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GWAS of Cerebrospinal Fluid Tau Levels Identifies Risk Variants for Alzheimer's Disease

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SUMMARY

Cerebrospinal fluid (CSF) tau, tau phosphorylated at threonine 181 (ptau), and $A\beta_{42}$ are established biomarkers for Alzheimer's disease (AD) and have been used as quantitative traits for genetic analyses. We performed the largest genome-wide association study for cerebrospinal fluid (CSF) tau/ptau levels published to date (n = 1,269), identifying three genome-wide significant loci for CSF tau and ptau: rs9877502 ($p = 4.89 \times 10^{-9}$ for tau) located at 3q28 between *GEMC1* and *OSTN*, rs514716 ($p = 1.07 \times 10^{-8}$ and $p = 3.22 \times 10^{-9}$ for tau and ptau, respectively), located at 9p24.2 within *GLIS3* and rs6922617 ($p = 3.58 \times 10^{-8}$ for CSF ptau) at 6p21.1 within the *TREM* gene cluster, a region recently

reported to harbor rare variants that increase AD risk. In independent data sets, rs9877502 showed a strong association with risk for AD, tangle pathology, and global cognitive decline ($p = 2.67 \times 10^{-4}$, 0.039, 4.86×10^{-5}, respectively) illustrating how this endophenotype-based approach can be used to identify new AD risk loci.

INTRODUCTION

AD is neuropathologically characterized by the presence of extracellular Aβ plaques and intracellular aggregates of hyperphosphorylated tau in the brain (Hardy and Selkoe, 2002). CSF $A\beta_{42}$ and tau levels have emerged as useful biomarkers for disease and endophenotypes for genetic studies of AD. CSF tau and tau phosphorylated at threonine 181 (ptau) are higher in AD cases compared with nondemented elderly controls (Shoji et al., 1998; Kawarabayashi et al., 2001; Strozyk et al., 2003; Sunderland et al., 2003; Hampel et al., 2004; Jia et al., 2005; Schoonenboom et al., 2005; Welge et al., 2009). It has been shown that genetic variants that increase risk for AD modify CSF $A\beta_{42}$ and tau levels, including pathogenic mutations in *APP*, *PSEN1*, and *PSEN2*, and the common variants in *APOE* (Kauwe et al., 2007, 2008, 2009; Ringman et al., 2008; Cruchaga et al., 2010). CSF ptau levels correlate with the number of neurofibrillary tangles and the load of hyperphosphorylated tau present in the brain (Buerger et al., 2006). Elevated CSF ptau levels are correlated with neuronal loss and predict cognitive decline and conversion to AD in subjects with mild cognitive impairment (de Leon et al., 2004; Buerger et al., 2006; Andersson et al., 2007). Enigmatically, CSF tau levels are normal or low in other tauopathies such as progressive supranuclear palsy, so the precise relationship between the burden of tau pathology as well as the extent of neurodegeneration and the levels of CSF tau remain to be fully clarified (Hu et al., 2011). This notwithstanding, CSF tau levels may be a useful marker to identify genetic variants implicated not only with risk for Alzheimer's disease but also age at onset (Kauwe et al., 2008) or rate of progression (Shoji et al., 1998; Cruchaga et al., 2010). Previous GWAS for CSF tau and ptau levels (Han et al., 2010; Kim et al., 2011) have been conducted in much smaller samples and have shown robust association with markers on chromosome 19 surrounding *APOE* but failed to detect additional genome-wide significant associations. We have conducted a genome-wide association study (GWAS) for CSF tau and ptau using a sample that is more than three times the size of previous studies and have successfully detected loci that show novel genome-wide significant association signals.

RESULTS

Variability in CSF Tau and Ptau Levels Explained by Common Variants

Before performing any analysis, we performed stringent quality control (QC) in both the genotype and the phenotype data. For the phenotype data we confirmed that the tau and ptau level followed a normal distribution after log transformation. We also performed a stepwise regression analysis to identify the covariates showing a significant association with these endophenotypes. We performed a GWAS on 1,269 unrelated individuals recruited through the Knight-ADRC at Washington University, the Alzheimer's Disease Neuroimaging Initiative, a biomarker Consortium of Alzheimer Disease Centers coordinated by University of Washington and the University of Pennsylvania (Table 1 and see Table S1 available online). While there are differences in the absolute levels of the biomarker measurements between the different studies that likely reflect differences in the methods used for quantification (regular ELISA versus Luminex), both methods measure the same analytes but yield different absolute levels. In addition, CSF ptau and tau levels in the different studies show similar characteristics. CSF ptau and tau levels show a 10- to 17-fold difference in each data set, are normally distributed after log transformation, and have similar covariates in each data set (see statistical analyses).

Table 1. Summary of Sample Characteristics

	Knight-ADRC	ADNI	UW	UPenn
n	501	394	323	51
Age (years); mean ± SD (range)	69 ± 9 (46–91)	75 ± 6.9 (55–91)	67 ± 10 (45–88)	67 ± 9 (50–86)
APOE ε4+ (%)	37	49	43	61
CDR 0 (%)	73	27	61	2
Male (%)	42	60	48	33
Ptau	64 ± 35 (17–229)	34 ± 18 (7–115)	37 ± 17 (5–83)	35 ± 24 (6–116)
Tau	355 ± 229 (85–1,624)	98 ± 57 (28–495)	77 ± 35 (27–204)	81 ± 60 (17–351)
$A\beta_{42}$	587 ± 247 (154–1,293)	170 ± 56 (53–300)	216 ± 73 (64–366)	177 ± 59 (78–298)

Age at lumbar puncture (LP), percentage of males, percentage of *APOE4* allele carriers, and clinical dementia rating (CDR) at LP date for each sample. For each phenotype, the mean in pg/ml with the standard deviation and range is shown. Charles F. and Joanne Knight Alzheimer's Disease Research Center at University of Washington (Knight-ADRC), Alzheimer's Disease Neuroimaging Initiative (ADNI), and for the University of Washington, Seattle (UW). Cerebrospinal fluid (CSF); case-control (CC). See also Table S1.

To maximize our statistical power we performed a single-stage GWAS with our combined sample (Dubé et al., 2007; Rohlfs et al., 2007; Kraft and Cox, 2008). The sample includes 687 elderly nondemented individuals and 591 individuals with a clinical diagnosis of AD (Tables 1 and S1). We used linear regression to test the additive genetic model of each single nucleotide polymorphism (SNP) for association with CSF biomarker levels after adjustment for age, gender, site, and the three principal component factors from population stratification analysis. A total of 5,815,690 imputed and genotyped SNPs were included in these analyses. The inclusion of clinical dementia rating (CDR) or case/control status did not change the results significantly. No evidence of systematic inflation of p values was found (λ = 1.003 for ptau, and 1.009 for tau). To estimate the proportion of variance in CSF tau and ptau levels explained by genetic variants we used a genome-partitioning analysis (Yang et al., 2011).

Table 2. Genome-wide Significant SNPs for CSF Tau and Ptau

CHR	SNP	MAF	Closest Gene	SNP Type/Location	Tau	Ptau	$A\beta_{42}$	SNP Type
19	rs769449	0.190	APOE	Intron	1.95×10^{-16}	2.56×10^{-18}	9.02×10^{-47}	Imputed
19	rs12972970	0.193	PVRL2	Intron	8.24×10^{-16}	1.28×10^{-15}	3.74×10^{-40}	Imputed
19	rs34342646	0.193	PVRL2	Intron	5.54×10^{-16}	1.17×10^{-15}	4.17×10^{-40}	Imputed
19	rs34404554	0.208	TOMM40	Intron	3.58×10^{-16}	1.33×10^{-16}	1.01×10^{-39}	Imputed
19	rs11556505	0.210	TOMM40	Synonymous	3.48×10^{-16}	1.68×10^{-16}	1.06×10^{-39}	Imputed
19	rs2075650	0.213	TOMM40	Intron	4.28×10^{-16}	5.81×10^{-16}	2.21×10^{-39}	Typed
19	rs71352238	0.201	TOMM40	Intergenic	1.78×10^{-16}	2.46×10^{-16}	5.68×10^{-39}	Imputed
19	rs12972156	0.187	PVRL2	Intron	3.03×10^{-15}	2.31×10^{-15}	6.35×10^{-37}	Imputed
3	rs9877502	0.386	SNAR-I	Intergenic	4.98×10^{-09}	1.68×10^{-07}	0.022	Imputed
3	rs1316356	0.362	SNAR-I	Intergenic	2.80×10^{-07}	1.96×10^{-07}	0.102	Typed
9	rs514716	0.136	GLIS3	Intron	1.07×10^{-08}	3.22×10^{-09}	0.026	Imputed
9	rs622536	0.114	GLIS3	Intron	6.68×10^{-07}	4.50×10^{-08}	0.029	Imputed
9	rs59860681	0.114	GLIS3	Intron	6.68×10^{-07}	4.50×10^{-08}	4.66×10^{-3}	Imputed
9	rs623295	0.114	GLIS3	Intron	6.68×10^{-07}	4.50×10^{-08}	4.66×10^{-3}	Imputed
9	rs624290	0.114	GLIS3	Intron	6.68×10^{-07}	4.50×10^{-08}	4.66×10^{-3}	Typed
6	rs6922617	0.064	NCR2	Intergenic	2.55×10^{-05}	3.58×10^{-08}	3.69×10^{-3}	Typed
6	rs11966476	0.067	NCR2	Intergenic	1.59×10^{-05}	4.97×10^{-08}	3.99×10^{-3}	Imputed

Standardized log-transformed CSF tau and ptau values were tested for association with the SNP in an additive model using PLINK, including age, gender, site, and the third first component factors for population stratification. The table only shows the genome-wide significant SNPs or the most significant genotyped SNPs for each locus. All novel loci that show association with CSF tau and/or ptau show a weak association with CSF $A\beta_{42}$ levels. See also Tables S2, S3,S6, and S7.

Approximately 7% (ptau) and 15% (tau) of the variability in the CSF levels of these proteins are explained by variants included on the GWAS chip plus the imputed SNPs. In this study SNPs in the *APOE* region show a genome-wide significant association with CSF tau and ptau (Tables 2 and 3) and explain just 0.25%–0.29% of the variability in CSF tau and ptau, suggesting that most of the genetic variability in CSF tau and ptau levels is explained by other genetic variants.

APOE Variants Affect CSF Tau and Ptau Levels Independently of $A\beta_{42}$

Prevailing hypotheses suggest that *APOE* ε4 exerts its pathogenic effects through an Aβ-dependent mechanism (Castellano et al., 2011). However, several SNPs in the *APOE* region were genome-wide significant with both tau and ptau (rs769449; $p = 1.96 \times 10^{-16}$ and 2.56×10^{-18}, respectively; Tables 2 and 4; Figure 1). To determine whether *APOE* SNPs influence CSF tau and ptau levels independently of Aβ pathology, and disease status we performed analyses including CSF $A\beta_{42}$ levels, or CDR as covariates in a regression model. When clinical status was included as a covariate the *APOE* SNP rs769449 was still the most significant signal ($p = 1.23 \times 10^{-12}$; Table 4). When CSF $A\beta_{42}$ levels were included in the model we also found a strong, but less significant, association for rs769449 with CSF ptau levels ($p = 3.22 \times 10^{-05}$). Analyses of tau follow the same pattern (Table 4) suggesting that at least part of the tau/ptau-*APOE* association is due to the underlying association of *APOE* with $A\beta_{42}$ levels. When the sample was stratified by clinical status, rs769449 showed a strong and similar effect size in both cases (n = 519; Beta: 0.067; $p = 3.38 \times 10^{-6}$) and in controls (n = 687; Beta: 0.075, $p = 1.54 \times 10^{6}$) with CSF ptau levels (Table S2). Several studies have suggested that up to 30% of elderly nondemented control samples meet neuropathological criteria for AD (Price and Morris, 1999; Schneider et al., 2009). It has also been shown that individuals with CSF $A\beta_{42}$ levels less than 500 pg/ml in the Knight-ADRC-CSF, and 192 pg/ml in the ADNI series have evidence of Aβ deposition in the brain, as detected by PET-PIB (Fagan et al., 2006; Jagust et al., 2009). Individuals with CSF $A\beta_{42}$ levels below these thresholds could be classified as preclinical AD cases with the presumption that some evidence of fibrillar Aβ deposits would be detected (Fagan et al., 2006; Jagust et al., 2009). When we used these thresholds, rs769449 showed a significant association with CSF tau and ptau in both strata, although the effect size was almost two-fold higher in individuals with high $A\beta_{42}$ levels (n = 416; Beta: 0.072; $p = 6.58 \times 10^{-5}$, for CSF tau levels) than in individuals with low $A\beta_{42}$ levels (n = 478; Beta: 0.035; $p = 1.83 \times 10^{-2}$, for CSF tau levels; Table S2). These results indicate that the residual association of SNPs in the *APOE* region is not dependent on clinical status or the presence of fibrillar Aβ pathology and clearly suggests that DNA variants in the *APOE* gene region influence tau pathology independently of Aβ or AD disease status.

To analyze whether there is more than one independent signal in the *APOE* gene region, *APOE* genotype was included in the model as a covariate (Table 4; additional figures on https://hopecenter.wustl.edu/data/Cruchaga_Neuron_2013). The association for the SNPs located in the *APOE* region was reduced drastically (p values between 0.02 and 0.008), suggesting that most of the association in this locus is driven by *APOE* genotype.

Table 3. Genome-Partitioning Analysis

	Default Model		Without *APOE* SNPs[a]			Without *chr 3* SNPs[a]			Without *chr 6* SNPs[a]			Without *chr 9* SNPs[a]			Without TopLoci[a]		
	Vp	V(G)/Vp	V(G)	V(G)/Vp	dif	V(G)	V(G)/Vp	dif	V(G)	V(G)/Vp	dif	V(G)	V(G)/Vp	dif	V(G)	V(G)/Vp	dif
ptau	0.0473	0.0032	6.79%	0.0031	6.45%	0.33%	0.0028	5.93%	0.85%	0.0031	6.65%	0.14%	0.0031	5.84%	0.14%	0.0025	5.33%	1.45%
tau	0.0550	0.0083	15.14%	0.0082	14.86%	0.28%	0.0079	14.32%	0.82%	0.0083	15.07%	0.08%	0.0082	15.04%	0.96%	0.0076	13.86%	1.28%

We used the algorithm GCTA (genome-wide complex trait analysis) to estimate the proportion of phenotypic variance explained by genome-wide SNPs[16].
[a]The SNPs located in each locus with a p value lower than 1×10^{-5} were excluded from the analysis. A total of 13 SNPs were excluded for the APOE locus, 81 for chr 3, 14 for chr 6, and 9 for chr 9.

Loci Associated with CSF Tau and Ptau Levels

Outside the *APOE* region, we detected genome-wide significant association with three loci for CSF tau, ptau, or both at 3q28, 9p24.2, and 6p21.1. Several SNPs in each locus showed highly significant p values (Figure 1). For all loci, at least one SNP was directly genotyped (Table 2) and each of the data sets contributed to the signal, showing similar effect sizes and direction (Table S3), suggesting that these are real signals and unlikely to be the result of type I error.

The strongest association for CSF tau, after *APOE*, is rs9877502 ($p = 4.98 \times 10^{-09}$), located on 3q28 between *GEMC1* and *OSTN* and the noncoding RNA *SNAR-I* (Figures 1 and 2). Fifty-five intragenic SNPs located between *SNAR-I* and *OSTN*, showed a p value lower than 9.00×10^{-05} (additional information on https://hopecenter.wustl.edu/data/Cruchaga_Neuron_2013). Other genes located in this region, include *IL1-RAP*, *UTS2D*, and *CCDC50*, all of which are highly expressed in the brain. Bioinformatic analyses indicate that the most significant SNP in this locus and 33 SNPs in linkage disequilibrium (LD) with rs9877502 are located in transcription factor binding sites and some of these SNPs are also part of a transcription factor matrix, suggesting that rs9877502 or a linked variant could influence the expression of one or more of the genes located in this region.

Rs514716, located at 9p24.2 in an intron of *GLIS3*, shows genome-wide significant association with both CSF tau and ptau levels (Figure 2). The minor allele G (MAF = 0.136) is associated with lower CSF tau ($\beta = -0.071$; $p = 1.07 \times 10^{-8}$) and ptau levels ($\beta = -0.072$; $p = 3.22 \times 10^{-9}$). Seven additional intronic SNPs show genome-wide significant association with CSF ptau levels or p values lower than 9.00×10^{-05} for CSF tau levels (additional information on https://hopecenter.wustl.edu/data/Cruchaga_Neuron_2013). We used the HapMap and the 1,000 genome project data to identify all of the SNPs in linkage disequilibrium (LD, $R^2 > 0.8$) with rs514716. A total of nine SNPs were identified, all of them intronic. Our bioinformatic analysis indicated that none of these SNPs disrupt a core splice site, but all of them are located in a conserved region.

Finally, for CSF ptau levels, several, relatively rare SNPs (MAF = 0.06), located at 6p21.1, within the *TREM* gene cluster show genome-wide significant p values (Figure 2). As in the case of the other genome-wide signals, at least one SNP in the region was directly genotyped (rs6922617, $\beta = -0.094$; $p = 3.58 \times 10^{-8}$; Table 2), and all of the CSF series contributed to the association (Table S5). In this region, there was an additional peak driven by rs6916710 (MAF = 0.39; $p = 1.58 \times 10^{-4;}$ $\beta = -0.034$) located in intron 2 of *TREML2*. In a recent study, we found a rare functional variant (R47H, rs75932628) in *TREM2*, which substantially increases risk for AD (Guerreiro et al., 2012). Based on these results, we genotyped rs75932628 in the Knight-ADRC and ADNI series to test whether this variant is associated with CSF levels. *TREM2 R47H* (rs75932628) showed strong association with both CSF tau (MAF = 0.01; $p = 6.9 \times 10^{-4;}$ $\beta = 0.19$) and ptau levels ($p = 2.6 \times 10^{-3;}$ $\beta = 0.16$). As expected the minor allele (T) of rs75932628 is associated with higher CSF tau and ptau levels. The effect size (β) for the R47H variant was twice that of rs6922617 and rs6916710 (Table 5), while the less significant p value is explained by the lower

Table 4. Most but Not All of the CSF Ptau Association with *APOE* Is Driven by $A\beta_{42}$ Levels

		Default Model[a]		+$A\beta_{42}$ in the Model		+CDR in the Model		+APOE in the Model	
CHR	SNP	Ptau	Tau	Ptau	Tau	Ptau	Tau	Ptau	Tau
19	rs769449	**2.57×10^{-18}**	**1.95×10^{-16}**	3.22×10^{-05}	6.39×10^{-06}	**1.23×10^{-12}**	**1.04×10^{-10}**	1.29×10^{-02}	9.07×10^{-03}
19	rs34404554	**1.33×10^{-16}**	**8.24×10^{-16}**	2.91×10^{-05}	1.72×10^{-06}	**1.87×10^{-12}**	**3.19×10^{-10}**	1.43×10^{-02}	1.34×10^{-02}
19	rs11556505	**1.68×10^{-16}**	**5.54×10^{-16}**	3.86×10^{-05}	1.58×10^{-06}	**2.83×10^{-12}**	**3.00×10^{-10}**	1.77×10^{-02}	1.55×10^{-02}
19	rs71352238	**2.46×10^{-16}**	**3.58×10^{-16}**	5.33×10^{-05}	1.58×10^{-06}	**1.13×10^{-11}**	**2.04×10^{-10}**	1.37×10^{-02}	9.46×10^{-03}
19	rs2075650	**5.83×10^{-16}**	**3.48×10^{-16}**	8.82×10^{-05}	8.22×10^{-07}	**1.01×10^{-11}**	**3.88×10^{-10}**	2.24×10^{-02}	2.03×10^{-02}
19	rs34342646	**1.18×10^{-15}**	**4.28×10^{-16}**	9.36×10^{-05}	1.04×10^{-06}	**7.99×10^{-11}**	**5.87×10^{-10}**	1.25×10^{-02}	9.58×10^{-03}
19	rs12972970	**1.28×10^{-15}**	**1.78×10^{-16}**	9.96×10^{-05}	1.04×10^{-06}	**7.83×10^{-11}**	**5.94×10^{-10}**	1.25×10^{-02}	9.74×10^{-03}
19	rs12972156	**2.31×10^{-15}**	**3.03×10^{-15}**	6.72×10^{-05}	1.53×10^{-06}	**1.21×10^{-10}**	**1.10×10^{-09}**	9.26×10^{-03}	8.37×10^{-03}
3	rs9877502	1.68×10^{-07}	**4.98×10^{-09}**	5.62×10^{-07}	7.24×10^{-07}	2.47×10^{-07}	**4.39×10^{-08}**	4.60×10^{-07}	**7.77×10^{-09}**
9	rs514716	**2.99×10^{-09}**	**1.07×10^{-08}**	4.14×10^{-07}	7.43×10^{-07}	**3.76×10^{-08}**	**1.38×10^{-08}**	**2.00×10^{-08}**	2.30×10^{-07}
6	rs6922617	**3.58×10^{-08}**	2.55×10^{-05}	2.34×10^{-06}	4.03×10^{-04}	3.49×10^{-07}	1.22×10^{-04}	1.66×10^{-06}	4.61×10^{-05}

[a]Age, gender, series, and PC are included in all the analyses as covariates. Bold numbers represent p values that pass the genome-wide significant threshold.

MAF, and sample size. To determine whether the associations seen with these three SNPs represent one signal or several independent associations we analyzed the linkage disequilibrium between the SNPs and performed conditional analyses. When rs6922617, rs6916710, or rs75932628 were included as a covariate in the model the other SNPs remained significant (Table 5). In our population, none of these SNPs were in LD with each other (Table S3 and additional information on https://hopecenter.wustl.edu/data/Cruchaga_Neuron_2013). Together these results suggest that these three SNPs are tagging three independent signals within the *TREM* gene cluster that influence CSF ptau levels, and at least in the case of *TREM2*-R47H, AD risk.

Conditional analysis was also performed for the other genome-wide significant loci to test whether the association signal at each locus is driven by a single effect or by multiple independent effects and to determine whether the identified loci interact with each other. For the other loci, the signal for the conditioned SNP (and other SNPs in the same locus) totally disappeared confirming that the association at each locus represents a single signal. Conditioning on the genome-wide significant SNPs did not dramatically change the signals in other parts of the genome (additional information on https://hopecenter.wustl.edu/data/Cruchaga_Neuron_2013), suggesting that there is not strong interaction between these loci and the rest of the genome.

To evaluate the specificity of these genome-wide significant loci we also examined whether the SNPs were associated with another AD biomarker, CSF Aβ42 levels. Only SNPs within the *APOE* region showed genome-wide association with CSF tau and CSF Aβ42 (rs2075650 $p = 1.83 \times 10^{-40}$). For the other regions, the p values for association with CSF Aß42 were modest: 0.02 for rs9877502, 0.03, for rs514716, and for 3.6×10^{-3} rs6922617. Furthermore, the correlation between the variants that give p values $< 10^{-4}$ for either phenotype was low ($r^2 = 0.07$). Together these results confirm the specificity of our results and that CSF tau/ptau and CSF Aß42 can be used as endophenotypes to identify genetic variants that influence different facets of the AD phenotype.

Gene Expression Analysis

To further characterize these associations we evaluated gene expression levels in three different ways. First, we determined whether the expression levels of the identified genes are associated with case-control status. Second, we determined whether the SNPs associated with CSF tau/ptau levels also affect tau (*MAPT*) gene expression levels in brain; and third, we tested whether the SNPs were associated with expression levels of the candidate genes within each locus. To do this, we analyzed *MAPT*, *GEMC1*, *IL1RAP*, *OSTN*, and *FOXP4* gene expression using cDNA from the frontal lobes of 82 AD cases and 39 nondemented individuals obtained through the Knight-ADRC Neuropathology Core. In addition, *MAPT*, *RFX3*, *SLC1A1*, and *PPAPDC2* gene expression were analyzed using publically available data from 486 late onset Alzheimer's disease cases and 279 neuropathologically clean individuals form the GSE15222 data set (Myers et al., 2007). We found strong association for *RFX3* ($p = 1.39 \times 10^{-9}$; $\beta = 0.42$), *SLC1A1* ($p = 1.01 \times 10^{-4}$; $\beta = -0.28$), and *PPAPDC2* ($p = 4.80 \times 10^{-3}$; $\beta = -0.35$), all located in the chromosome 9 region of association, with case-control status. We also found a nominally significant association of *IL1RAP* (Chr. 3; $p = 0.04$; $\beta = -0.18$) with case-control status but not for *MAPT*, *GLIS3*, *GEMC1*, *OSTN*, or *FOXP4* (Table S5). None of the SNPs associated with CSF tau/ptau levels showed an association with *MAPT* gene expression levels suggesting that they impact CSF tau levels by a post-transcriptional mechanism. Rs9877502 (chr. 3) showed nominally significant association with *IL1RAP* expression ($p = 0.02$; $\beta = -0.17$), but not with other genes in the same locus: *GEMC1* ($p = 0.54$; $\beta = -0.09$), and *OSTN* ($p = 0.87$; $\beta = -0.02$; Table S5).

Impact of the Identified Loci on Other AD Phenotypes

Because the purpose of this endophenotype-based approach is to identify variants implicated in disease, we tested whether the most significant SNP from each locus shows association with risk for AD, tau pathology, or rate of cognitive decline. For the SNP located on 3q28 between *GEMC1* and *OSTN*, each copy

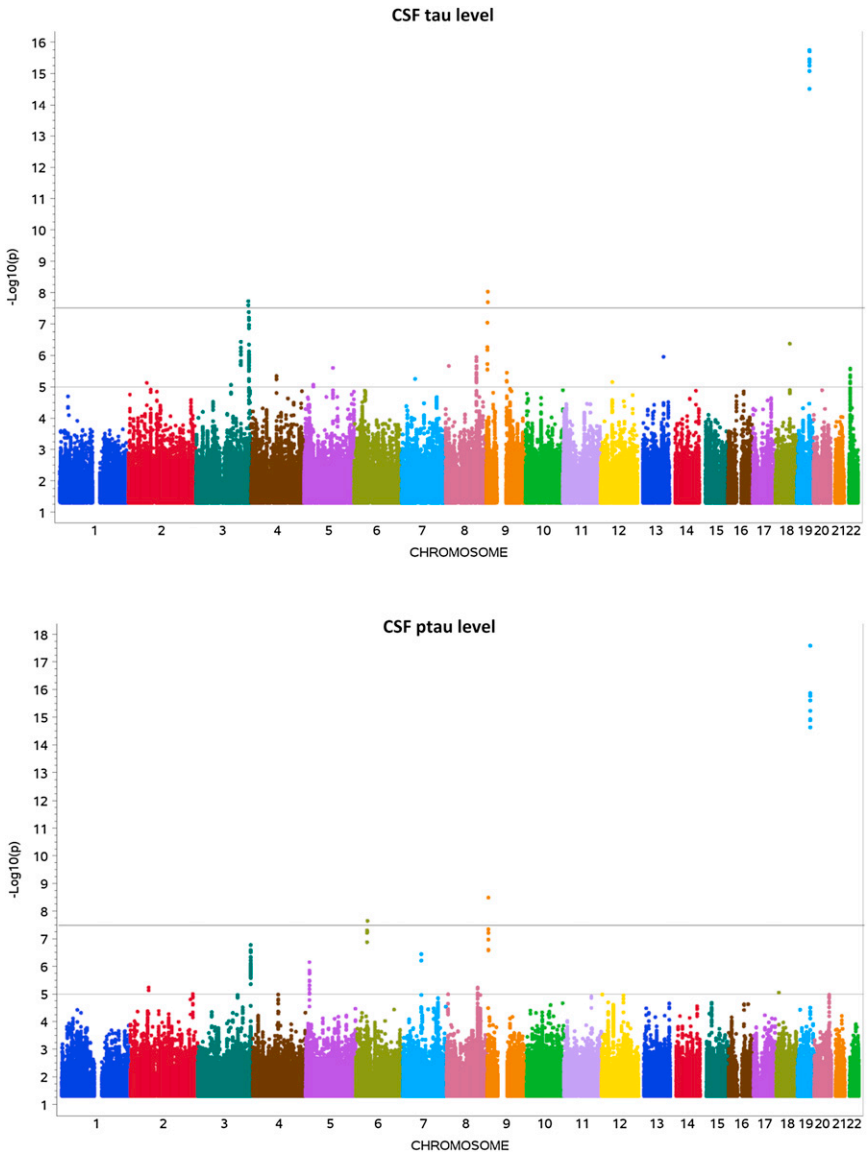

Figure 1. Genome-wide Signal Intensity (Manhattan) Plots Showing the Individual p Values (Based on Fixed-Effects Meta-analysis) against Genomic Position

The results for the association of CSF tau (top) and ptau (bottom) levels with 5,815,690 SNPs are shown. Within each chromosome, shown on the x axis, the results are plotted left to right from the p-terminal end. Horizontal dashed lines indicate p value thresholds of 1×10^{-5} and 5×10^{-8} (genome-wide significance).

of the rs9877502-A allele (minor allele frequency [MAF] = 0.386) is associated with higher CSF tau levels (regression coefficient [β] = 0.052). Genotypes for rs9877502 were not available for the case-control series, but rs1316356, which is in LD with rs9877502 (D′ = 1, R^2 = 0.932) showed a strong association with AD risk (β = 0.81; $p = 2.67 \times 10^{-4}$). Further, in an independent analysis leveraging two prospective cohorts, the Religious Orders Study and Rush Memory and Aging Project, rs9877502 was associated with global cognitive decline (n = 1,593; β = −0.014; $p = 4.6 \times 10^{-5}$), and in deceased subjects, this variant was associated with burden of neurofibrillary tangles at autopsy (n = 651; β = 0.055; p = 0.014) (Table 6). Importantly, these associations showed the predicted direction of effect for these phenotypes based on the CSF tau levels: the allele associated with lower tau levels is predicted to be protective for disease risk, associated with lower tau pathology, and with slower cognitive decline.

There was also some evidence that the SNPs associated with CSF tau and ptau levels in the 6p21.1 locus are also associated with risk for AD. A rare (MAF = 0.01) functional coding variant with large effect size (odds ratio > 2) for AD risk was recently reported (Guerreiro et al., 2012). This rare SNP (*TREM2*-R47H, rs75932628) was also associated with CSF ptau levels at $p = 2.6 \times 10^{-3}$ (Table 4). For the other locus we failed to detect significant association with risk for AD, tau pathology or cognitive decline, although the direction of the effect was in the expected direction based on the CSF levels (Table 6).

Pathway Analyses

We performed a pathway analysis to determine whether signals that do not achieve genome-wide significance ($p < 1.0 \times 10^{-04}$) are enriched for sets of biologically related genes, represented as gene ontology terms (GO), and Kyoto Encyclopedia of genes and genomes (KEGG). Gene ontology terms for lipid transport and metabolism are significant for tau and ptau (Table S6). Furthermore, the KEGG pathway *Type II diabetes mellitus* is also significant for ptau (enriched by *MAPK9* and *IRS2*) and tau (enriched by *MAPK9*, *IRS2*, and *MAPK1*). These results and the association of genetic variants in *GLIS3*, implicated in diabetes, with CSF tau levels support previous data suggesting that diabetes could influence risk for AD.

DISCUSSION

We have previously shown that using CSF tau and ptau levels as endophenotypes it is possible to identify genetic variants implicated in AD (Kauwe et al., 2008, 2010, 2011; Cruchaga et al., 2011, 2012). This study represents the largest GWAS for CSF tau and ptau levels performed to date. Two other GWAS using the ADNI data (n = 394) have been reported previously. In these smaller studies only the *APOE* locus showed genome-wide significant association with CSF $A\beta_{42}$ and tau levels. By using a

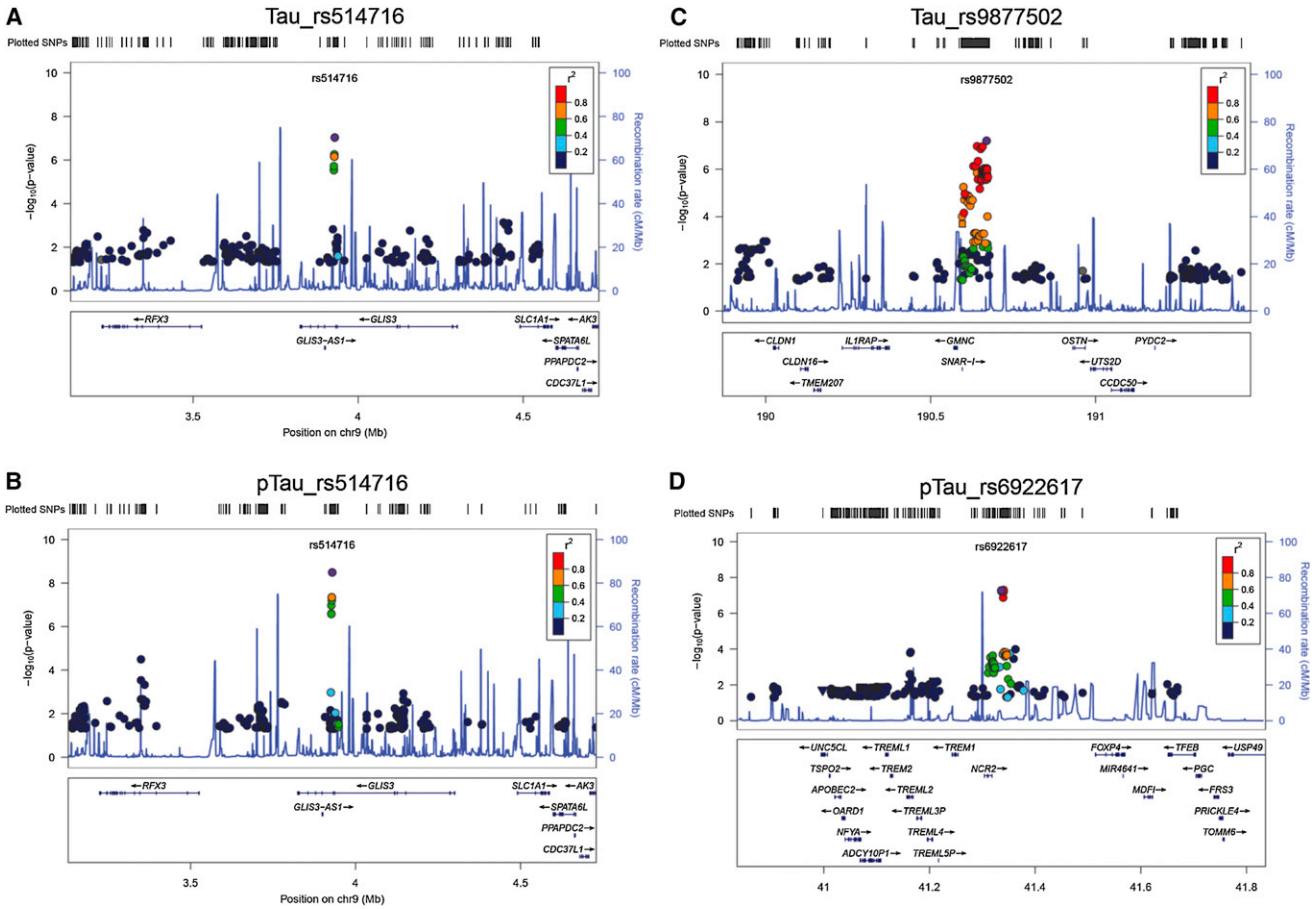

Figure 2. Regional Plots for Associations with CSF Tau and Ptau at Genome-wide Significance

Plots are centered on the most significant SNP at a given locus along with the combined-analysis results for SNPs in the region surrounding it (typically ± 400 kb). Symbols are colored according to the LD of the SNP with the top SNP. The light blue line represents the estimated recombination rate. Gene annotations are shown as dark green line. See also Tables S5 and S6.

threefold larger sample size than these studies we were able to identify four independent genome-wide significant loci, including *APOE* (Table 2). We calculated that common variants tagged by SNPs on the GWAS chip explain 6.45% and 15.14% of the overall variability in CSF ptau and tau levels, respectively. The four genome-wide significant loci identified in this study explain 1.45% of CSF ptau and 1.28% of CSF tau variability (Table 3). Together these four loci explain 22% and 9% of the genetic component for CSF ptau and tau levels, respectively, indicating additional variants and genes associated with CSF tau and ptau levels may be identified in future, using larger data sets and different approaches such as whole-genome sequencing.

A single-stage GWAS, rather than a two stage GWAS approach using the largest series as the discovery series, with follow up of the most significant SNPs in the rest of the samples, was used to maximize power (Dubé et al., 2007; Rohlfs et al., 2007; Kraft and Cox, 2008). There are several indications that the identified genome-wide significant loci are real signals and not artifacts from the analysis or type I errors. First, several SNPs in each locus show highly significant p values (Figure 1), and at least one SNP in each locus was directly genotyped (Table 2), eliminating the possibility that the signal is the result of an imputation error. Second, each of the genome-wide significant loci is the result of a strong and consistent association in each data set. This is especially important, because a priori, the absolute values for the CSF biomarker traits are significantly different between series, which could lead to the identification of false positives. The fact that the SNPs show similar effect sizes and the same direction of effect in each data set indicates that we were able to correct for any potential series-bias and represents an internal replication of each of the associations. If we had performed a two-stage analysis we would have identified these same four loci. Finally, for three (chr. 19, APOE and 3q28 and 6p21.1) of the four genome-wide significant loci we also found that the SNPs associated with CSF levels are also associated with risk for disease, tau pathology, and/or cognitive decline. Importantly, all of these associations are in the direction predicted by the CSF tau and ptau associations. The alleles associated with lower tau and ptau levels (which would be considered protective) are associated with lower risk for AD, lower tangle counts and slower memory decline.

Table 5. Association of the *TREM2* Gene Cluster with CSF Tau and Ptau Levels

			Tau p Value (Beta)				Ptau p Value (Beta)			
	MAF	n	Default Model	Cond. on R47H	Cond. on rs6916710	Cond. on rs6922617	Default model	Cond. on R47H	Cond. on rs6916710	Cond. on rs6922617
TREM2-R47H	0.01	815	6.9×10^{-4} (0.19)	—	1.3×10^{-3} (0.18)	5.3×10^{-4} (0.19)	2.6×10^{-3} (0.16)	—	4.7×10^{-3} (0.15)	2.0×10^{-3} (0.16)
rs6916710	0.39	815	8.2×10^{-3} (−0.03)	0.015 (−0.03)	—	9.3×10^{-3} (−0.03)	6.4×10^{-3} (−0.03)	0.011 (−0.03)	—	7.0×10^{-3} (−0.03)
rs6922617	0.06	815	4.1×10^{-5} (−0.10)	3.2×10^{-5} (−0.10)	4.6×10^{-5} (−0.09)	—	6.1×10^{-6} (−0.10)	4.9×10^{-6} (−0.10)	6.5×10^{-6} (−0.10)	—

The *TREM2 R47H* variant (rs75932628) was genotyped in the Knight-ADRC and ADNI series by Sequenom. The association of *TREM* gene cluster variant with CSF levels was performed with PLINK including, age, gender, series, and principal component factors as covariates. See also Table S4.

As in the previously published GWAS for CSF tau/ptau levels, we found that the *APOE* locus was the strongest signal for CSF tau and ptau (Han et al., 2010; Kim et al., 2011; Table 2). SNPs in this locus explain between 0.25% and 0.29% of the variability in CSF tau and ptau levels (Table 3). *APOE* is a known genetic risk factor for AD and most functional studies have focused on Aβ-dependent mechanisms. To determine whether or not the association of *APOE* SNPs with CSF tau and ptau levels was dependent of Aβ pathology we performed analyses including CSF $A\beta_{42}$ levels as a covariate. We also stratified our samples by case control status and by low or high CSF $A\beta_{42}$ levels. In all of these analyses, we found that the association between *APOE* SNPs and tau or ptau levels remained significant (Table 4 and S2), suggesting that *APOE* may also affect tau pathology via an Aβ-independent mechanism. Several other studies support this hypothesis. APOE shows isoform specific differences in its interaction with tau in vitro (Gibb et al., 2000; Zhou et al., 2006), and in transgenic mice neuron-specific differences in APOE isoform proteolysis are associated with increased tau phosphorylation (Brecht et al., 2004) and pathology (Andrews-Zwilling et al., 2010). These data provide additional evidence that *APOE* could also influence risk for AD through a tau-dependent mechanism, independent of effects on Aβ. When *APOE* genotype was included as a covariate, some SNPs in the *APOE* locus showed a moderate association with CSF tau/ptau levels (rs769449; $p = 9.07 \times 10^{-03}$), indicating that most of the association is driven by *APOE* genotype, but suggesting that there may be additional variants in this region that modify CSF tau levels and risk for AD, independently of *APOE* genotype.

SNPs within the 3q28 locus showed association with CSF tau/ptau levels and a range of AD phenotypes including AD risk in the case control data set, tangle pathology, and rate of cognitive decline providing four independent sources of evidence that variants in this region influence risk for AD through a tau-dependent mechanism. Bioinformatic analysis did not reveal any strong putative functional SNP. However, the genes located in this region (*GEMC1*, *OSTN* and the noncoding RNA *SNAR-I*, *IL6RAP*, *UTS2D*, and *CCDC50)* are highly expressed in brain and involved in neuronal synaptogenesis (Yoshida et al., 2012). The most significant SNP in this locus and 33 SNPs in LD with rs9877502 are located in transcription factor binding sites and some of these SNPs are also part of a transcription factor matrix (additional information on https://hopecenter.wustl.edu/data/Cruchaga_Neuron_2013), suggesting that rs9877502 or a linked variant could influence the expression of one or more of the genes located in this region. Based on the results of these bioinformatic analyses we performed several gene-expression experiments. *IL1RAP* showed a nominally significant association with case-control status ($p = 0.04$). In addition rs9877502 showed a significant association with *IL1RAP* expression in frontal cortex ($p = 0.02$; Table S5).

The lack of association with risk for AD in the ADGC GWAS for the most significant SNP in the 6p21.1 locus may reflect insufficient power because the SNP has a low minor allele frequency (MAF = 0.06). This hypothesis is supported by our recent identification of a rare functional coding variant (*TREM2*- R47H, rs75932628) in the same locus which substantially increases risk for AD (Guerreiro et al., 2012), and is also associated with CSF ptau levels in the present study. Interestingly, the genome-wide significant signal (tagged by rs6922617) is not in LD with rs75932628. Conditional analyses in this region identified another independent SNP (Figure 2; Table 5), located in an intron of *TREML2* that is associated with CSF tau and ptau levels. These data suggest that in this region there are at least three independent signals modifying CSF tau levels and risk for AD. Six *TREM*-family genes (*TREM1*, *TREM2*, and *TREML1* to *TREML4*) are located in this region suggesting that several variants in genes with similar function may affect risk for AD in an independent manner. The genome-wide significant SNP in this locus (rs11966476; $p = 4.79 \times 10^{-8}$), is located in a regulatory element and could modify the expression of *FOXP4*, *TREML3*, *TREML4*, or *TREM1* (Figure 2). Unfortunately, these genes were not included in the GSE15222 data set and Taqman assays for these genes were out of the dynamic range so we were unsuccessful in analyzing expression levels in brain tissue. Despite this, data from the Allen Brain Atlas suggests that these genes are expressed in the brain. *TREM2* was expressed at higher levels in brain tissue from AD cases compared to controls ($p = 1.35 \times 10^{-5}$), as predicted in our previous studies (Guerreiro et al., 2012).

For the 9p24.2 locus, we did not observe significant association with risk for AD. This could be because these SNPs affect another aspect of AD such as disease duration or age at onset. Alternatively, these SNPs could affect CSF clearance or protein half-life without affecting risk for AD. If this were the case, we would expect that the same locus would be associated with levels of other CSF proteins. To test this, we looked at the association of all of the SNPs identified in this study at the

Table 6. Association of the Top Loci for CSF Tau and Ptau with Risk for AD, Tangle Counts, and Cognitive Decline

				CSF (n = 1,269)		Risk for AD (n = 22,771)		Tangles (n = 651)		Global Cognitive Decline (n = 1,593)	
Chr.	Rs #	Phen.	Minor Allele	Beta	p Value	Beta	p Value	Beta	p Value	Beta	p Value
19	rs2075650	tau	G	0.074	4.29×10^{-16}	0.985	6.22×10^{-184}	0.266	9.33×10^{-10}	−0.049	1.22×10^{-11}
		ptau	G	0.081	4.92×10^{-16}						
3	rs9877502	tau	A	0.052	4.98×10^{-09}	0.081*	2.67×10^{-04}*	0.055	0.014	−0.014	4.64×10^{-05}
		ptau	A	0.050	1.68×10^{-07}						
9	rs514716	tau	G	−0.071	1.07×10^{-08}	−0.022	0.518	−0.036	0.360	0.004	0.479
		ptau	G	−0.072	2.99×10^{-09}						
6	rs6922617	tau	A	−0.094	2.55×10^{-05}	−0.0174	0.677	−0.029	0.547	0.007	0.330
		ptau	A	−0.093	3.58×10^{-08}						

The association of the most significant SNPs for CSF tau and ptau for risk for AD, tangle count, or cognitive decline is shown. p values and direction for risk for disease were extracted from the previously published GWAS for AD (Naj et al., 2011). Association with tangle counts and memory decline was performed in the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP) as previously reported (De Jager et al., 2012; Keenan et al., 2012). *The p value for risk for disease is for rs1316356, which is in high LD with rs9877502. In all cases the beta is calculated in reference to the minor allele.

genome-wide significance level with other CSF biomarkers. We did not observe association between these SNPs and CSF levels of either APOE or Aβ (Cruchaga et al., 2012), suggesting that these loci are specific for CSF tau levels and are not associated with CSF clearance or protein half life in general. Finally, the lack of association of these loci with AD risk could indicate that the association with this locus is a type I error. The most significant SNPs in this locus are located in intron 7 of *GLIS3*, a gene which is highly expressed in brain. However, these SNPs (rs514716) are not associated with *GLIS3* expression in our relatively small series of brain samples (82 AD cases and 39 nondemented individuals). Both common and rare variants in this gene have been associated with risk for diabetes (Barker et al., 2011; Dimitri et al., 2011). There are several studies linking AD with glucose metabolism and diabetes (Accardi et al., 2012). In fact, a meta-analysis combining data from eight studies, observed an association between diabetes mellitus and increased risk for AD (OR: 1.51, 95%; CI = 1.31–1.73) (Bertram et al., 2013). In addition, our pathway analysis independently identified a diabetes pathway (path: hsa04930, p value for ptau = 6.60×10^{-03}, and tau = 8.00×10^{-04}; Table S6), because of an enrichment of significant SNPs in *MAPK9*, *IRS2*, and *MAPK1*. Two independent analyses in this study therefore suggest that diabetes-related genes may influence CSF tau and ptau levels, and ultimately risk for AD. These data all provide supportive evidence for common variants in this locus that influence AD pathogenesis.

Finally, because SNPs identified in this study were associated with CSF tau/ptau levels, we tested whether these SNPs are also associated with *MAPT* gene expression. None of the genome-wide significant SNPs showed association with *MAPT* expression in the brain and *MAPT* expression was not associated with case-control status in our brain series, the GSE15222, or any other published work on gene expression in brain (Webster et al., 2009; Zou et al., 2012). These results suggest that the SNPs identified in this study influence CSF tau/ptau protein levels posttranscriptional mechanism. Tau protein undergoes several posttranslational modifications including acetylation, glycosylation; and phosphorylation. These changes are thought to play an important role in tau-related pathogenesis (Farías et al., 2011; Marcus and Schachter, 2011). It is possible that the genes identified in this study modify tau protein levels through posttranslational modification rather than gene expression.

Together these results clearly demonstrate the utility of using these endophenotypes to identify AD risk variants and variants associated with the rate of decline in symptomatic AD cases. The use of these endophenotype allowed us to identify risk variants that were not identified by GWAS because either those variants did not pass the stringent multiple test correction applied in the GWAS or were not covered in the earlier studies, because of their relatively low MAF. A second advantage of this approach is that in contrast to GWAS hits from case control studies the endophenotype predicts a specific biological hypothesis for the pathogenic effect, which can be directly tested.

In summary, we have detected four genetic loci associated with CSF levels of tau, and ptau. One of them, in *APOE*, is already known to be associated with CSF tau and $A\beta_{42}$ (Kauwe et al., 2007, 2008, 2011; Cruchaga et al., 2010, 2011) as well as risk for AD. The other three are novel loci. The top hit for CSF tau (rs9877502; 3q28) also exhibited association with risk for AD ($p = 2.67 \times 10^{-4}$), tangle pathology ($p = 0.01$), and global memory decline ($p = 4.86 \times 10^{-5}$). SNPs in the 6q21.1 locus are in the *TREM* gene cluster close to *TREM2,* a gene in which a rare variant has recently been reported to substantially increase risk for AD (Guerreiro et al., 2012). The other genome-wide significant locus identified in this study did not show association with risk for disease, tangle pathology or memory decline. The lack of association with other AD phenotypes could be because these SNPs have a weaker impact on these phenotypes, or because they affect other aspects of AD, such as disease duration or age at onset. Alternatively, the sample size for the data sets used in the pathology and memory decline studies may not provide enough statistical power. Overall, these results illustrate how genetic studies of disease endophenotypes are an effective approach for identifying disease risk loci that is complementary to case-control association studies.

EXPERIMENTAL PROCEDURES

Subjects and Phenotypes

CSF tau, ptau, and $A\beta_{42}$ were measured in 1,269 individuals. There were 501 samples from research participants enrolled in longitudinal studies at the Knight-ADRC, 394 in ADNI, 323 in studies at the University of Washington (UW), and 51 in studies in University of Pennsylvania (UPenn). CSF collection and $A\beta_{42}$, tau, and ptau181 measurements were performed as described previously (Fagan et al., 2006). Table 1 shows the demographic data and description of the CSF biomarkers in each data set. The samples were genotyped using Illumina chips. Cases received a diagnosis of dementia of the Alzheimer's type (DAT), using criteria equivalent to the National Institute of Neurological and Communication Disorders and Stroke-Alzheimer's Disease and Related Disorders Association for probable AD (McKhann et al., 1984). Controls received the same assessment as the cases but were nondemented. All individuals were of European descent and written consent was obtained from all participants.

While there are differences in the absolute levels of the biomarker measurements between the studies that likely reflect differences in the methods used for quantification (regular ELISA versus Luminex), ascertainment, and/or in handling of the CSF after collection, CSF ptau levels in the Knight-ADRC, ADNI, UW, and UPenn samples show similar characteristics (Table S1). CSF ptau and tau show a 10-fold difference between individuals in each data set and have similar covariates in each data set.

The Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP) recruit participants without known dementia who agree to annual clinical evaluations and sign an Anatomic Gift Act donating their brains at death. The full cohort with genotype data included 1,708 subjects (817 ROS and 891 MAP). The mean age at enrollment was 78.5 years and 30.9% were male. At the last evaluation, 24.9% met clinical diagnostic criteria for AD and 21.8% had mild cognitive impairment. The summary measure of global cognitive performance was based on annual assessments of 17 neuropsychiatric tests. A nested autopsy cohort consisted of 651 deceased subjects (376 ROS and 275 MAP); mean age at death was 81.5 years and 37.6% were male. Proximate to death, 40.9% of subjects included in the autopsy cohort met clinical diagnostic criteria for AD. Bielschowsky silver stain was used to visualize neurofibrillary tangles in tissue sections from the midfrontal, middle temporal, inferior parietal, and entorhinal cortices, and the hippocampal CA1 sector. A quantitative composite score for neurofibrillary tangle pathologic burden was created by dividing the raw counts in each region by the standard deviation of the region specific counts and then averaging the scaled counts over the five brain regions to create a single standardized summary measure. Additional details of the ROS and MAP cohorts as well as the cognitive and pathologic phenotypes are described in prior publications (De Jager et al., 2012; Keenan et al., 2012).

Genotyping

The Knight-ADRC and UW samples were genotyped with the Illumina 610 or the Omniexpress chip. The ADNI samples were genotyped with the Illumina 610 chip, and the UPenn sample with the Omniexpress. Prior to association analysis, all samples and genotypes underwent stringent quality control (QC). Genotype data were cleaned by applying a minimum call rate for SNPs and individuals (98%) and minimum minor allele frequencies (0.02). SNPs not in Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$) were excluded. The QC cleaning steps were applied for each genotyping array separately. We tested for unanticipated duplicates and cryptic relatedness among samples using pairwise genome-wide estimates of proportion identity-by-descent. When a pair of identical samples or a pair of samples with cryptic relatedness was identified, the sample from the Knight-ADRC or samples with a higher number of SNPs passing QC were prioritized. Eigenstrat (Price et al., 2006) was used to calculate principal component factors for each sample and confirm the ethnicity of the samples. Rs7412 and rs429358 which define the *APOE* $\varepsilon 2/\varepsilon 3/\varepsilon 4$ isoforms were genotyped using Taqman genotyping technology, as previously described (Koch et al., 2002; Cruchaga et al., 2009, 2010, 2011, 2012; Kauwe et al., 2010).

DNA from ROS and MAP subjects was extracted from whole blood, lymphocytes, or frozen postmortem brain tissue and genotyped on the Affymetrix Genechip 6.0 platform, as previously described (Keenan et al., 2012). Following standard QC procedures, imputation was performed using MACH software (version 1.0.16a) and HapMap release 22 CEU (build 36) as a reference.

Imputation in Illumina Data Sets

The 1,000 genome data (June 2011 release) and the Beagle software were used to impute up to 6 million SNPs. SNPs with a Beagle R^2 of 0.3 or lower, a minor allele frequency (MAF) lower than 0.02, out of Hardy-Weinberg equilibrium ($p < 1 \times 10^{-6}$), a call rate lower than 95% or a Gprobs score lower than 0.90 were removed. A total of 5,815,690 SNPs passed the QC process. To confirm the accuracy of our imputation we genotyped 23 SNPs, included the most significant SNPs, using Sequenom. All of the SNPs, showed a concordance rate between imputed and directly genotyped calls greater than 97.9% except rs1024718 which was 93.33% (Table S7).

Statistical Analyses

Association of CSF ptau with the genetic variants was analyzed as previously reported (Cruchaga et al., 2010, 2011; Kauwe et al., 2011). Our analysis included a total of 5,815,690 imputed and genotyped variants. CSF tau and ptau values were log transformed to approximate a normal distribution. Because the CSF biomarker levels were measured using different platforms (Innotest plate ELISA versus AlzBia3 bead-based ELISA, respectively), we were not able to combine the raw data. For the combined analyses we standardized the mean of the log transformed values from each data set to zero. No significant differences in the transformed and standardized CSF values for different series were found.

We used Plink to analyze the association of SNPs with CSF biomarker levels. Age, gender, site, and the three principal component factors for population structure were included as covariates. The calculated genomic inflation factor was $\lambda = 1.003$, and 1.009, for tau and ptau, respectively (Figure S1). In order to determine whether the association of *APOE* with CSF tau levels was driven by case-control status, we included clinical dementia rating (CDR) or CSF $A\beta_{42}$ as a covariate in the model or stratified the data by case control status. We also performed analyses including *APOE* genotype and CDR as covariates.

Association with Risk for Alzheimer's Disease

p values for the most significant SNPs for the association with CSF tau and ptau were included here from the previously published GWAS for AD, consisting of 11,840 controls and 10,931 cases (Naj et al., 2011).

Genome Partitioning

We used the algorithm GCTA (genome-wide complex trait analysis) to estimate the proportion of phenotypic variance explained by genome-wide and imputed SNPs (Yang et al., 2011).

Association with Cognitive Decline and Neurofibrillary Pathology

Analyses of SNP effects on global cognitive decline in ROS and MAP were performed as in prior publications (De Jager et al., 2012). Briefly, we first fit linear mixed effects models using the global cognitive summary measure in order to characterize individual paths of change, adjusted for age, sex, years of education, and their interactions with time. At least two longitudinal measures of cognition were required for inclusion in these analyses, for which data on 1,593 subjects was available. We then used these person-specific, residual cognitive decline slopes as the outcome variable in our linear regression models, with each SNP of interest coded additively relative to the minor allele, and further adjusted for study membership (ROS versus MAP) and the first three principal components from population structure analysis. For analyses of neurofibrillary tangle burden, linear regression was used to relate SNPs to the pathologic summary measure, adjusting for age at death, study membership, and three principal components. Because the data were skewed, square-root of the scaled neurofibrillary tangle burden summary score was used in analyses.

Bioinformatics Analyses

We used Pupasuite (Conde et al., 2006), the SNP Function Portal (http://brainarray.mbni.med.umich.edu/Brainarray/Database/SearchSNP/), the SNP

Function annotation portal (http://brainarray.mbni.med.umich.edu/Brainarray/Database/SearchSNP/snpfunc.aspx), and the SNP and CNV Annotation Database (http://www.scandb.org) to perform the SNP annotation and to identify the putative functional SNPs.

Pathway Analysis

We applied the method ALIGATOR (Holmans et al., 2009) to identify the gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched by SNP with significant association. This method performs an overrepresentation analysis, evaluating the significance for each category of genes while correcting for gene size, number of SNPs genotyped per gene, overlapping genes, and linkage disequilibrium between SNPs. It selects the set of genes, which are tagged by SNPs that are more significant than a specific threshold (p values < 1.0E-04). The pruning process that eliminates SNPs in linkage disequilibrium is performed by considering only the most significant SNP among all of the SNPs that have $r^2 > 0.2$ and are within 1 Mb. Moreover, we removed all of the genes that are in the *APOE* region (1 Mb up/downstream) (Jones et al., 2010). The significance of each term and pathway is calculated by comparing the number of significant genes to the number of genes expected by chance. For this purpose, the algorithm generates 5,000 sets of genes, by randomly selecting SNPs until a list of n tagged genes is formed. The excess of significantly overrepresented sets of genes (Holmans et al., 2009) is calculated by applying a bootstrap method (1,000 permutations).

Gene Expression Analysis

Analyses of association between SNPs and gene expression was carried out using cDNA from the frontal lobes of 82 AD cases and 39 nondemented individuals obtained through the Washington University Knight-Alzheimer Disease Research Center (WU-ADRC) Neuropathology Core. Total RNA was extracted from the frontal lobe using the RNeasy mini kit (QIAGEN) following the manufacturer's protocol. cDNAs were prepared from the total RNA, using the High-Capacity cDNA Archive kit (ABI). Gene expression was analyzed by real-time PCR, using an ABI-7500 real-time PCR system. Real-time PCR assays were used to quantify *MAPT*, *GLIS3*, *GEMC1*, *IL1RAP*, *OSTN*, and *FOXP4* cDNA levels using Taqman assays. GADPH, MAP2, AIF, and GFAP were used as reference genes. Each real-time PCR run included within-plate duplicates. Real-time data were analyzed using the comparative Ct method. The Ct values of each sample were normalized with the Ct value for the housekeeping genes. We also used the GEO data set GSE15222 (Myers et al., 2007) to analyze the association of *MAPT*, *RFX3*, *SLC1A1*, and *PPAPDC2* genes and case-control status. None of the other genes (*GLIS3*, *GEMC1*, *IL1RAP*, *OSTN*, *FOXP4*) were found in this data set. This data set includes genotype and expression data from 486 late onset Alzheimer's disease cases and 279 neuropathologically clean individuals. Association of mRNA levels with case control status or the different SNPs was carried out using ANCOVA. Stepwise regression analysis was used to identify the potential covariates (postmortem interval, age at death, site, and gender) and significant covariates were included in the analysis. SNPs were tested using an additive model with minor allele homozygotes coded as 0, heterozygotes coded as 1, and major allele homozygotes coded as 2.

ADNI Material and Methods

Data used in the preparation of this article were obtained from the ADNI database (www.loni.ucla.edu/ADNI). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations, as a $60 million, 5 year public-private partnership. The Principal Investigator of this initiative is Michael W. Weiner, MD. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the US and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research—approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years, and 200 people with early AD to be followed for 2 years. For up-to-date information, see www.adni-info.org.

SUPPLEMENTAL INFORMATION

Supplemental Information includes seven tables, a supplemental author list, and Supplemental Acknowledgments and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2013.02.026.

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