively normal controls in alpha2M levels (Akuffo, et al., 2008, Giometto, et al., 1988, Licastro, et al., 1995).

The promoter SNP (rs226379) analyzed in the present study is located near a previously described positive-transcription regulatory element that differentially regulates the *A2M* gene during replicative senescence (Li, et al., 2011). The findings from this previous study suggest that *A2M* regional regulatory elements may be involved in the regulation of alpha2M expression in a temporal or spatial manner (Li, et al., 2011). Both of the *A2M* SNPs analyzed in the present study are located within transcription factor ChIP sequence sites described by ENCODE, which further suggests that these SNPs may influence *A2M* gene expression (Rosenbloom, et al., 2010).

The *A2M* gene lies in an AD linkage region on chromosome 12 (12p13.31) (Blacker, et al., 1998, Liao, et al., 1998). Within this *A2M* region, other studies have investigated two polymorphisms (rs3832852 and rs669) defined by a 5-bp insertion/deletion at a splice site of exon 18 (Matthijs and Marynen, 1991). *A2M* polymorphisms rs3832852 and rs669 show contrasting results in different AD populations (Blacker, et al., 1998, Liao, et al., 1998, Panza, et al., 2006, Saunders, et al., 2003). The SNP rs669 has been associated with argyrophilic grain disease (Ghebremedhin, et al., 2002), a disease that may have neurofibrillary lesions that are typical of AD (Thal, et al., 2005), but meta-analyses of

rs3832852 and rs669 association studies show no significant allele frequency differences between AD cases and controls (Corder, et al., 1993), even after stratification by *APOE* ε4 (Koster, et al., 2000, Panza, et al., 2006). Most genetic studies of *A2M* genotypes, including those evaluating an *A2M* haplotype, suggest a weak association with AD (Edwards, et al., 2009, Flachsbart, et al., 2010). Haplotype analyses of *A2M* polymorphisms in *APOE* ε4 non-carriers (Verpillat, et al., 2000) support initial reports of an association with AD and lend some support to the results described in the present study, where *APOE* ε4 non-carriers who carry the *A2M* rare allele have high CSF $A\beta_{42}$ levels.

Previous reports suggest that the level of alpha2M expression is critical in amyloid deposition (Cucullo, et al., 2003, Ozawa, et al., 2011), whereas the evidence we present here suggests that *A2M* locus 5′ and 3′ genetic variation is associated with the modulation of CSF $A\beta_{42}$ levels in controls but not in AD patients. If SNPs within the regulatory regions of the *A2M* gene influence *A2M* expression, a corresponding increase in alpha2M may lead to efficient clearance of Aβ and thus to higher CSF $A\beta_{42}$ levels. Furthermore, cognitively normal controls with an *A2M* rs226379 A allele or an rs1805667 T allele may clear Aβ more efficiently and may therefore have higher CSF $A\beta_{42}$ levels than controls without these alleles. In contrast, it has been reported that the modulation of Aβ clearance and deposition leads to decreasing CSF $A\beta_{42}$ levels in the early stages of cognitive decline and, subsequently, to very low CSF $A\beta_{42}$ levels in AD (Fagan, et al., 2006, Tapiola, et al., 2009, Visser, et al., 2009). In our study, and in reports by others, it appears that low CSF $A\beta_{42}$ levels in AD may contribute to a lack of a significant association between genotype (i.e., *A2M* or *APOE*) and CSF $A\beta_{42}$ levels (Peskind, et al., 2006, Pirttila, et al., 1996).

In conclusion, to our knowledge, the present study is the first to identify an association between CSF $A\beta_{42}$ levels and SNPs in the *A2M* gene. Cognitively normal *APOE* ε4 non-carriers with the *A2M* rare allele were found to have the highest CSF $A\beta_{42}$ levels. Given that low CSF $A\beta_{42}$ levels are associated with increased Aβ deposition in the brain, these results suggest that genetic variation within the *A2M* gene influences CSF $A\beta_{42}$ levels and that *A2M* rare allele carriers may be protected against Aβ deposition.

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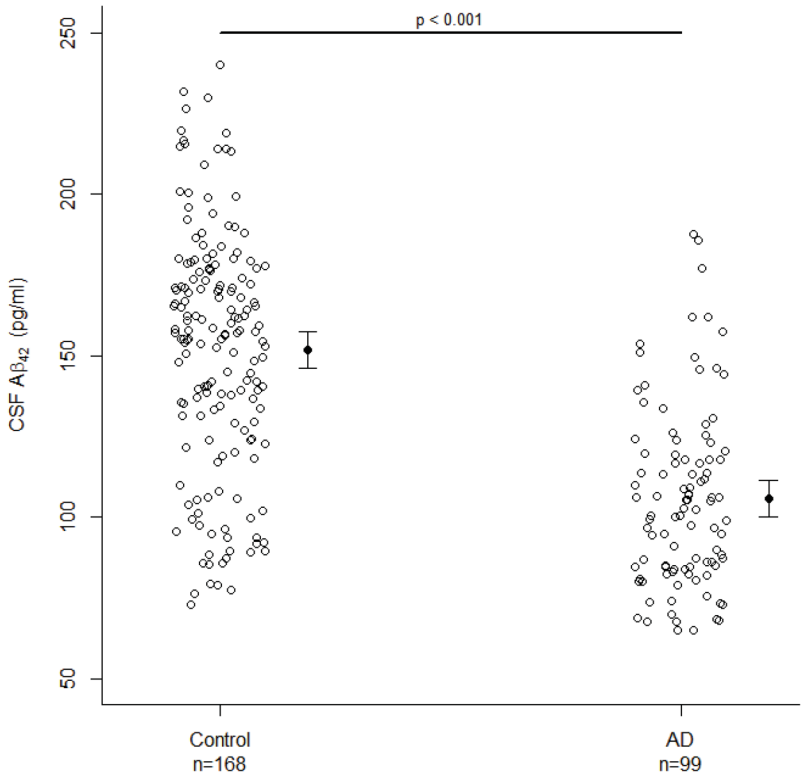


Figure 1.
Cerebrospinal fluid (CSF) $A\beta_{42}$ levels in cognitively normal control subjects and Alzheimer's disease (AD) patients.

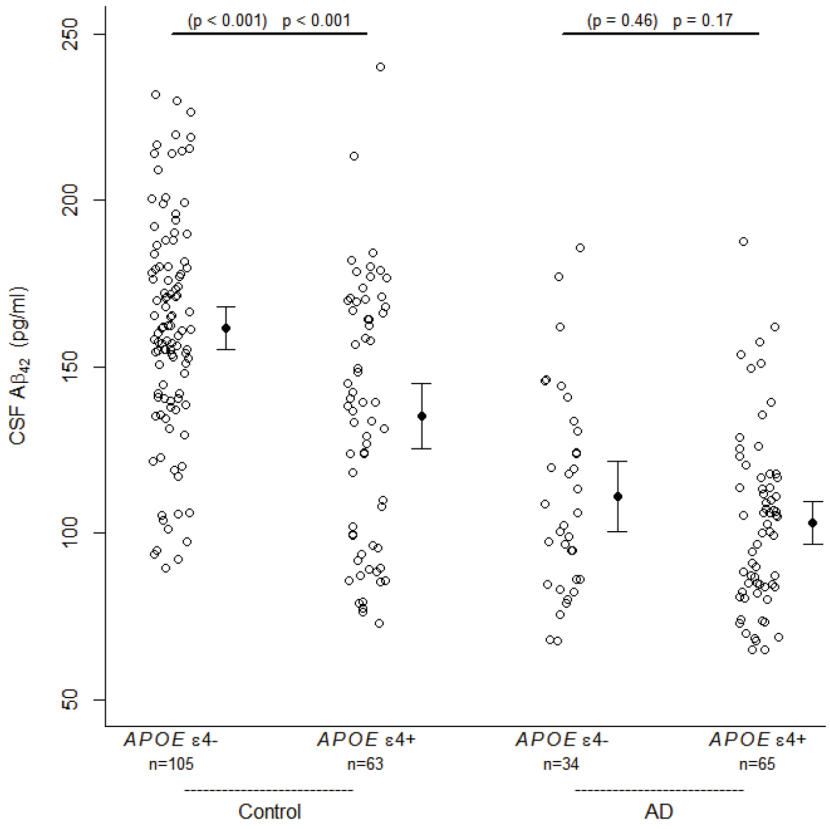


Figure 2.

Cerebrospinal fluid (CSF) $A\beta_{42}$ levels in cognitively normal control subjects and Alzheimer's disease (AD) patients by *APOE* ε4 status. Differences between *APOE* groups were tested with and without adjusting for gender, race, and age. Adjusted *p* values are in parentheses.

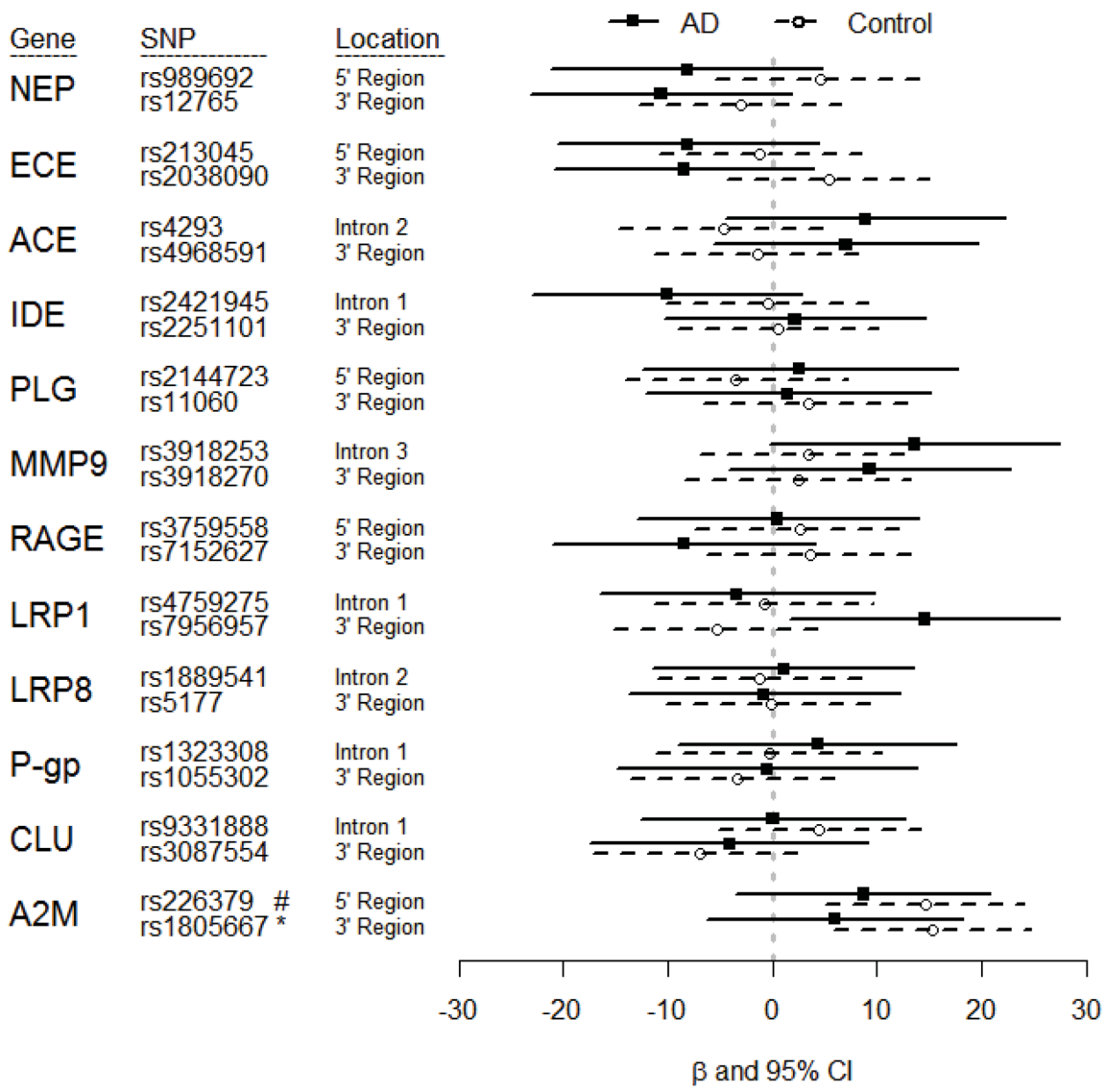
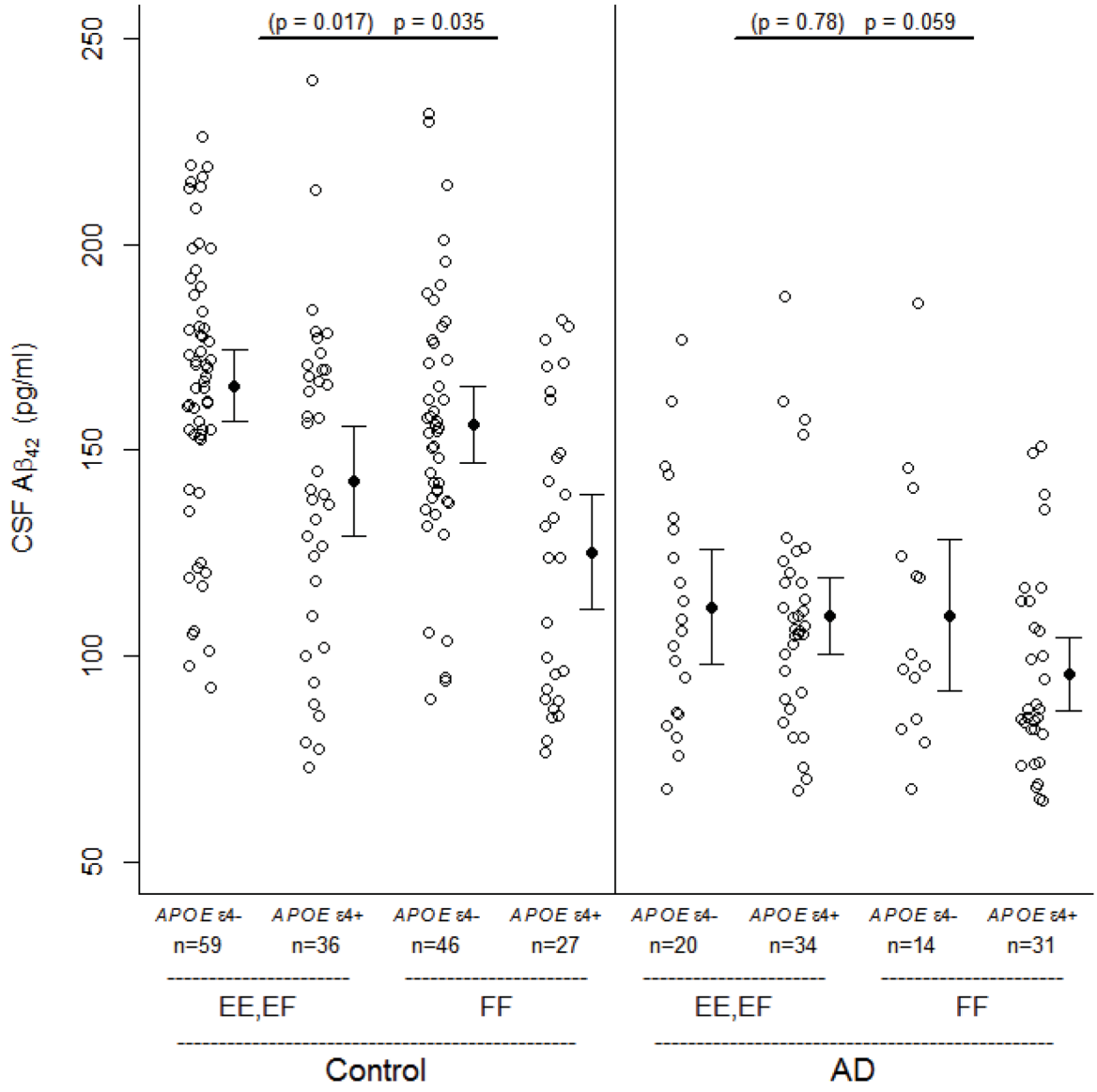


Figure 3.

Beta coefficients for cerebrospinal fluid (CSF) $A\beta_{42}$ levels in cognitively normal control subjects and Alzheimer's disease (AD) patients per collapsed genotype. See the *Statistical analysis* section for an explanation of how SNP effects were modeled. A confidence interval which does not cross the vertical line at zero indicates that the difference in genotypes is significant (p value < 0.05) before adjusting for multiple comparisons. A beta coefficient (solid square for AD, circle for controls) to the right of the vertical line represents higher CSF $A\beta_{42}$ levels for the collapsed genotype group that contains minor alleles (EE or EF). # Single-nucleotide polymorphism (SNP) effect of A2M SNP rs226379 in controls: $p = 0.003$; Holm correction for multiple comparisons $p = 0.06$. * SNP effect of A2M SNP rs1805667 in controls: $p = 0.002$; Holm correction for multiple comparisons $p = 0.04$.



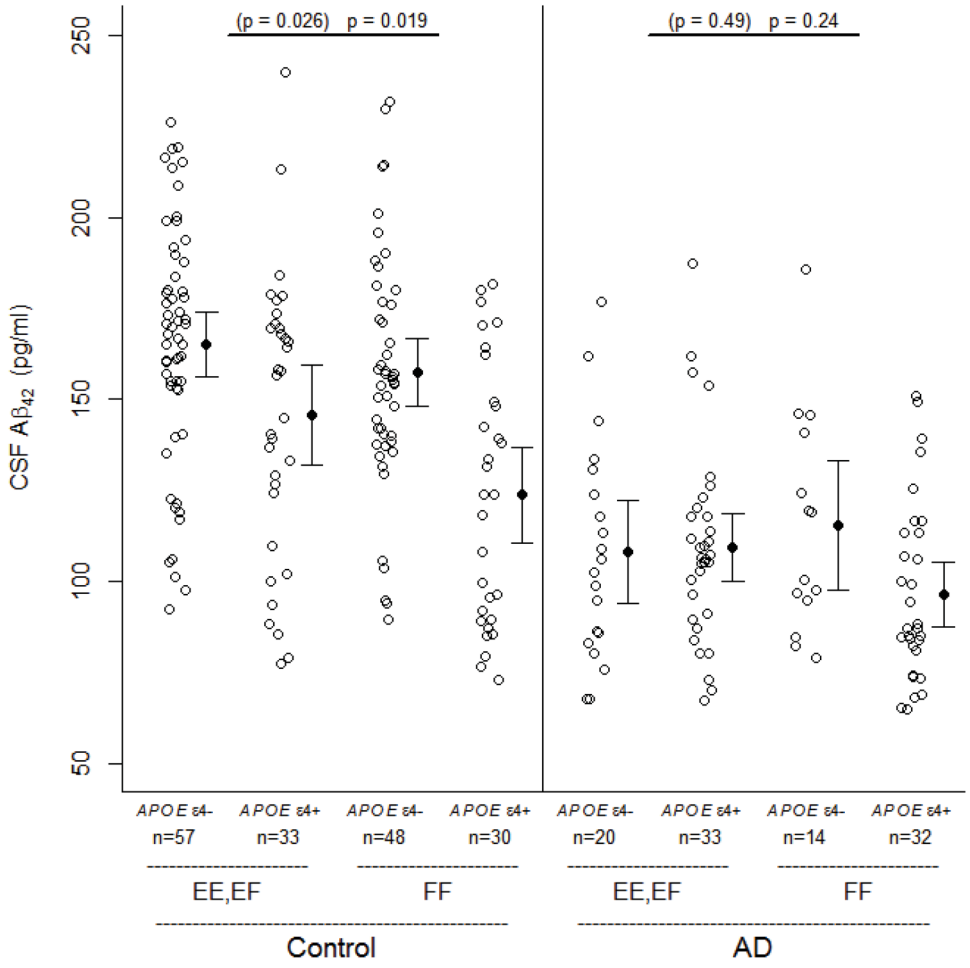


Figure 4.

Cerebrospinal fluid (CSF) $A\beta_{42}$ levels in cognitively normal control subjects and Alzheimer's disease (AD) patients stratified by *APOE* ε4 and *A2M* single-nucleotide polymorphisms (SNPs). *A2M* SNPs: rs226379 (EE, EF vs. FF: AA, AG vs. GG) (Panel A) and rs1805667 (EE, EF vs. FF: TT, TG vs. GG) (Panel B). Differences between SNP groups were tested separately for control participants and AD patients and were tested with and without adjusting for gender, race, age, and *APOE* ε4 status. Adjusted *p* values are in parentheses. *P* values do not take into account multiple comparisons.

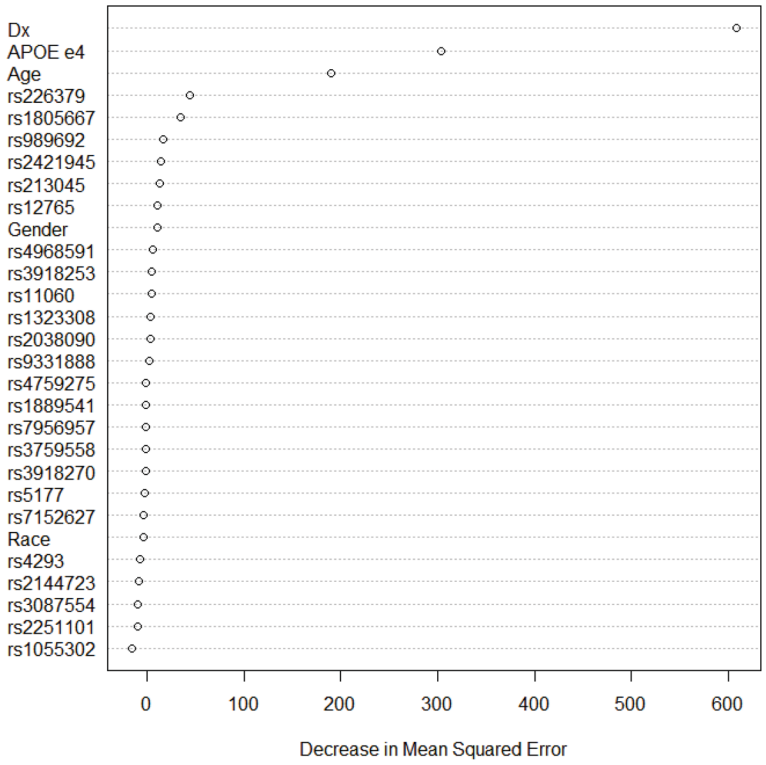


Figure 5.
Random forest variable importance plot for predicting cerebrospinal fluid (CSF) $A\beta_{42}$ levels, using disease status, gender, race, age, *APOE* ε4 status, and the 24 single-nucleotide polymorphisms (SNPs) (collapsed genotypes). Percent variance explained = 40%.

Table 1

Sample Description

	Controls (n = 168)	AD (n = 99)	*P* value[a]
Age	67 (9; 52–88)	71 (10; 52–87)	< 0.001
Female	103 (61%)	45 (45%)	0.02
Caucasian	153 (91%)	96 (97%)	0.11
APOE ε4+	63 (38%)	65 (66%)	< 0.0001
Age at onset		66 (9; 46–82)	
$A\beta_{42}$ (pg/ml)	152 (37; 73–240)	106 (28; 65–18f8)	< 0.0001

Mean, standard deviation, and range shown for continuous variables; sample size and percent shown for categorical variables.

AD, Alzheimer's disease; *APOE* ε4+, at least one *APOE* ε4 allele

[a] *P* value based on the two-sample t-test for continuous variables and the chi-square test for categorical variables.

Table 2

Genotype Frequency Distribution

					Frequency						P-values			
					AD			Controls			Genotype		Collapsed Genotype	
Aβ Degradation Genes	**Gene Name**	**Gene Location**	**SNP**	**SNP Location**	**EE**	**EF**	**FF**	**EE**	**EF**	**FF**	**Unadj.**	**Adj.**	**Unadj.**	**Adj.**
NEP	Neprilysin	3q25.1-q25.2	rs989692	5′ Region	0.21	0.43	0.35	0.18	0.48	0.34	0.701	0.511	0.813	0.743
			rs12765	3′ Region	0.06	0.47	0.46	0.10	0.31	0.60	**0.024**	0.164	**0.038**	**0.020**
ECE	Endothelin-Converting Enzyme	1p36.1	rs213045	5′ Region	0.11	0.46	0.42	0.11	0.36	0.52	0.238	0.297	0.116	0.154
			rs2038090	3′ Region	0.09	0.38	0.53	0.08	0.36	0.55	0.902	0.498	0.654	0.519
ACE	Angiotensin I converting enzyme	17q23.3	rs4293	Intron 2	0.23	0.44	0.32	0.17	0.50	0.33	0.462	0.657	0.944	0.911
			rs4968591	3′ Region	0.16	0.41	0.42	0.12	0.51	0.38	0.311	0.461	0.426	0.305
IDE	Insulin-degrading enzyme	10q23-q25	rs2421945	Intron 1	0.14	0.49	0.36	0.12	0.45	0.42	0.634	0.728	0.342	0.468
			rs2251101	3′ Region	0.06	0.47	0.46	0.08	0.40	0.52	0.520	0.716	0.401	0.565
PLG	Plasminogen	6q26	rs2144723	5′ Region	0.29	0.48	0.22	0.19	0.52	0.29	0.135	0.006	0.255	0.115
			rs11060	3′ Region	0.23	0.45	0.31	0.24	0.40	0.35	0.716	0.524	0.525	0.334
MMP9	Matrix metallopeptidase 9	20q11.2-q13.1	rs3918253	Intron 3	0.21	0.52	0.27	0.27	0.39	0.33	0.149	0.701	0.301	0.250
			rs3918270	3′ Region	0.01	0.29	0.70	0.02	0.24	0.74	0.618	0.539	0.468	0.497
Aβ Clearance Genes														
RAGE	Receptor for advanced glycation end products	14q32	rs3759558	5′ Region	0.01	0.30	0.69	0.04	0.33	0.64	0.384	0.700	0.407	0.765
			rs7152627	3′ Region	0.05	0.36	0.59	0.04	0.35	0.61	0.805	0.522	0.660	0.580
LRP1	Low density lipoprotein receptor- related protein 1	12q13-q14	rs4759275	Intron 1	0.15	0.48	0.36	0.13	0.57	0.30	0.441	0.588	0.312	0.254
			rs7956957	3′ Region	0.11	0.52	0.37	0.17	0.44	0.39	0.347	0.999	0.757	0.771
LRP8	Low density lipoprotein receptor- related protein 8	1p34	rs1889541	Intron 2	0.09	0.37	0.54	0.14	0.40	0.46	0.398	0.455	0.262	0.760
			rs5177	3′ Region	0.20	0.43	0.36	0.19	0.45	0.36	0.953	0.853	0.915	0.984
ABCB1	ATP-binding cassette, sub-family B (P-glycoprotein)	7q21.12	rs13233308	Intron 1	0.20	0.46	0.33	0.29	0.43	0.29	0.307	0.077	0.414	0.251
			rs1055302	3′ Region	0.02	0.24	0.74	0.02	0.31	0.67	0.502	0.381	0.266	0.256
CLU	Clusterin (apolipoprotein J)	8p21-p12	rs9331888	Intron 1	0.12	0.45	0.42	0.10	0.40	0.50	0.485	0.155	0.231	0.249
			rs3087554	3′ Region	0.05	0.26	0.69	0.05	0.27	0.68	0.991	0.588	0.968	0.689
A2M	α2-macroglobulin	12p13.31	rs226379	5′ Region	0.10	0.44	0.45	0.12	0.44	0.43	0.832	0.690	0.750	0.745
			rs1805667	3′ Region	0.11	0.42	0.46	0.13	0.40	0.46	0.880	0.882	0.995	0.942

Unadjusted (Unadj.) and adjusted (Adj.) *p* values are provided. Adjusted *p* values are for covariates: age, gender, race, and *APOE* ε4 status. *P* values are not corrected for multiple comparisons.

AD, Alzheimer's disease; SNP, single-nucleotide polymorphism