Published in final edited form as:

Neurobiol Aging. 2011 March ; 32(3): 556.e13–556.e23. doi:10.1016/j.neurobiolaging.2010.10.020.

APP Processing Genes and Cerebrospinal Fluid APP Cleavage Product Levels in Alzheimer's Disease

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Abstract

The aim of this exploratory investigation was to determine if genetic variation within APP or its processing enzymes correlates with APP cleavage product levels: APP α , APP β or A β 42, in cerebrospinal fluid (CSF) of cognitively normal subjects or Alzheimer's disease (AD) patients. Cognitively normal control subjects (n=170) and AD patients (n=92) were genotyped for 19 putative regulatory tagging SNPs within nine genes (*APP*, *ADAM10*, *BACE1*, *BACE2*, *PSEN1*, *PSEN2*, *PEN2*, *NCSTN* and *APH1B*) involved in the APP processing pathway. SNP genotypes were tested for their association with CSF APP α , APP β , and A β 42, AD risk and age-at-onset while taking into account age, gender, race and *APOE* ϵ 4. After adjusting for multiple comparisons a significant association was found between *ADAM10* SNP rs514049 and APP α levels. In controls, the rs514049 CC genotype had higher APP α levels than the CA,AA collapsed genotype, whereas the opposite effect was seen in AD patients. These results suggest that genetic variation within *ADAM10*, an APP processing gene, influences CSF APP α levels in an AD specific manner.

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Disclosure Statement for Authors: There are no actual or potential conflicts of interest.

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Keywords

APP; ADAM10; BACE1; BACE2; PSEN1; PSEN2; PEN2; NCSTN; APOE4; Alzheimer's; Cerebrospinal Fluid

Introduction

Amyloid precursor protein (APP) is an integral membrane protein of unknown function expressed in many tissues including neurons. APP has been implicated as a regulator of synapse formation (Priller et al., 2006) and neuronal plasticity (Turner et al., 2003). However, APP is best known as the protein whose cleavage generates amyloid beta (A β), a peptide that is the primary component of the amyloid plaques found in Alzheimer's disease (AD).

Rare coding mutations in APP are a cause of autosomal dominant early onset AD (Goedert et al., 2006). The majority of these mutations alter processing of APP so that the relative levels of A β 42 are increased (Scheuner et al., 1996; Walker et al., 2005). Triplication of the APP gene, due to chromosome 21 trisomy in Down's Syndrome, is associated with increased APP expression and amyloid plaque formation (Englund et al., 2007; Giaccone et al., 1989; Lemere et al., 1996). In addition, APP promoter polymorphisms have been associated with AD (Guyant-Marchal et al., 2007; Lv et al., 2008).

Many proteins are involved in the post-translational cleavage of APP into A β . APP undergoes cleavage by at least two pathways. In one pathway, cleavage by the enzyme α -secretase produces an APP α fragment. A number of proteins have been implicated as having α -secretase activity, including ADAM10, ADAM9 and ADAM17 (Deuss et al., 2008; Postina, 2008). ADAM10 has been well characterized and its levels are reported to be decreased in AD CSF, therefore it was chosen as the ADAM gene to analyze in this investigation (Colciaghi et al., 2002; Postina et al., 2004; Qin et al., 2009).

In another pathway, APP cleavage by β -secretase produces a soluble APP β fragment and a C terminal fragment (β CTF) bound to the membrane which subsequently can undergo γ -secretase cleavage to produce an A β peptide ranging in size from 35–42 amino acids. BACE1 has β -secretase activity and CSF BACE1 levels have been measured in AD compared to controls with contradicting results (Ewers et al., 2008; Wu et al., 2008; Zetterberg et al., 2008). BACE2 also has β -secretase activity (Stockley and O'Neill, 2007; Sun et al., 2005).

γ -secretase is a protein complex composed of several subunits including presenilin (either presenilin 1 or presenilin 2), nicastrin, APOE4 and PEN2 (Baulac et al., 2003; Francis et al., 2002; Kimberly et al., 2003). Each γ -secretase subunit protein is uniquely involved in APP cleavage, the tissue specificity, or the stability of the protein complex (Lee et al., 2004; Prokop et al., 2004; Shirotani et al., 2004; Watanabe et al., 2005; Zhang et al., 2005). Autosomal dominant early onset familial AD (EOFAD) occurs before the age of 60, has a strong family history, and is caused by rare mutations in the presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*) or the APP genes (Goedert and Spillantini, 2006). *PSEN1* missense mutations are the most common cause of EOFAD (Theuns et al., 2000). Little is known about the influence of genetic variation within non-coding regions (putative regulatory elements) of these AD relevant proteins which play an important role in the post-translational cleavage of APP.

Both soluble APP and A β are normally present in the brain and CSF (Seubert et al., 1992). Decreasing CSF A β 42 levels are associated with age and *APOE* ϵ 4 genotype in cognitively normal adults (Peskind et al., 2006). CSF A β 42 levels are significantly decreased in AD and mild cognitive impairment compared to controls (Galasko et al., 1998; Motter et al., 1995; Sunderland et al., 2003). CSF A β 42 levels are correlated with both AD status and A β deposition in the brain (Fagan et al., 2006; Tapiola et al., 2009; Visser et al., 2009). The APP cleavage products; APP α and APP β , are normally present in CSF but comparisons between AD patients and controls have shown conflicting results (Diniz et al., 2008; Farlow et al., 1992; Galasko et al., 1998; Hampel et al., 2004; Hampel et al., 2008; Motter et al., 1995; Nakamura et al., 1994; Olsson et al., 2003; Palmert et al., 1990; Sunderland et al., 2004; Van Nostrand et al., 1992).

Given that CSF A β 42 levels are associated with AD, age and *APOE* ϵ 4, as described above, we hypothesized that genetic variation within non-coding regions of APP, by influencing the amount of APP protein (substrate) available for cleavage, would correlate with CSF levels of APP cleavage products (APP α , APP β , A β 42). Furthermore, we hypothesized that other genes encoding secretase genes responsible for cleavage of APP (*ADAM10*, *BACE1*, *BACE2*, *PSEN1*, *PSEN2*, *APH1B* and *PEN2*) also would correlate with APP cleavage product levels. Specifically, the aim of this investigation was to determine if genetic variation both 5' and 3' of *APP*, *ADAM10*, *BACE1*, *BACE2*, *PSEN1*, *PSEN2*, *PEN2*, *NCSTN* and *APH1B* correlated with CSF APP α , APP β , A β 42, AD age-at-onset or AD risk. A total of 19 SNPs were analyzed while taking into account age, gender, race and *APOE* ϵ 4. The main novel finding of this investigation was that the SNP rs541049, within the α -secretase gene, *ADAM10*, correlates with CSF APP α levels.

Methods

Subjects

All procedures were approved by the institutional review boards of the participating institutions. Subjects were 173 healthy cognitively normal control subjects age 52–88 years and 96 AD patients 52–87 years old with an age-at-onset of 46–82. After removing seven subjects due to missing values that included failed genotyping and CSF measures, 170 controls and 92 AD patients remained in the analysis (Table 1). Following informed consent, all subjects underwent extensive evaluation that consisted of medical history, family history, physical and neurologic examinations, laboratory tests, and neuropsychological assessment; information was obtained from subjects and from informants for all patients.

All control subjects had normal cognition and underwent 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, and 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). Most AD patients had a CDR score of 1, only a few had a CDR score of 2 and no AD patients had a CDR score of 3.

No AD subjects had a known AD-causing mutation and none had a family history of AD that would suggest familial AD.

Cerebrospinal Fluid

All CSF samples were collected in the morning after an overnight fast using the Sprotte 24-g atraumatic spinal needle with the patient in either the lateral decubitus or sitting position. (Peskind et al., 2005; Peskind et al., 2006) Samples were aliquoted at the bedside and frozen immediately on dry ice and stored at -80°C until assayed. Concentrations of A β 42 in the 9th ml of collected CSF were measured using a sensitive multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use—only reagents) immunoassay kit—based reagents. (Shaw et al., 2009) Concentrations of APP α and APP β in the 10th ml of collected CSF were measured by immunoassays (Johnson-Wood et al., 1997).

Only a subset of CSF APP α and APP β were measured because the initial agreement between investigators was to measure CSF APP α and APP β in a certain number of consecutive samples. CSF A β 42 was measured in all samples. Intra-assay coefficient of variation was $<10\%$ for all assays.

Genes and SNP selection

The nine studied genes were chosen for their biologically characterized role in the APP processing pathway. SNPs were also chosen according to the following criteria: 1) The SNP was located within a known or putative regulatory region of the gene. 2) The SNP had a minor allele frequency (MAF) of ≥ 0.1 in HapMap Caucasian (CEU) population and a minor genotype frequency in our study sample of ≥ 0.01 . 3) The SNP genotyping assay was commercially available. 4) When necessary, tagging SNPs were chosen to capture putative regulatory regions. Based on these criteria, a total of 19 SNPs were selected (see Table 2). An additional SNP (rs429358) was also genotyped to determine *APOE* $\epsilon 4$ status.

SNP Genotyping

Genomic DNA was genotyped using TaqMan allelic discrimination detection on 384 well plates as previously described (Bekris et al., 2008). Briefly, 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.

Statistical Analysis

We compared SNP genotype frequencies (EE vs. EF vs. FF, where E denotes the major allele and F denotes the minor allele) between AD patients and control subjects using the chi-squared test. To adjust for age, gender, race, and *APOE* $\epsilon 4$ status ($\epsilon 4$ positive versus negative), we performed logistic regression using disease status as the response variable, and SNP and these covariates as predictor variables. The inclusion of non-Caucasian individuals raises the possibility of population substructure differences. Thus race was taken into account in all analyses. However, to assess the possible influence of population substructure the data was analyzed both with and without taking into account race. The significance of the results, when race was not taken into account, did not change (data not shown). We also compared frequencies of collapsed genotype groups (presence or absence of the minor allele; i.e., EE vs. EF or FF) between AD patients and control subjects after adjusting for covariates (Table 2). All subsequent analyses involving SNPs were based on the collapsed genotype group.

The distribution of CSF APP α , APP β , and A β 42 protein levels was compared between AD patients and control subjects initially using t-tests (Figure 1). We then examined the relationship between SNPs and CSF protein levels while taking into account gender, age, race and *APOE* ϵ 4 status using linear regression. Each of the initial linear regression models (one for each SNP) included the CSF protein level as the dependent variable and the predictor variables gender, age, race, *APOE* ϵ 4 status, disease status, and SNP, as well as a disease status by SNP interaction term to allow the effect of SNP to possibly vary between disease groups (Figure 2). In the case where a significant SNP effect was found, we also investigated the model that included two-way interactions between the significant SNP and each of the other SNPs (i.e., 18 additional terms), as well as three-way interactions between the SNP pairs and disease status (18 additional terms). We investigated the relationship between SNPs and age of onset in AD patients using linear regression models with age of onset as the response variable and SNP as a predictor variable, along with gender, age, race, and *APOE* ϵ 4 status. Statistical analyses were performed in SPSS (version 14) and R (version 2.8.1; R Development Core Team, 2009; <http://www.R-project.org>). When correcting for multiple comparisons the Holm (1979) method was used.

Results

SNP Genotype Frequency

Nineteen SNPs from nine APP processing genes were genotyped (Table 2). Comparison of genotype frequency between AD and controls indicated that only the *BACE1* SNP rs11601511 was significantly different ($p = 0.046$) when genotypes were collapsed into two groups, (the major genotype (EE) and the minor genotypes (EF, FF)), and after adjusting for age, gender, race, and *APOE* ϵ 4 status. However, significance is lost after adjustment for multiple comparisons.

CSF APP Cleavage Product Levels

CSF APP cleavage product levels (APP α , APP β , and A β 42) were compared between cognitively normal control subjects and AD patients. CSF APP α , APP β and A β 42 levels were lower in AD patients compared to controls ($p = 0.049$, 0.028 and <0.001 , respectively) (Figure 1, Panels A, B and C). For APP α , the mean (SD) for control subjects and AD patients was 28.5 (9.4) and 25.1 (8.2) nM, respectively, and the 95% confidence interval (95% CI) was [0.01, 6.7]. For APP β , the mean (SD) for control subjects and AD patients was 17.7 (5.1) and 15.5 (5.0) nM, respectively, and the 95% CI [0.2, 4.0]. For A β 42, the mean (SD) for control subjects and AD patients was 152 (38) and 106 (28) pg/ml, respectively, and the 95% CI [36, 54].

SNP Genotype Effect on CSF APP Cleavage Product Levels

Each APP processing gene SNP was tested individually for its effect on CSF APP cleavage product levels in AD patients and controls. Figure 2 shows the effect of SNP on protein level for each of the 19 SNPs based on the linear regression model described in the Methods section. For each disease group, where disease group is defined as without disease (control subjects) or with disease (AD patients), the figure shows either an increase or decrease in protein level for the minor SNP group (EF, FF) compared to the major SNP group (EE), along with 95% confidence intervals unadjusted for multiple comparisons. For example, APP α levels are higher for minor allele carriers of the rs514049 SNP within the AD group, but are lower within the control group (Figure 2).

For CSF APP α levels, one SNP (rs514049) was nominally significant within AD patients ($p = 0.047$), and three SNPs were nominally significant within control subjects: rs214484 ($p = 0.044$), rs2040273 ($p = 0.032$), and rs514049 ($p = 0.001$), as indicated by the confidence

interval line not crossing the vertical line at 0 in Figure 2. Three SNPs showed nominally significant differential effects between control subjects and AD patients (i.e., significant SNP by disease group interaction terms): rs514049 ($p = 0.001$), rs2305421 ($p = 0.025$), and rs638405 ($p = 0.028$). However, after controlling for multiple comparisons, only the differential effect between control subjects and AD patients of the *ADAM10* rs514049 SNP (asterisk in Figure 2) remained significant ($p_{\text{Holm}} = 0.014$), and none of the other SNPs showed significant effects within either disease group or when disease groups were combined.

For CSF APP β levels, two SNPs were nominally significant within AD patients: rs2305421 ($p = 0.020$) and rs1295652 ($p = 0.034$), and three SNPs were nominally significant in controls: rs214484 ($p = 0.028$), rs12149 ($p = 0.025$), and rs362354 ($p = 0.031$). Three SNPs showed nominally significant differential effects between control subjects and AD patients: rs514049 ($p = 0.039$), rs2305421 ($p = 0.019$), and rs1295652 ($p = 0.011$). However, after controlling for multiple comparisons, none of these remained significant, and none of the SNPs showed significant effects within either disease group or when disease groups were combined.

For CSF A β 42 levels, one SNP was nominally significant within AD patients: rs11601511 ($p = 0.030$), and a second SNP was nominally significant within controls: rs1295652 ($p = 0.047$). Two SNPs showed nominally significant differential effects between control subjects and AD patients: rs514049 ($p = 0.019$) and rs362408 ($p = 0.049$). Again, after controlling for multiple comparisons, none of these remained significant.

Effect of rs514049 Genotype on CSF APP α Levels

The *ADAM10* rs514049 SNP is the only SNP, out of the 19 APP processing gene SNPs tested, that remained significant when testing for between group (AD compared to control) SNP effect on CSF APP cleavage product levels (CSF APP α levels), after correcting for multiple comparisons ($p_{\text{Holm}} = 0.014$). The nature of this difference is summarized in Figure 3. For example, for AD patients, the CA,AA collapsed genotype group has a higher mean CSF APP α level than the CC genotype, whereas the reverse is true for control subjects. Differences between genotype and disease groups were analyzed with or without adjusting for gender, race, age, and *APOE* $\epsilon 4$ status. Unadjusted p-values are based on the two-sample t-test and adjusted p-values are based on the linear model described in the methods section (Figure 3).

Similarly, there was a significant association with APP α levels when all three rs514049 genotypes (not collapsed genotypes) were analyzed within the control group with an increasing trend in APP α levels for C alleles carriers (p-value, <0.001). An opposite but non-significant trend was seen for the AD group (p-value = 0.108) (data not shown).

In addition, even though great care was taken to exclude possible prodromal AD cases from the cognitively normal control group using standard psychiatric AD testing (see methods section), it is possible that the cognitively normal control sample contains subjects with prodromal AD pathology. Thus, analyses to investigate the possible inclusion of prodromal AD cases within the control sample were performed, including using CSF A β 42 levels as a covariate (data not shown) and taking into account *APOE* $\epsilon 4$ status. Significant associations between CSF APP α levels and rs514049 remained when using CSF A β 42 levels as a covariate (data not shown) and while taking into account *APOE* $\epsilon 4$ status.

Interaction between rs514049 and other SNPs

To assess the possible combined influence of the *ADAM10* SNP and any of the other 18 APP processing gene SNPs on CSF APP cleavage product levels, we utilized a linear

regression model that consisted of the initial model as well as including two-way interactions between rs514049 and each of the other 18 SNPs, as well as three-way interactions between the SNP pairs and disease status. None of these two-way or three-way interactions were statistically significant (data not shown).

SNP Effect on AD age-at-onset

Each SNP was tested individually for its effect on AD age-at-onset. None of the SNPs showed a significant association with AD age-at-onset (data not shown). In addition, if the AD group was restricted to an age-at-onset of >60 or 65 years the significant association between CSF APP α levels and rs514049 SNP remained (data not shown).

Discussion

The aim of this investigation was to determine if genetic variation within or near *APP*-processing-related genes correlate with CSF APP cleavage product levels. This investigation is important and novel because it involves the analysis of several *APP* processing genes in parallel using the same sample set thus eliminating the variability and uncertainty associated with across study comparisons of individual genes.

The main interesting finding of this investigation was that the rs514049 SNP significantly correlates with CSF APP α levels. In controls, the rs514049 CC genotype is associated with increased APP α levels whereas in contrast, in the AD group, the CC genotype is associated with decreased APP α levels. The differential effect of rs514049 on CSF APP α levels between AD and controls remained significant after multiple comparisons. In addition, an association with APP α levels when all three rs514049 genotypes (not collapsed genotypes) were analyzed within the control group showed an increasing trend for C allele carriers whereas, in contrast, an opposite trend was seen for the AD group. These results suggest a fundamental difference in CSF APP α production between AD and controls that is strongly associated with the C allele of rs514049.

Since the C allele of rs514049 is located immediately 5' to the *ADAM10* core promoter region, it may be speculated that trans-acting factors or other modifying factors specific to the microenvironment of the AD brain modulate *ADAM10* promoter activity. Several SNPs exist within the *ADAM10* promoter which spans nucleotides from -2179 to -1 where nucleotides -508 to -300, are described as the core promoter, and Sp1, USF, and retinoic acid-responsive elements appear to modulate promoter activity. (Prinzen et al., 2005) Interestingly, successive deletion of the first half of the *ADAM10* 5'-UTR region of the promoter, increases of ADAM10 protein levels in HEK293 cells, whereas mRNA levels are not changed, suggesting that the 5'-UTR represses the rate of ADAM10 translation. In addition, there is enhanced α -secretase activity and consequently reduced A β levels in the conditioned medium of HEK293 cells expressing both APP and the 5'-UTR-*ADAM10* deletion construct. (Lammich et al., 2010)

Intriguingly, the rs514049 SNP is located within a predicted CREB/cJun transcription factor site at -644 (Prinzen et al., 2005) and is also located within a DNase hot spot (region of regulatory protein binding) reported by the ENCODE project, further implicating the importance of this SNP in gene regulation (Rosenbloom et al., 2010). The association between cAMP-response element-binding protein (CREB) and memory related processes has been extensively studied. (Benito and Barco, 2010) AD mouse models of neurodegeneration show dysregulation of CREB. (Gong et al., 2004; Ma et al., 2007) Molecular network analysis suggests aberrant CREB-mediated gene regulation in the AD hippocampus. (Sato et al., 2009) CREB was the principal transcription factor found to exhibit the most significant relevance to molecular networks. (Sato et al., 2009)

Evidence suggests that the *ADAM10* gene plays an important role in the development of AD relevant processes. Overexpression of ADAM10 in HEK293 cells first identified its function as an APP cleaving α -secretase. (Lammich et al., 1999) Neuronal overexpression of ADAM10 in mice transgenic for human APP [V717I] increases the secretion of APP α , reduces the production of A β peptides, prevents amyloid plaque formation and alleviates cognitive deficits. (Postina et al., 2004) In contrast, mice with a dominant negative mutant of ADAM10 have lower APP α levels, accompanied by an enhanced plaque formation (Postina et al., 2004; Schroeder et al., 2009)

In addition, *ADAM10* has been proposed as a potential AD therapeutic target because vitamin A has been reported to influence *ADAM10* promoter activity (Prinzen et al., 2005) and acitretin, which is a synthetic retinoid drug, lowers A β peptide production and enhances APP α secretion in an AD mouse model. (Tippmann et al., 2009)

Furthermore, there is evidence that modifying factors can modulate α -secretase activity in the AD brain. Both APP α and ADAM10 protein are decreased in AD CSF compared to control CSF (Colciaghi et al., 2002). S100 expression in the brain may selectively promote α -secretase activity (ADAM10) in AD (Peskind et al., 2001; Qin et al., 2009). S100 proteins contain 2 EF-hand calcium-binding motifs, are present in the cytoplasm and/or nucleus in many cell types, and appear to be involved in the regulation of cellular processes such as cell cycle progression and differentiation; however their specific function in AD is still unclear (Eckert et al., 2004). Thus, the present exploratory investigation and previous evidence described here lend support to the idea that effectors of ADAM10 regulation may contribute to AD related pathology.

Other neurodegenerative diseases may be influenced by ADAM10. For example, ADAM10 may play a role in prion disease. The prion protein has been described as a substrate for ADAM10 cleavage (Taylor et al., 2009) and mice overexpressing ADAM10 show an increased survival time after prion exposure. (Endres et al., 2009) In addition, Parkinson's disease patients have elevated levels of CSF A β 42 compared to AD patients (Zhang et al., 2008) as well as modulated levels associated with Parkinson's disease cognitive impairment, (Alves et al., 2010; Montine et al., 2010; Siderow et al., 2010) suggesting that APP processing genes, such as *ADAM10*, may play an important role in Parkinson's disease.

Several SNPs show nominal associations with CSF APP cleavage product levels in our study (Figure 2). However, several of these associations did not remain significant after taking into account multiple comparisons. It is possible that by correcting for multiple comparisons, and thus categorizing these nominal results as false positives, important associations with CSF APP cleavage product levels may have been missed. For example, it may be important to note that the *ADAM10* rs514049 SNP, in addition to a significant association with CSF APP α levels, also showed a nominally significant differential effect in CSF A β 42 levels between AD and controls (Figure 2). In addition, control CC genotype carriers had lower CSF A β 42 levels and higher CSF APP α levels than CA, AA genotype carriers where the reverse was true for AD. Furthermore, CSF APP β levels correlate with the *ADAM10* SNP, rs2305421 in AD patients which may implicate an important association between *ADAM10* and APP β levels. Similarly, recently it has been reported that an *ADAM10* central nervous system conditional knockout has decreased APP α , APP β and A β 42 levels in the brain (Jorissen et al., 2010) suggesting that ADAM10 deficiency and the effects on APP processing may result in changes in cellular localization or trafficking of β -secretase or APP instead of competition between the two secretases. However, in contrast, an AD mouse model that over-expresses ADAM10, amyloid accumulation is decreased (Postina et al., 2004), suggesting that when there is an increase in ADAM10 there is less β -secretase cleavage of APP, implicating α -secretase and β -secretase competitive cleavage.

Thus, it remains to be determined whether similar trends seen in our study for both APP α and APP β supports a general or distinct effect on how ADAM10 levels or genotype effect APP processing.

An advantage of this investigation, in contrast to genome wide associations, is that a small number of SNPs (n=19), within the context of a very specific hypothesis, were tested and restricted to a specific biological connection between genetic variation and production of the APP cleavage products in CSF. A limitation of this investigation's approach was that only a few SNPs were used to capture putative regulatory genetic variation within and surrounding the genes of interest. Thus these results must be approached with caution since many important SNPs may have been missed.

In addition, a positive SNP may represent a surrogate marker for a true functional SNP that was not analyzed in this study. The *ADAM10* rs514049 SNP found positive here may be a surrogate marker in linkage disequilibrium with a functional SNP that affects *ADAM10* structure and thus protein function, instead of modulation of expression levels. Interestingly, potential late-onset AD associated rare mutations, Q170H and R181G, in the *ADAM10* gene have been reported to attenuate α -secretase and elevate A β levels in cell-based studies (Kim et al., 2009). However, evidence against a role for these rare mutations in late-onset AD has also been reported (Cai et al., 2010). Thus further functional studies are needed to fine map the specific contribution of the genetic variation within and surrounding the *ADAM10* gene on both ADAM10 protein activity and gene expression levels.

In conclusion, these results taken together with previous studies, may suggest that the discrepancy between AD and control *ADAM10* rs514049 CC carrier APP α levels is the result of modifying factors specific to the pathology of the AD brain that modulate C allele expression differentially compared to A alleles. Further hypotheses may be generated by this exploratory investigation, including that decreased CSF APP cleavage product levels seen in AD are in part driven by *ADAM10* genetic variation. Characterization of the functional influence of *ADAM10* genetic variation on *ADAM10* expression may lead to a better understanding of AD pathogenesis, help find novel AD specific biomarkers and identify AD therapeutic targets.

Acknowledgments

This work is supported in part by the U.S. Department of Veterans Affairs, Office of Research and Development Clinical Research and Development Program, the Biomedical Laboratory Research Program and NIH Grants 2P50AG005136-27 and 1P50NS062684-01A1. Additional support includes University of Washington Alzheimer's Disease Research Center NIH P50-AB005136 and University of California San Diego Alzheimer's Disease Research Center AGO 5131.

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Figure 1. Mean CSF APP Cleavage Product Levels

Each graph shows CSF APP α , APP β , or A β 42 mean level by group (cognitively normal controls versus AD patients), along with 95% confidence intervals. Each dot represents the CSF APP cleavage product level for one subject. AD patients have a significantly lower mean level than controls for CSF APP α ($p = 0.049$; 95%CI [0.01, 6.7]) (panel A), CSF APP β levels ($p = 0.028$; 95% CI [0.2, 4.0]) (panel B) and CSF A β 42 levels ($p < 0.001$; 95% CI [36, 54]) (panel C).

Figure 2. SNP Effect on CSF APP cleavage product levels

The effect of a SNP within APP processing genes on CSF APP cleavage product level for each disease group (AD versus controls) was analyzed using linear regression, where CSF APP cleavage product level was the outcome variable and the independent variables were age, gender, race, APOE $\epsilon 4$ status, disease status, SNP collapsed genotype (presence or absence of the minor allele; i.e., EF and FF vs. EE), and a disease status by SNP interaction term to allow the effect of SNP to possibly vary between disease groups. A confidence interval which does not cross the vertical line at zero indicates that the difference in genotypes is significant ($p < 0.05$) before adjustment for multiple comparisons for that group. A beta coefficient (solid square for AD, circle for controls) to the right of the vertical line represents higher CSF APP cleavage product levels for the collapsed genotype group that contains minor alleles (EF and FF). After correcting for multiple comparisons, the only significant SNP effect was for the ADAM10 rs514049 SNP, which showed a differential effect on CSF APP α levels in AD patients versus control subjects (asterisk; $p = 0.014$).

Figure 3. Effect of rs514049 Genotype on CSF APPα levels

Dots represent CSF APPα level for each subject and are grouped into rs514049 major genotype (CC) or collapsed genotype (CA, AA) groups for both controls and AD. For AD patients, the CA, AA collapsed genotype group has a mean CSF APPα level that is 6.0 nM greater than the mean for the CC genotype, whereas the CC genotype has higher levels in the control subjects. Significant differences between group CSF APPα level were tested with and without adjusting for gender, race, age and APOE ε4. Adjusted p-values are in parentheses.

Table 1
Population description for cognitively normal control subjects and Alzheimer’s disease patients (AD)

Gender, race, *APOE* $\epsilon 4$ status and age at the time of sampling, as well as AD age-at-onset, are described.

	Controls		AD	
	n =	%	n =	%
Females	100	0.59	43	0.47
Caucasian	156	0.92	89	0.97
<i>APOE</i> $\epsilon 4+$	61	0.36	65	0.71
Age Mean (Range)	67 (52–88)		72 (52–87)	
Age-at-onset Mean (Range)			67 (46–82)	

Table 2
SNP Description. Genotypes ‘E’ and ‘F’ represent major and minor alleles, respectively

For each SNP genotype, the frequency distribution for AD patients (n=92) and control subjects (n=170) is shown. Differences in frequency distribution between AD and controls was tested with both the chi-square test (“Unadjusted Genotype p-value”) and also using logistic regression to adjust for age, gender, race, and APOE ε4 status (“Adjusted Genotype p-value”). No SNP genotype frequency distribution shows a significant difference between AD and controls. Differences between AD and control collapsed genotype frequencies (EE vs. EF, FF) were analyzed adjusting for age, gender, race and APOE ε4 status (“Adjusted Collapsed Genotype p-value”).

Gene ID	Chromosome Location	SNP	NCBI Gene Location	AD Genotype Frequency			Control Genotype Frequency			Unadjusted Genotype p-value	Adjusted Genotype p-value	Adjusted Collapsed Genotype p-value	
				EE	EF	FF	EE	EF	FF				
Amyloid Precursor Protein													
APP	21q21.3	rs466448	5' region	G/a	0.29	0.47	0.24	0.25	0.50	0.25	0.717	0.904	0.858
		rs2830101	intron 1	G/a	0.49	0.40	0.11	0.45	0.42	0.14	0.741	0.861	0.780
		rs214484	intron 17	A/c	0.38	0.53	0.09	0.48	0.41	0.11	0.172	0.169	0.137
		rs2040273	3' region	A/g	0.32	0.58	0.11	0.41	0.44	0.15	0.092	0.052	0.051
α-Secretase													
ADAM10	15q22.1	rs514049	5' region	C/a	0.40	0.39	0.21	0.32	0.46	0.22	0.369	0.470	0.285
		rs2305421	Intron 13	A/g	0.75	0.23	0.02	0.71	0.25	0.04	0.730	0.511	0.304
β-Secretase													
BACE1	11q23–24	rs573801	5' region	G/a	0.63	0.30	0.07	0.54	0.42	0.04	0.145	0.083	0.157
		rs11601511	intron 0	G/c	0.64	0.34	0.02	0.75	0.22	0.02	0.138	0.133	0.046
		rs638405	exon 5	C/g	0.41	0.38	0.21	0.34	0.49	0.16	0.210	0.210	0.161
BACE2	21q22.3	rs734757	intron 1	C/t	0.32	0.50	0.18	0.38	0.43	0.19	0.494	0.373	0.262
		rs12149	exon 9	C/t	0.32	0.45	0.24	0.26	0.46	0.28	0.645	0.411	0.213
γ-Secretase													
PSEN1	14q24.3	rs362354	intron 2	A/g	0.51	0.45	0.04	0.60	0.35	0.05	0.336	0.257	0.207
		rs362408	3' region	G/a	0.76	0.22	0.02	0.76	0.22	0.02	0.974	0.999	0.971
PSEN2	1q42.2	rs1295652	5' region	A/g	0.59	0.36	0.05	0.56	0.37	0.06	0.914	0.356	0.273
		rs2802268	3' region	T/g	0.62	0.34	0.04	0.55	0.39	0.05	0.580	0.307	0.274
PEN2 (PSENEN)	19q13.1	rs2293688	intron 2	C/g	0.45	0.42	0.13	0.40	0.47	0.13	0.746	0.458	0.319
NCSTN	1q22-q23	rs7540865	intron 5	G/t	0.51	0.42	0.07	0.48	0.41	0.11	0.472	0.561	0.400
APHB	15q22.2	rs35408871	intron 4	G/a	0.75	0.23	0.02	0.74	0.23	0.04	0.828	0.948	0.759

Gene ID	Chromosome Location	SNP	NCBI Gene Location	Alleles	AD Genotype Frequency			Control Genotype Frequency			Unadjusted Genotype p-value	Adjusted Genotype p-value	Adjusted Collapsed Genotype p-value
					EE	EF	FF	EE	EF	FF			
		rs2068143	intron 4	G/a	0.54	0.40	0.05	0.60	0.33	0.07	0.481	0.557	0.425