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Tau Phosphorylation Pathway Genes and Cerebrospinal Fluid Tau Levels in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is characterized by the presence in the brain of amyloid plaques, consisting predominately of the amyloid β peptide ($A\beta$), and neurofibrillary tangles, consisting primarily of tau. Hyper-phosphorylated-tau (p-tau) contributes to neuronal damage, and both p-tau and total-tau (t-tau) levels are elevated in AD cerebrospinal fluid (CSF) compared to cognitively normal controls. Our hypothesis was that increased ratios of CSF phosphorylated-tau levels relative to total-tau levels correlate with regulatory region genetic variation of kinase or phosphatase genes biologically associated with the phosphorylation status of tau. Eighteen SNPs located within 5' and 3' regions of 5 kinase and 4 phosphatase genes, as well as two SNPs within regulatory regions of the *MAPT* gene were chosen for this analysis. The study sample consisted of 101 AD patients and 169 cognitively normal controls. Rs7768046 in the *FYN* kinase gene and rs913275 in the *PPP2R4* phosphatase gene were both associated with CSF p-tau and t-tau levels in AD. These SNPs were also differentially associated with either CSF t-tau (rs7768046) or CSF p-tau (rs913275) relative to t-tau levels in AD compared to controls. These results suggest that rs7768046 and rs913275 both influence CSF tau levels in an AD-associated manner.

Keywords

FYN; PPP2R4; MAPT; AD; CSF

INTRODUCTION

Alzheimer's disease is definitively diagnosed at autopsy according to the level and distribution of both amyloid plaques and neurofibrillary tangles (NFT) in the brain [Braak and Braak, 1991]. NFTs consist primarily of insoluble hyperphosphorylated microtubule-associated protein tau. Tau is a cytosolic protein expressed in neurons where it is involved in the stabilization of microtubules in the axon. Tau protein activity is post-translationally modulated by phosphorylation at multiple sites [Sato-Harada et al., 1996]. The list of kinases and phosphatases that actively phosphorylate and de-phosphorylate tau is long and constantly evolving [Hanger et al., 2009; Spire-Jones et al., 2009; Martin et al., 2011; Wang et al., 2012]. Hyperphosphorylation of tau destabilizes the microtubule network which impairs axonal transport and leads to NFT formation and neuronal death [Hanger et al., 2009; Spire-Jones et al., 2009; Martin et al., 2011; Wang et al., 2012].

The influence of phosphorylated-tau (p-tau) on neurodegeneration is thought to be due to a loss of a functional (and/or the gain of a toxic functional) mechanism which leads to the generation of multimeric intraneuronal phosphorylated tau species. As many as 85 putative phosphorylation sites of p-tau have been described [Hanger et al., 2009; Spire-Jones et al., 2009; Martin et al., 2011; Wang et al., 2012] and enzyme-linked immunosorbent assays (ELISAs) have been developed for at least five of them. Studies examining different forms of p-tau in the early diagnosis of AD and in the differentiation from other causes of dementia have shown that as tau phosphorylated threonine 181 (p-tau 181) as well as the tau phosphorylation sites; p-tau 231–235, or p-tau 396–404, offer at least equivalent diagnostic utility for AD as compared with total-tau (t-tau) [Rosenbloom et al., 2010; van Harten et al., 2011].

CSF t-tau and p-tau 181 are elevated in AD compared to cognitively normal control subjects in a manner specific to neurodegenerative disease types [Arai et al., 1997; Price and Morris, 1999; Itoh et al., 2001; Lewczuk et al., 2004; Fagan et al., 2006]. Elevated CSF t-tau levels however do not appear to be specific to AD; they are also seen in stroke [Hesse et al., 2001] and traumatic brain injury [Abdi et al., 2006]. Taken together these findings suggest that CSF t-tau and p-tau levels reflect different pathogenic processes in the brain; t-tau the degree of neuronal damage and p-tau the phosphorylation state of tau, and thus possibly the formation of NFTs.

Given that p-tau is the major component of NFTs found in AD brain and that CSF t-tau and p-tau levels are associated with AD, we hypothesized that genes involved in the phosphorylation (kinases) or de-phosphorylation (phosphatases) of tau may be associated with t-tau or p-tau levels in AD CSF differentially compared to cognitively normal controls. Eighteen SNPs, tagging putative regulatory regions and located within both the 5' and 3' regions of 5 kinase and 4 phosphatase genes, as well as SNPs within the *MAPT* gene, were chosen for this analysis. Two SNPs were found to be associated with CSF tau levels in AD.

METHODS

Subjects

For the tau phosphorylation gene analysis, subjects were 169 healthy cognitively normal controls and 101 AD patients (Table I). All procedures were approved by the institutional

review boards of the participating institutions. A separate *MAPT* association analysis was performed to take into account *MAPT* H1 and H2 haplotypes. In the *MAPT* analysis, subjects were 149 healthy cognitively normal controls between 52–88 years old and 83 AD patients between 52–87 years old with an age-at-onset range of 46–82 years. After removing *MAPT*H2 positive subjects as defined by the SNP rs1800547, 90 healthy controls and 64 AD patients remained for analysis of *MAPT*SNP association with CSF t-tau and p-tau levels. Almost all subjects in the *MAPT* association analysis (88 of 90 controls and 63 of 64 AD patients) were also subjects in the tau phosphorylation gene analysis. Following informed consent, all subjects underwent extensive evaluation that consisted of medical history, family history, physical and neurologic examination, 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 autosomal dominant AD.

Cerebrospinal Fluid

All CSF samples were collected in the morning after an overnight fast using the Sprotte 24-g traumatic spinal needle with the patient in either the lateral decubitus or sitting position [Peskind et al., 2005, 2006]. Samples were aliquoted at the bedside and frozen immediately on dry ice and stored at –80°C until assayed. Total-tau and phosphorylated (181) tau were measured in the 9th ml of collected CSF using a sensitive multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) [Shaw et al., 2009]. CSF Aβ42 was also measured but was not further evaluated in this investigation (Table I). Intra-assay coefficient of variation was <10% for all assays.

Genes and SNP Selection

The tau phosphorylation gene analysis used SNPs from nine genes that were chosen for their biologically characterized role in tau phosphorylation. 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 20 SNPs were selected (18 associated with tau phosphorylation genes and two within regulatory regions of the *MAPT* gene; see Table II). An additional SNP (rs429358) was also genotyped to determine *APOE* ε4 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, Carlsbad, CA), 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 (FF vs. EE vs. EF, where F denotes the major allele and E denotes the minor allele) between AD patients and control subjects using the chi-squared test. To adjust for age, gender, race, and *APOE* ϵ 4 status (ϵ 4 positive vs. negative), we performed logistic regression using disease status as the response variable, and SNP and these covariates as predictor variables. We also compared frequencies of collapsed genotype groups (absence or presence of the minor allele; i.e., FF vs. EE and EF) between AD patients and control subjects after adjusting for covariates (Table II). All subsequent analyses involving SNPs were based on the collapsed genotype group.

CSF t-tau and p-tau protein levels, as well as the ratio of these levels, were compared between AD patients and control subjects initially using *t*-tests (Fig. 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 on disease (Fig. 2). For the *t*-tests and the linear regression models, CSF p-tau and t-tau protein levels and the p-tau/t-tau ratio were all log-transformed to induce homoscedasticity.

In the case where significant SNP effects were found, we also investigated the model that included two-way interactions between the significant SNPs, as well as three-way interactions between the SNP pairs and disease status. 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 2.12.2; 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

Eighteen SNPs from nine tau phosphorylation-related genes were genotyped (Table II). Comparison of the genotype frequency between AD and controls indicated that only rs3755557 in the GSK3B gene was significantly different ($P = 0.041$) when genotypes were collapsed into two groups, [the major genotype (FF) vs. the minor genotypes (EF, EE)], and after adjusting for age, gender, race, and *APOE* ϵ 4 status. However, significance was lost after correcting for multiple comparisons.

CSF Tau Levels

CSF p-tau, t-tau, and p-tau/t-tau were compared between cognitively normal control subjects and AD patients. CSF p-tau and t-tau levels were significantly higher in AD patients compared to controls (P -value < 0.0005 ; Fig. 1, Panels A and B). CSF p-tau relative to t-tau

levels (p-tau/t-tau) were significantly lower in AD patients compared to controls (P -value <0.0005) (Fig. 1, Panel C). For p-tau, the mean (SD) for control subjects and AD patients was 1.66 (0.16) and 1.90 (0.21) $\log_{10}(\text{pg/ml})$, respectively, and the 95% confidence interval (95% CI) for the difference was (0.19, 0.28) $\log_{10}(\text{pg/ml})$. For t-tau, the mean (SD) for control subjects and AD patients was 1.64 (0.23) and 1.96 (0.28) $\log_{10}(\text{pg/ml})$, respectively, and the 95% CI for the difference was (0.26, 0.38) $\log_{10}(\text{pg/ml})$. For the $\log_{10}$ (p-tau/t-tau) ratio, the mean (SD) for control subjects and AD patients was 0.02 (0.14) and -0.07 (0.15), respectively, and the 95% CI for the difference was $(-0.12, -0.05)$.

SNP Genotype Effect on Tau Levels

Each SNP was tested individually for its effect on CSF tau levels in AD patients and controls. Figure 2 shows the effect of the SNP on p-tau or t-tau levels for each of the 18 tau phosphorylation-related SNPs and two SNPs within regulatory regions of the *MAPT* gene, 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 figures show either an increase or decrease in protein level (or ratio) for the collapsed minor allele carriers (EE, EF) compared to major genotype group (FF), along with 95% confidence intervals unadjusted for multiple comparisons. For example, CSF t-tau levels are significantly higher for minor allele carriers compared to major genotype carriers of rs7768046 in the *FYN* kinase gene within the AD group and remains significant after Holm correction for multiple comparisons ($P = 0.0007$, Holm corrected $P = 0.01$) while the difference between minor allele carriers and major genotype carriers is not significant within the control group (Fig. 2).

Figure 3 shows the effect of the SNP on p-tau relative to t-tau levels for each of the 18 tau phosphorylation-related SNPs, plus two SNPs within regulatory regions of the *MAPT* gene, based on the linear regression model described in the Methods Section.

Table III shows the SNP effects and P -values for all SNPs which showed nominally significant (as shown in Figs. 2 and 3; i.e., $P \leq 0.05$ before correcting for multiple comparisons). SNP by disease status interaction effects, or nominally significant SNP effects within at least one of the disease status groups. After adjusting for multiple comparisons, the only effects that remained significant were the SNP effects within AD patients for rs913275 in the *PPP2R4* phosphatase gene and rs7768046.

Effect of rs7768046 and rs913275 Genotype on CSF Tau Levels

One SNP in the *FYN* kinase gene (rs7768046) and one in the *PPP2R4* phosphatase gene (rs913275) remained significant after Holm correction for multiple comparisons. The nature of this difference is summarized in Figure 4. For example, within the AD patient group the rs7768046 and rs913275 GG, AG collapsed genotype group (G allele carriers) have higher mean CSF p-tau and t-tau levels than the AA genotype, whereas this is not the case for control subjects. In addition, within the AD patient group the rs7768046 and rs913275 GG, AG collapsed genotype group have lower mean CSF p-tau/t-tau levels than the AA genotype and the difference between AD and controls is restricted to the GG, AG collapsed genotype group (Fig. 4). The 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 (in parentheses) are based on the linear model described in the Methods Section (Fig. 4), but not corrected for multiple comparisons.

Interaction Between rs7768046 and rs913275

To assess the possible combined influence of rs7768046 and rs913275 on CSF tau levels, we utilized a linear regression model that consisted of the initial model with the addition of two-

way interactions between rs7768046 and rs913275 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. Only one SNP (rs873785 in the *GAK* kinase gene) showed nominal significance ($P = 0.049$), but significance was lost after correcting for multiple comparisons (Holm corrected $P = 1$; data not shown).

DISCUSSION

The abnormal phosphorylation of tau is a characteristic hallmark of AD and NFTs. The aim of this investigation was to determine if genetic variation within or near genes that encode kinases or phosphatases, with reported involvement in the phosphorylation status of tau, correlate with CSF t-tau or p-tau levels. This is a novel investigation because it involves the analysis of several tau phosphorylation-related genes in parallel using the same subjects, thus eliminating the variability and uncertainty associated with across study comparisons of individual genes. In addition, the hypothesis of this investigation focuses on a specific biological pathway implicated in the pathogenesis of AD which eliminates the large scale analysis of SNPs required in GWAS, further decreasing uncertainty.

The main finding of this investigation is that, after corrections for multiple comparisons, two SNPs, one within a kinase gene (*FYN*: rs7768046) and the other within a phosphatase gene (*PPP2R4*: rs913275), significantly correlate with CSF tau levels. These results suggest that genetic variation within genes that encode proteins implicated in the maintenance of tau phosphorylation influence tau levels measured in CSF.

Minor allele carriers for rs7768046 and rs913275 (i.e., G allele carriers: AG, GG collapsed genotype) were found to have higher t-tau and p-tau levels compared to the AA genotype within AD subjects but not within cognitively normal controls (with and without adjusting for gender, age, race, and *APOE* $\epsilon 4$ status). These differences remain significant after adjustment for multiple comparisons for t-tau but not p-tau. This difference in tau levels is further reflected in significantly lower ratios of p-tau/t-tau levels for rs7768046 and rs913275 G allele carriers compared to the AA genotype within AD subjects but not within cognitively normal subjects. Taken together, these results suggest a difference in CSF phosphorylated tau production between AD and cognitively normal controls that are related to the G allele of rs7768046 and the G allele of rs913275.

The genetic association found between *FYN*(rs7768046) and CSF tau levels are supported by a previous report by Cruchaga et al. [2010] that describes an association between rs7768046 and CSF p-tau levels in their initial series. Genetic associations between *FYN* SNPs and alcohol dependence [Schumann et al., 2003; Pastor et al., 2009], schizophrenia cognitive performance [Rybakowski et al., 2007] and allergic asthma [Szczepankiewicz et al., 2008] have been reported, but were not found for AD in a Japanese population [Watanabe et al., 2004].

A genetic association between CSF tau levels and rs1868402 in *PPP3R1*, another kinase gene investigated here, was reported in by Cruchaga et al. [2010], but was not found in our study. Interestingly, there was partial overlap between the Cruchaga et al. study sample and our study sample suggesting that variability in study sample contributes to the ability to detect a genetic association with CSF tau levels in AD and emphasizes the need to approach genetic biomarker association study results with caution.

FYN is a tyrosine kinase that has been implicated in the phosphorylation of tau and AD pathology [Ittner and Gotz, 2011; Yang et al., 2011]. *FYN* has been reported to be elevated in AD [Shirazi and Wood, 1993; Ho et al., 2005] and appears to affect AD pathology and cognition in animal models [Chin et al., 2004, 2005; Roberson et al., 2011]. Rs7768046 is located within intron 1 of the *FYN* kinase gene. Since rs7768046 is located near the core promoter region as well as within a dense ENCODE predicted H3K4Me1 histone mark promoter-enhancer region it may be speculated that trans-acting factors or other modifying factors specific to the microenvironment of the AD brain modulate *FYN* expression [Rosenbloom et al., 2010].

Rs913675 is located within the 3' region of the *PPP2R4* gene. The *PPP2R4* gene encodes protein phosphatase 2A (PP2A), a major tau phosphatase in the human brain that has been implicated in the hyperphosphorylation of tau in AD [Liu et al., 2005; Iqbal et al., 2009; Qian et al., 2010]. The association between *PPP2R4* genetic variation and CSF tau levels is novel and to our knowledge has not been reported previously. Since rs913275 is located within a putative regulatory region for the gene, as well as within a dense ENCODE predicted H3K4Me1 histone mark promoter-enhancer region it may be speculated that trans-acting factors or other modifying factors specific to the microenvironment of the AD brain modulate its expression [Rosenbloom et al., 2010].

Interestingly, the *FYN* and *PPP2R4* SNP effect within the AD patient group, but not the control subjects, suggests that both *FYN* and *PPP2R4* may be influenced by modifying factors specific to the pathology of the AD brain that modulate G allele expression differentially compared to A alleles.

An advantage of this investigation, in contrast to GWAS, is that a small number of SNPs (n = 20), within the context of a specific hypothesis, were tested and restricted to a specific biological relationship between genetic variation and production of the phosphorylated tau in CSF. A limitation of this investigation's approach is that only a few SNPs were used to capture putative regulatory genetic variations within and surrounding the kinase and phosphatase genes of interest. The tau phosphorylation status is regulated by many known and unknown kinase and phosphatase proteins that were not taken into account in this investigation. Thus, these results must be approached with caution since many important genes and 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. Thus, the *FYN* and *PPP2R4* SNPs found to have effects in this study, may be surrogate markers in linkage disequilibrium with functional SNPs that affect gene structure and thus protein function instead of modulation of expression levels. To elucidate a functional influence of genetic variation within the *FYN* and *PPP2R4* regulatory regions, functional studies that fine map the specific contribution of the genetic variations, both within and surrounding these genes, on expression levels are required. In addition, these findings need to be further replicated because they were found to be only marginally significant after adjusting for multiple comparisons.

A disadvantage of this investigation is that only p-tau phosphorylation site threonine 181 was measured. Multiple previously described tau phosphorylation sites were not measured [Sato-Harada et al., 1996; Lebouvier et al., 2009; Martin et al., 2011; Wang et al., 2012]. For example, *FYN* has been reported to phosphorylate tyrosine 18, not threonine 181 [Lebouvier et al., 2009]. Therefore, assessment of p-tau tyrosine 18 may be of greater interest in relation to a genetic association between CSF p-tau and *FYN* genetic variation. Furthermore, it may be speculated that the lack of significant association between *FYN* SNPs and CSF p-tau may be related to the phosphorylation site, but does not explain the association between the *FYN*

SNP and CSF t-tau levels. The correlation between *FYN* and *PPP2R4* SNPs and CSF t-tau levels in AD may implicate that the activity by these genes does not impact tau phosphorylation but instead may influence CSF total tau levels. Previous reports suggest that multiple proteins are influenced by *FYN* and *PPP2R4* [Qian et al., 2010; Usardi et al., 2011]. For example, it has been reported that tyrosine phosphorylation of tau by *FYN* may alter the cellular localization of tau [Usardi et al., 2011]. In addition, indirect effects on phosphatase activity by *PPP2R4* on t-tau levels have been reported to occur via activation of *GSK-3beta* [Qian et al., 2010].

In summary, in this exploratory investigation, CSF total-tau levels in AD were found to correlate with SNPs tagging putative regulatory regions within *FYN* and *PPP2R4*. These results suggest that the proteins encoded by these genes influence AD CSF tau levels. Characterization of the functional influence of *FYN* and *PPP2R4* genetic variation on expression levels of these genes, as well as their impact on tau levels, may lead to a better understanding of AD pathogenesis, help find novel AD-specific biomarkers, and identify AD therapeutic targets.

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FIG. 1.

CSF tau levels in AD compared to Controls. CSF log 10 p-tau levels (Panel A). CSF log 10 t-tau levels (Panel B). CSF log 10 p-tau/t-tau levels (Panel C).

FIG. 2.

Beta coefficients for CSF phosphorylation tau and total tau levels in AD and controls for Tau phosphorylation pathway gene collapsed genotypes. The effect of a SNP for each disease group (AD or controls) was analyzed using linear regression, where CSF biomarker level was the outcome variable and the independent variables were age, gender, race, APOE $\epsilon 4$ status, disease status and SNP collapsed genotype (presence or absence of the minor allele; i.e., EF and EE vs. FF). 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 biomarker levels for the collapsed genotype group that contains minor alleles (EF, EE). Significant SNP P-values are shown in Table III.

FIG. 3.

Beta coefficients for CSF phosphorylation tau relative to total tau levels in AD and controls for tau phosphorylation pathway gene collapsed genotypes. The effect of a SNP for each disease group (AD or controls) was analyzed using linear regression, where CSF biomarker 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 EE vs. FF). 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 biomarker levels for the collapsed genotype group that contains minor alleles (EF, EE). Significant SNP P-values are shown in Table III.

FIG. 4.

CSF tau levels in controls and AD patients stratified by collapsed genotype for SNPs rs 7768046 (FF = AA; EE, EF = GG, AG) (Panel A) and rs913675 (FF = AA; EE, EF = GG, AG) (Panel B). Unadjusted P-values are based on the two-sample t-test while adjusted P-values take into account gender, race, age, and APOE ϵ 4 status (parentheses) are based on a linear model.

TABLE I

Sample Description

	Controls	AD	P-value
n	169	101	
Mean age (range)	68 (52–88)	71 (52–87)	
Mean age-at-onset (range)		66 (46–82)	
Mean phosphorylated-tau (range)	49.6 (15.0–140.0)	88.7 (23.0–282.0)	<0.0001
Mean total-tau (range)	50.1 (9.0–168.0)	110.9 (17.0–438.0)	<0.0001
Mean A β 42 (range)	151.6 (73.0–240.0)	105.7 (65.0–240.0)	<0.0001
% Male	39	54	0.0190
% <i>APOE</i> ϵ 4+	37	66	<0.0001
% Caucasian	91	97	0.1000

TABLE II

Genotype Frequency Description

Gene name	Chromosome	SNP	Alleles	Location	NCBI (CEU), genotype frequency			AD (n = 101), genotype frequency			Control (n = 169), genotype frequency			Chi-sq. unadjusted genotype P-value	Chi-sq. adjusted genotype ^a P-value	Adjusted collapsed genotype ^d P-value
					EE	EF	FF	EE	EF	FF	EE	EF	FF			
Protein Kinases																
<i>GSK3B</i>	3q13.33	rs3755557	T/a	5' Region	0.02	0.33	0.65	0.01	0.17	0.82	0.01	0.25	0.74	0.247	0.037	0.041
		rs9826659	A/g	Intron 11	0.13	0.44	0.43	0.04	0.49	0.48	0.11	0.38	0.51	0.055	0.616	0.675
		rs1549759	G/a	Intron 5	0.07	0.20	0.73	0.05	0.27	0.68	0.02	0.30	0.69	0.312	0.173	0.419
		rs2069459	T/g	Intron 9	0.15	0.47	0.38	0.20	0.33	0.48	0.17	0.42	0.41	0.310	0.899	0.374
<i>PRKCA</i>	17q24.2	rs9910577	C/t	Intron 2	0.20	0.48	0.32	0.25	0.47	0.29	0.23	0.46	0.31	0.890	0.668	0.658
		rs4791035	G/c	3' Region	0.27	0.40	0.33	0.22	0.50	0.29	0.23	0.49	0.28	0.967	0.691	0.479
<i>FYN</i>	6q21	rs7768046	A/g	Intron 1	0.16	0.49	0.35	0.25	0.44	0.32	0.17	0.49	0.35	0.261	0.512	0.981
		rs1621289	T/c	Last intron	0.07	0.46	0.48	0.18	0.46	0.37	0.09	0.49	0.42	0.092	0.238	0.558
<i>GAK</i>	4p16	rs873785	G/a	Intron 1	0.15	0.46	0.39	0.11	0.55	0.34	0.07	0.54	0.39	0.451	0.486	0.637
		rs6964	C/t	3' Region	0.07	0.51	0.41	0.08	0.38	0.54	0.09	0.44	0.47	0.471	0.303	0.289
Protein phosphatases																
<i>PPP3R1</i>	2p15	rs6546366	C/a	5' Region	0.23	0.42	0.35	0.10	0.45	0.46	0.08	0.48	0.44	0.825	0.910	0.776
		rs1868402	C/t	Last intron	0.06	0.48	0.46	0.07	0.39	0.54	0.06	0.42	0.52	0.839	0.996	0.864
PTEN	10q23.3	rs1234224	A/g	Intron 2	0.13	0.52	0.35	0.14	0.51	0.35	0.14	0.40	0.46	0.150	0.575	0.248
		rs478839	A/g	3' Region	0.15	0.44	0.41	0.13	0.37	0.50	0.17	0.40	0.43	0.469	0.538	0.417
<i>PPP2R4</i> (<i>PP2A</i>)	9q34	rs10988217	G/a	Intron 4	0.43	0.44	0.13	0.16	0.50	0.34	0.19	0.46	0.36	0.696	0.965	0.629
		rs913275	A/g	3' Region	0.05	0.47	0.48	0.09	0.45	0.47	0.12	0.40	0.49	0.630	0.912	0.524
<i>PIN1</i>	19p13	rs889162	C/t	Intron 2	0.05	0.31	0.64	0.05	0.32	0.63	0.06	0.28	0.66	0.824	0.914	0.962
		rs2010457	C/t	Intron 3	0.14	0.37	0.49	0.09	0.45	0.47	0.11	0.49	0.40	0.568	0.180	0.129
Gene name	Chromosome	SNP	Alleles	Location	NCBI (CEU), genotype frequency			AD (n = 83), genotype frequency			Control (n = 149), genotype frequency			Chi-sq. unadjusted genotype ^d P-value	Chi-sq. adjusted genotype ^d P-value	Adjusted collapsed genotype ^d P-value
					EE	EF	FF	EE	EF	FF	EE	EF	FF			
Tau (with H2)	17q21.1	rs1800547	A/g	Intron 4	0.01	0.40	0.59	0.04	0.19	0.77	0.04	0.36	0.60	0.030	0.036	0.016
MAPT		rs7521	A/g	3' Region	0.19	0.49	0.33	0.27	0.49	0.24	0.20	0.48	0.32	0.368	0.361	0.867

Gene name	Chromosome	SNP	Alleles	Location	NCBI (CEU), genotype frequency			AD (n = 101), genotype frequency			Control (n = 169), genotype frequency			Chi-sq. unadjusted genotype P-value	Chi-sq. adjusted genotype ^a P-value	Adjusted collapsed genotype ^a P-value
					EE	EF	FF	EE	EF	FF	EE	EF	FF			
		rs1467967	G/a	Intron 1	0.09	0.42	0.49	0.19	0.35	0.46	0.13	0.43	0.44	0.352	0.729	0.674
Gene name	Chromosome	SNP alleles	Location		AD (n = 64), genotype frequency			Control (n = 90), genotype frequency			Unadjusted genotype P-value			Adjusted genotype ^a P-value	Adjusted collapsed genotype ^a P-value	
					EE	EF	FF	EE	EF	FF	EE	EF	FF			
Tau (without H2)	17q21.1	rs7521	A/g	3' Region	0.20	0.45	0.34	0.20	0.45	0.34	0.20	0.47	0.33	0.986	0.718	0.638
MAPT		rs1467967	G/a	Intron 1	0.25	0.36	0.39	0.22	0.46	0.32	0.25	0.46	0.32	0.483	0.796	0.356

^a Adjusted for covariates: age, gender, race, and *APOE ε4* status. *P*-values are not multiple comparison corrected.

TABLE III

SNP Effect on CSF Biomarker Levels

Biomarker	Gene	SNP	SNP by disease status	SNP within AD	SNP within controls
Log ₁₀ (p-tau)	<i>PRKCA</i>	rs9910577	0.0441 (0.8387)	0.0509 (0.9157)	0.4438 (1.0000)
	<i>FYN</i>	rs7768046	0.0163 (0.3263)	0.0203 (0.4066)	0.3648 (1.0000)
	<i>PPP2R4</i>	rs913275	0.8410 (1.0000)	0.0447 (0.8501)	0.0243 (0.4860)
	<i>GAK</i>	rs6964	0.0797 (1.0000)	0.5039 (1.0000)	0.0421 (0.7995)
Log ₁₀ (t-tau)	<i>CDK5</i>	rs1549759	0.4188 (1.0000)	0.5674 (1.0000)	0.0428 (0.8556)
	<i>FYN</i>	rs7768046	0.0050 (0.1020)	0.0007 (0.0141) ^a	0.8723 (1.0000)
	<i>PPP2R4</i>	rs10988217	0.1435 (1.0000)	0.0069 (0.1236)	0.2640 (1.0000)
	<i>PPP2R4</i>	rs913275	0.0832 (1.0000)	0.0024 (0.0451) ^a	0.2629 (1.0000)
Log ₁₀ (p-tau/t-tau)	<i>FYN</i>	rs7768046	0.0741 (1.0000)	0.0032 (0.0630)	0.3536 (1.0000)
	<i>PPP2R4</i>	rs10988217	0.0781 (1.0000)	0.0142 (0.2560)	0.7615 (1.0000)
	<i>PPP2R4</i>	rs913275	0.0050 (0.0997)	0.0057 (0.1087)	0.3115 (1.0000)

P-values and Holm multiple comparison corrected *P*-values (shown in parentheses) are based on linear regression models that include gender, age, race, *APOE* $\epsilon 4$ status, disease status, and collapsed SNPs.

^aSignificant after Holm (multiple comparisons taken into account).