Genome-wide association study of CSF biomarkers $A\beta_{1\text{-}42}$, t-tau, and p-tau_{181p} in the ADNI cohort

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Supplemental data at www.neurology.org

ABSTRACT

Objectives: CSF levels of $A\beta_{1\text{-}42}$, t-tau, and p-tau_{181p} are potential early diagnostic markers for probable Alzheimer disease (AD). The influence of genetic variation on these markers has been investigated for candidate genes but not on a genome-wide basis. We report a genome-wide association study (GWAS) of CSF biomarkers ($A\beta_{1\text{-}42}$, t-tau, p-tau_{181p}, p-tau_{181p}/$A\beta_{1\text{-}42}$, and t-tau/$A\beta_{1\text{-}42}$).

Methods: A total of 374 non-Hispanic Caucasian participants in the Alzheimer's Disease Neuroimaging Initiative cohort with quality-controlled CSF and genotype data were included in this analysis. The main effect of single nucleotide polymorphisms (SNPs) under an additive genetic model was assessed on each of 5 CSF biomarkers. The *p* values of all SNPs for each CSF biomarker were adjusted for multiple comparisons by the Bonferroni method. We focused on SNPs with corrected $p < 0.01$ (uncorrected $p < 3.10 \times 10^{-8}$) and secondarily examined SNPs with uncorrected *p* values less than 10^{-5} to identify potential candidates.

Results: Four SNPs in the regions of the *APOE*, *LOC100129500, TOMM40*, and *EPC2* genes reached genome-wide significance for associations with one or more CSF biomarkers. SNPs in *CCDC134*, *ABCG2*, *SREBF2*, and *NFATC4*, although not reaching genome-wide significance, were identified as potential candidates.

Conclusions: In addition to known candidate genes, *APOE*, *TOMM40*, and one hypothetical gene *LOC100129500* partially overlapping *APOE*; one novel gene, *EPC2*, and several other interesting genes were associated with CSF biomarkers that are related to AD. These findings, especially the new *EPC2* results, require replication in independent cohorts. ***Neurology*® 2011;76:69–79**

GLOSSARY

$A\beta_{1\text{-}42}$ = amyloid-β 1-42 peptide; **AD** = Alzheimer disease; **ADNI** = Alzheimer's Disease Neuroimaging Initiative; **GWAS** = genome-wide association study; **LD** = linkage disequilibrium; **LOAD** = late-onset Alzheimer disease; **MAF** = minor allele frequency; **MCI** = mild cognitive impairment; **p-tau_{181p}** = tau phosphorylated at the threonine 181; **QC** = quality control; **SNP** = single nucleotide polymorphism; **t-tau** = total tau.

Alzheimer disease (AD) is the most common form of dementia, affecting an estimated 5.3 million Americans. Amyloid-β 1-42 peptide ($A\beta_{1\text{-}42}$), total tau (t-tau), and tau phosphorylated at the threonine 181 (p-tau_{181p}), measured in CSF samples, are potential diagnostic biomarkers for AD.[1-3] $A\beta_{1\text{-}42}$ is decreased and t-tau and p-tau_{181p} are increased in the CSF of patients with AD.[4] Baseline $A\beta_{1\text{-}42}$ has been shown to be a good predictor of the 12-month change in

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cognitive measures, successfully predicting the 12-month progression rate of participants with mild cognitive impairment (MCI).[5] In addition to these 3 biomarkers, p-tau$_{181p}$/Aβ_{1-42} and t-tau/Aβ_{1-42} ratios have been used to effectively distinguish patients with AD from healthy controls.[1,6] Genetic factors have been shown to play a key role in late-onset AD (LOAD) pathology, with a high heritability of 58%–79%,[7] and there is evidence for the influence of selected genes on CSF biomarker levels.[1,8-10] Alzheimer's Disease Neuroimaging Initiative (ADNI) is a multicenter project to assess whether serial MRI, PET, genetic factors such as single nucleotide polymorphisms (SNPs), other biological markers, and clinical and neuropsychological assessments can be combined to improve early diagnosis and predict progression of MCI and early AD. We performed a genome-wide association study (GWAS) to investigate genetic influences on three important CSF biomarkers (Aβ_{1-42}, t-tau, and p-tau$_{181p}$) and 2 ratios (p-tau$_{181p}$/Aβ_{1-42} and t-tau/Aβ_{1-42}) in the ADNI cohort. We hypothesized that *APOE* and the adjacent gene, *TOMM40*, would be strongly associated with CSF biomarkers and sought to discover additional genes that may be related to amyloid and tau pathophysiology in AD and MCI.

METHODS

Alzheimer's Disease Neuroimaging Initiative. Data used in this study were obtained from the ADNI database (www.loni.ucla.edu/ADNI). ADNI was launched in 2004 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, multiyear public-private partnership. The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California–San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations. ADNI includes more than 800 participants, aged 55 to 90, recruited from over 50 sites across the United States and Canada, including approximately 200 cognitively normal older individuals (i.e., healthy controls) to be followed for 3 years, 400 patients diagnosed with MCI to be followed for 3 years, and 200 patients diagnosed with early AD to be followed for 2 years. Longitudinal imaging, including structural 1.5-T MRI scans collected on the full sample and [^{11}C] PIB and [^{18}F]FDG PET imaging on a subset, and performance on neuropsychological and clinical assessments were collected at baseline and at follow-up visits in 6- to 12-month intervals. Of particular relevance to the present report, *APOE* and genome-wide genotyping is available on the full ADNI sample and longitudinal CSF markers were obtained for approximately half of the cohort. Further information about ADNI can be found in previous publications[11] and at www.adni-info.org.

Standard protocol approvals, registrations, and patient consents. This study was approved by institutional review boards of all participating institutions and written informed consent was obtained from all participants or authorized representatives.

Participants. In this study, 374 (AD = 96, MCI = 176, healthy controls = 102 at baseline) non-Hispanic Caucasian individuals from the ADNI cohort whose data met all quality control (QC) criteria were included. The restriction to non-Hispanic Caucasian participants served to reduce the likelihood of population stratification effects in the GWAS. Detailed QC steps for CSF[1] and genotype data[12] have been previously reported and are briefly described below.

CSF measurements and quality control. Baseline CSF samples were obtained from 416 ADNI subjects, enrolled at 56 participating centers using previously reported methods for CSF measurements as described.[1] In summary, baseline CSF samples were obtained in the morning after an overnight fast. Lumbar puncture was performed and CSF was collected into tubes provided to each site, then transferred into polypropylene transfer tubes followed by freezing on dry ice within 1 hour after collection, and shipped overnight to the ADNI Biomarker Core Laboratory at the University of Pennsylvania Medical Center on dry ice. Aliquots (0.5 mL) were prepared from these samples after thawing (1 hour) at room temperature and gentle mixing. The aliquots were stored in bar code–labeled polypropylene vials at −80°C.

Amyloid-β 1-42 peptide (Aβ_{1-42}), total tau (t-tau), and tau phosphorylated at the threonine 181 (p-tau$_{181p}$) were measured using the multiplex xMAP Luminex platform (Luminex Corp, Austin, TX) with Innogenetics (INNO-BIA AlzBio3; Ghent, Belgium; for research use-only reagents) immunoassay kit–based reagents. Among 416 samples, 410 samples passed quality control[1] and an additional subject later failed ADNI screening resulting in 409 valid CSF samples. The demographic, clinical, and *APOE* genotyping results of these samples were comparable with those of the entire ADNI cohort.[1]

Considering the relatively small number of samples for a GWAS, further quality control was performed to reduce the potential influence of extreme outliers on statistical results. Mean and SD of each of the 3 baseline CSF measures and 2 ratios (t-tau/Aβ_{1-42} and p-tau$_{181p}$/Aβ_{1-42}) were calculated, blind to diagnostic information and subjects who had at least one value greater or smaller than 4 SD from the mean value of each of 5 CSF variables were regarded as extreme outliers and removed from the analysis. This step removed 6 additional participants, resulting in 403 valid CSF samples.

Genotyping and quality control. Single nucleotide polymorphism (SNP) genotyping for more than 620,000 target SNPs was completed on all ADNI participants using the following protocol.[13] A total of 7 mL of blood was taken in EDTA-containing Vacutainer tubes from all participants and genomic DNA was extracted using the QIAamp DNA Blood Maxi Kit (Qiagen, Inc., Valencia, CA) following the manufacturer's protocol. Lymphoblastoid cell lines were established by transforming B lymphocytes with Epstein-Barr virus.[14] Genomic DNA samples were analyzed using the Human 610-Quad BeadChip (Illumina, Inc., San Diego, CA) according to the manufacturer's protocols (Infinium HD Assay; Super Protocol Guide; rev. A, May 2008). Before initiation of the assay, 50 ng of genomic DNA from each sample was examined qualitatively on a 1%

Table 1 Demographic information and summary statistics for CSF biomarkers

Baseline diagnosis	AD	MCI	HC	Total
No. of subjects	96	176	102	374
M/F	56/40	118/58	53/49	227/147
Baseline age, y, mean ± SD	75.05 ± 7.85	74.59 ± 7.59	75.92 ± 5.16	75.07 ± 7.09
APOE (ϵ4−/ϵ4+)	31/65	79/97	78/24	188/186
$A\beta_{1-42}$,[a] mean ± SD	143.64 ± 41.40	163.55 ± 53.76	205.06 ± 55.63	169.76 ± 56.26
t-tau,[a] mean ± SD	118.11 ± 53.61	98.30 ± 46.72	71.35 ± 31.23	96.03 ± 48.11
$p\text{-tau}_{181p}$,[a] mean ± SD	40.48 ± 18.49	34.90 ± 16.71	25.48 ± 15.14	33.76 ± 17.64
t-tau/$A\beta_{1-42}$,[a] mean ± SD	0.89 ± 0.45	0.69 ± 0.16	0.40 ± 0.28	0.66 ± 0.44
$p\text{-tau}_{181p}/A\beta_{1-42}$,[a] mean ± SD	0.31 ± 0.16	0.25 ± 0.16	0.15 ± 0.14	0.24 ± 0.16

Abbreviations: $A\beta_{1-42}$ = amyloid-β 1–42 peptide; AD = Alzheimer disease; HC = healthy controls; MCI = mild cognitive impairment; $p\text{-tau}_{181p}$ = tau phosphorylated at the threonine 181; t-tau = total tau.
[a] Analysis of variance of 3 diagnostic groups and post hoc pairwise *t* tests after Bonferroni correction were at $p < 0.05$ for all comparisons of each phenotype.

Tris-acetate-EDTA agarose gel to check for degradation. Degraded DNA samples were excluded from further analysis. Samples were quantitated in triplicate with PicoGreen® reagent (Invitrogen, Carlsbad, CA) and diluted to 50 ng/μL in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH 8.0). A total of 200 ng of DNA was then denatured, neutralized, and amplified for 22 hours at 37°C (this is termed the MSA1 plate). The MSA1 plate was fragmented with FMS reagent (Illumina) at 37°C for 1 hour, precipitated with 2-propanol, and incubated at 4°C for 30 minutes. The resulting blue precipitate was resuspended in RA1 reagent (Illumina) at 48°C for 1 hour. Samples were then denatured (95°C for 20 minutes) and immediately hybridized onto the BeadChips at 48°C for 20 hours. The BeadChips were washed and subjected to single base extension and staining. Finally, the BeadChips were coated with XC4 reagent (Illumina), desiccated, and imaged on the BeadArray Reader (Illumina). The Illumina BeadStudio 3.2 software was used to generate SNP genotypes from bead intensity data.

To restrict the present analysis to non-Hispanic Caucasians, these subjects were identified using ethnic and racial information from the clinical database. Among 403 subjects whose CSF sample passed the quality control, explained above, 374 were non-Hispanic Caucasian individuals with genotype data.

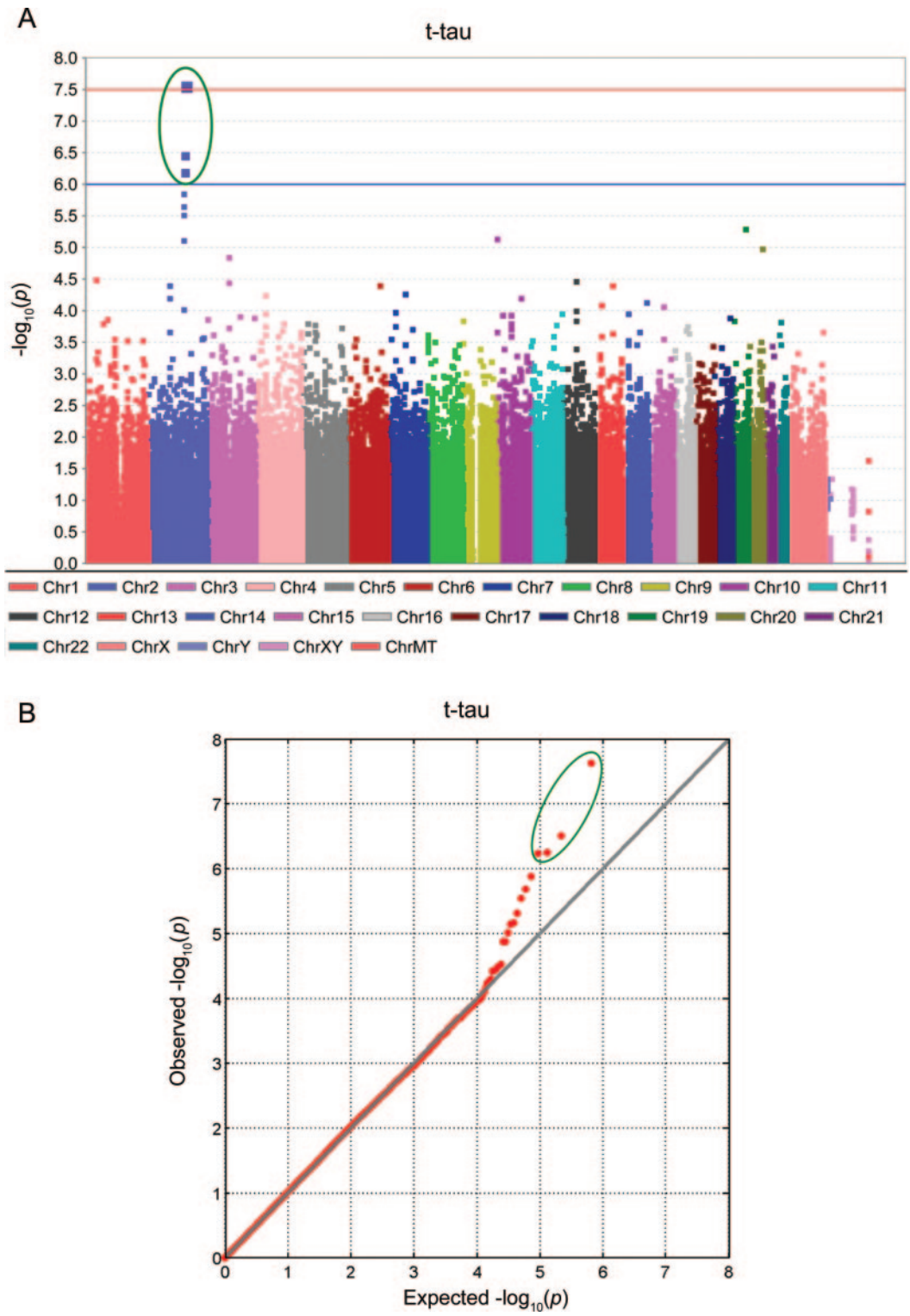
Standard QC assessment was performed on these 374 samples using the PLINK software package (http://pngu.mgh.harvard.edu/~purcell/plink/), release v 1.07,[15] as described previously.[12] Given the smaller size of the current sample (374) as compared to previous analyses, only SNPs with a minor allele frequency (MAF) greater than 20% were retained for analysis. This more stringent threshold was chosen to reduce the likelihood of false-positive results in the context of modest sample size. At the same time, elimination of relatively rare markers reduced the severity of the multiple comparison correction which in turn enhanced statistical power. After the QC procedure, all 374 participants remained in the analysis but only 322,557 out of 620,903 markers, including 2 *APOE* SNPs (rs429358, rs7412), were considered for analysis. The overall genotyping rate for the remaining dataset was ≥99.5%.

APOE genotype is an established risk factor for LOAD.[16] The 2 previously identified *APOE* SNPs (rs429358, rs7412) that define the ϵ2/ϵ3/ϵ4 alleles important for AD susceptibility were not available on the Illumina array. These SNPs were genotyped by PCR amplification followed by *Hha*I restriction enzyme digestion and Metaphor Gel and were available in the ADNI database.[17] They were added to ADNI genotype data based on the reported *APOE* ϵ2/ϵ3/ϵ4 status before the assessment of sample quality. One SNP (rs7412) was removed due to the low MAF (<20%). Also, *APOE* ϵ4 status was included in the statistical analysis as a dichotomous variable with ϵ4-positive classification indicating 1 or 2 ϵ4 alleles.

Statistical analyses. To examine the main effect of each SNP on the 5 CSF biomarkers, a separate GWAS was performed for each of the quantitative CSF variables using PLINK. We tested the additive genetic model, i.e., dose-dependent effect of the minor allele. Baseline age and sex had no significant influence on any of the CSF biomarkers and hence they were not included in the model. *APOE* ϵ4 status was entered as a covariate for analyses of other SNPs. To address the issue of multiple testing, Bonferroni correction was applied and SNPs with corrected $p < 0.01$ (uncorrected $p < 3.10 \times 10^{-8}$, i.e., 0.01/322,557 markers) were considered genome-wide significant. Manhattan and linkage disequilibrium (LD) plots were generated in Haploview v4.2 (http://www.broadinstitute.org/haploview/haploview)[18] and haplotype blocks were defined by 95% confidence bounds on D′. A block was created if 95% of informative comparisons were in strong LD.[19] Heat maps and hierarchical clustering[12,20] were employed for visualization of multiple statistical results and selecting important groups of genotypes and phenotypes for further analysis.

RESULTS Table 1 shows the demographic information for the final set of 374 non-Hispanic Caucasian participants and summary statistics for the 5 CSF biomarkers (3 baseline measurements and 2 ratios). The obtained genomic inflation factors[15] of all CSF biomarker associations (between 1.001 and 1.018) indicated low risk of confounding due to population stratification. All 5 CSF biomarkers were different ($p < 0.05$, after Bonferroni correction) across the 3 diagnostic groups. Figure 1 displays Manhattan (figure 1A) and quantile-quantile plots (figure 1B) of t-tau. Four SNPs in the regions of *APOE*, *LOC100129500*, *TOMM40*, and *EPC2* reached genome-wide significance after Bonferroni adjust-

Figure 1 Manhattan plot (A) and quantile-quantile plot (B) of total tau



Genomic inflation factor (based on median χ^2) is 1.01. In the Manhattan plot, the blue and red lines represent the $-\log_{10}(10^{-6})$ and $-\log_{10}(3.10 \times 10^{-8})$ threshold levels.

ment (corrected $p < 0.01$). Table 2 lists all SNPs whose p values reached the level of $p < 10^{-6}$ for any CSF biomarker and their annotation information. Four SNPs in the region of the *EPC2* gene, listed in table 2, were associated with t-tau at the threshold level of $p < 10^{-6}$. Figure e-1 (on the *Neurology*® Web site at www.neurology.org) shows heat maps of association pattern between SNPs and CSF biomarkers without (figure e-1A) and with (figure e-1B) *APOE* ϵ4 status as a covariate. rs429358 SNP (*APOE*)

Table 2 SNPs associated with CSF biomarkers at uncorrected *p* threshold of 10^{-6}

SNP	Chr	Position*	Closest RefSeq gene	Location relative to gene	Gene name	Associated phenotypes
rs2121433	2	149274330	*EPC2*	12.7 kb downstream	Enhancer of polycomb homolog 2	t-tau[b]
rs1374441	2	149275095	*EPC2*	13.5 kb downstream		t-tau[b]
rs4499362[a]	2	149284866	*EPC2*	23.3 kb downstream		t-tau[a,c]
						t-tau/$A\beta_{1\text{-}42}$[c]
rs10171238	2	149285199	*EPC2*	23.6 kb downstream		t-tau[c]
						t-tau/$A\beta_{1\text{-}42}$[c]
rs157580	19	50087106	*TOMM40*	Intron	Translocase of outer mitochondrial membrane 40 homolog	$A\beta_{1\text{-}42}$[b]
rs2075650[a]	19	50087459	*TOMM40*	Intron		$A\beta_{1\text{-}42}$[a,b]
						p-tau_{181p}/ $A\beta_{1\text{-}42}$[a,b]
						t-tau/$A\beta_{1\text{-}42}$[a,b]
rs429358[a]	19	50103781	*APOE*	Exon	Apolipoprotein E	$A\beta_{1\text{-}42}$[a,b]
						p-tau_{181p}[b]
						p-tau_{181p}/$A\beta_{1\text{-}42}$[a,b]
						t-tau/$A\beta_{1\text{-}42}$[a,b]
rs439401[a]	19	50106291	*LOC100129500*	Intron		$A\beta_{1\text{-}42}$[a,b]
rs7364180	22	40548802	*CCDC134*	Intron	Coiled-coil domain containing 134	$A\beta_{1\text{-}42}$[b]

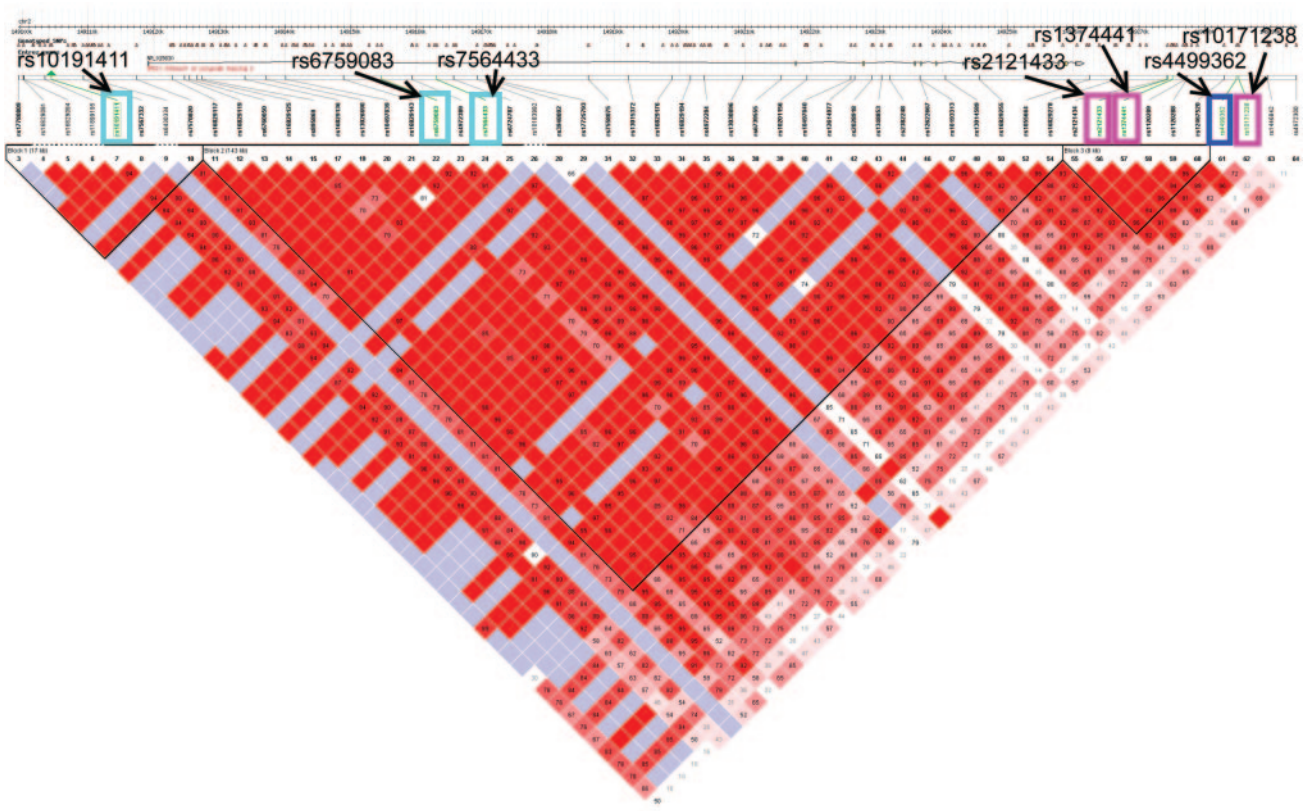
Abbreviations: $A\beta_{1\text{-}42}$ = amyloid-β 1–42 peptide; p-tau_{181p} = tau phosphorylated at the threonine 181; SNP = single nucleotide polymorphism; t-tau = total tau.
[a] SNPs and associated CSF phenotypes were at Bonferroni-corrected *p* threshold of 0.01.
[b] Significant without *APOE* adjustment.
[c] Significant with and without *APOE* adjustment.
* Positions are based on Genome Build 36.3.

was associated (corrected $p < 0.01$) with $A\beta_{1\text{-}42}$, t-tau/$A\beta_{1\text{-}42}$, and p-tau_{181p}/$A\beta_{1\text{-}42}$. rs2075650 (*TOMM40*) was associated with $A\beta_{1\text{-}42}$, t-tau/$A\beta_{1\text{-}42}$, and p-tau_{181p}/$A\beta_{1\text{-}42}$. rs439401 (*LOC100129500*) was associated with $A\beta_{1\text{-}42}$. rs4499362 (*EPC2*) was associated with t-tau. SNPs that did not reach genome-wide significance and whose uncorrected *p* values are between 10^{-6} and 10^{-5} are listed in table e-1 and were further investigated for indication of relevance to AD. LD among SNPs in the region of the *EPC2* gene (149095–149295 kb, HapMap v3.0 release 27 panel CEU) is shown in figure 2.

All genome-wide significant SNPs were analyzed further to examine possible interactions between baseline diagnosis and genotypes on associated CSF biomarkers. However, the cell sizes of one or more subgroups, defined by diagnosis and each SNP marker, were small, as indicated in figure 3. Therefore, no significant interactions were detected. However, the linear trend in mean CSF levels was observed within and across all diagnostic groups for all retained SNPs. Figure 3 shows the mean ± standard error for CSF biomarkers as a function of genotype and baseline diagnosis for the most significant SNP within each identified gene.

DISCUSSION A GWAS was performed on 374 ADNI CSF samples to investigate the influence of genetic variation on CSF biomarkers, $A\beta_{1\text{-}42}$, t-tau, and p-tau_{181p}. The use of quantitative traits in GWAS has been shown to have increased power over case-control designs.[21] The use of CSF biomarkers as quantitative traits in this study enabled us to identify a novel AD candidate gene in addition to examining the influence of well-known AD genes on CSF biomarker levels. Four SNPs in the regions of *APOE, LOC100129500, TOMM40*, and *EPC2* showed evidence of genome-wide association with one or more CSF biomarkers. *APOE* (rs429358) and *TOMM40* (rs2075650) are significantly associated with $A\beta_{1\text{-}42}$ and t-tau/$A\beta_{1\text{-}42}$, but not with t-tau. The significant association of these SNPs with t-tau/$A\beta_{1\text{-}42}$ seems to have been driven by $A\beta_{1\text{-}42}$. *APOE* is one of the most robust risk factors for LOAD.[22] The presence of one or more *APOE* ϵ4 alleles was associated with decreased levels of $A\beta_{1\text{-}42}$ in AD and healthy controls[10] and greater reduction in CSF $A\beta_{1\text{-}42}$ levels was observed with increasing number of *APOE* ϵ4 alleles in cognitively normal subjects[23] and in the ADNI cohort.[1] *LOC100129500* is a hypothetical gene that overlaps the *APOE* and *APOC1* genes. rs439401 lies

Figure 2 Linkage disequilibrium (LD) among single nucleotide polymorphisms (SNPs) in the region of *EPC2* at chromosome 2q23.1



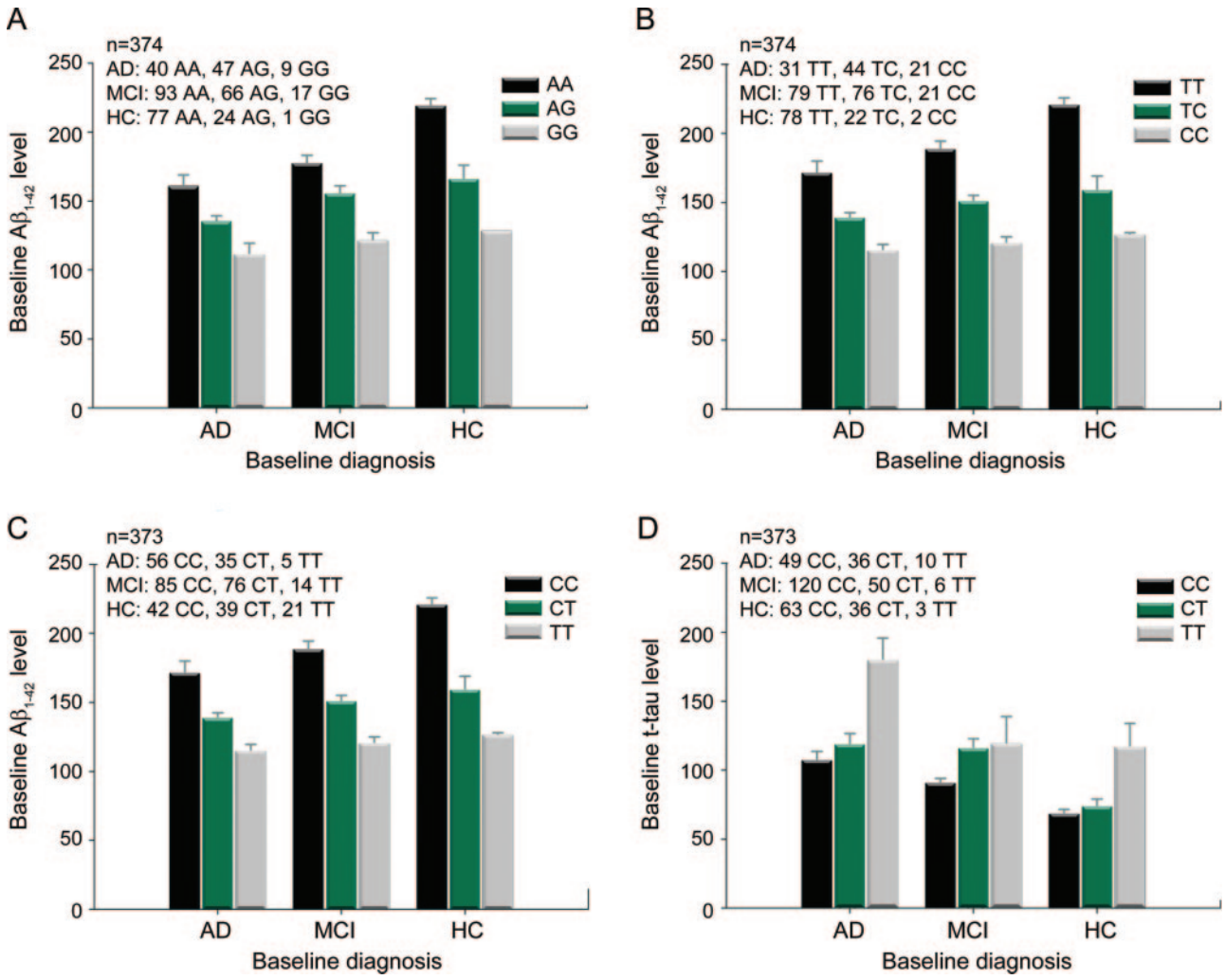
LD plot, showing D′, was created by Haploview v4.2 on chromosome 2 (149095–149295 kb, HapMap v3.0 release 27 panel CEU). SNPs, highlighted by blue, pink, and cyan rectangles, were at uncorrected *p* values less than 3.1×10^{-8}, 10^{-6}, 10^{-5}.

in the intron of this gene (figure e-2) and has been studied for the association with LOAD.[24] *TOMM40*, adjacent and approximately 15 kb upstream to *APOE*, was recently identified as a candidate gene for AD[17,25,26] and is associated with multiple neuroimaging phenotypes.[12] One exploratory study of healthy subjects without dementia examined the association between CSF apoE levels and SNPs in the region surrounding *APOE*.[27] Although *APOE* genotype did not predict CSF apoE level, these authors did find a strong association signal between several *TOMM40* SNPs and CSF apoE. The $A\beta_{1\text{-}42}$, t-tau, and p-tau_{181p} CSF biomarkers examined here were not investigated in the previous study[27] or analyzed by GWAS. A novel finding in the present study is the association of *EPC2* with t-tau level. *EPC2* (enhancer of polycomb homolog 2) belongs to the polycomb protein family and is involved in the formation of heterochromatin.[28] Dysregulation of epigenetic mechanisms and chromatin remodeling may play a role in neurodegenerative and cognitive disorders such as AD.[29] *EPC2* is one of the genes deleted in 2q23.1 microdeletion syndrome leading to severe mental retardation, short stature, and epilepsy and therefore *EPC2* may be causally involved in mental retardation.[30] The functional role of *EPC2* has not been fully characterized and its association with AD or neurodegeneration has not been previously reported. Although only one *EPC2* SNP (rs4499362) reached genome-wide significance, many other SNPs in this region reached the uncorrected *p* threshold levels of 10^{-6} or 10^{-5}. All SNPs, highlighted with colored rectangles in figure 3, are within 3 haplotype blocks (represented by black triangles), encompassing the *EPC2* gene. The possible role of this gene in AD pathogenesis warrants detailed investigation.

In addition, we found that one SNP (rs7364180) in *CCDC134* was associated with $A\beta_{1\text{-}42}$ (uncorrected $p < 10^{-6}$). This gene is associated with transcriptional activity of Elk1 and phosphorylation of Erk and JNK/SAPK,[31] but direct association of this gene with AD or neurodegeneration has also not been studied.

Among genes listed in table e-1, association of several genes with AD were previously studied, including *ABCG2* (ATP-binding cassette, subfamily G [WHITE], member 2), *NFATC4* (nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4), and *SREBF2* (sterol regulatory element binding transcription factor 2).[32-34] These

Figure 3 Mean CSF biomarker levels as a function of baseline diagnosis and genotype



Mean and standard errors of amyloid-β 1-42 peptide ($A\beta_{1-42}$) and total tau (t-tau) are shown for groups defined by baseline diagnosis and associated single nucleotide polymorphisms reaching genome-wide significance. Baseline $A\beta_{1-42}$ CSF level by diagnosis group and genotype: (A) *TOMM40* (rs2075650), (B) *APOE* (rs429358), (C) *LOC100129500* (rs439401), (D) *EPC2* (rs4499362). AD = Alzheimer disease; HC = healthy controls; MCI = mild cognitive impairment.

genes have not previously been associated with CSF biomarkers.

ABCG2 was found upregulated in AD brains and hypothesized as a gatekeeper at the blood–brain barrier for $A\beta_{1-40}$ peptide[32] and this gene is expressed in brain endothelial cell blood vessels[35] and the developing human CNS.[36] Morphologic changes occurring around amyloid plaques in AD was studied and it was found that an active form of phosphatase calcineurin and *NFATC4* was enriched in the nuclear fraction from the cortex of patients with AD.[33] This gene is expressed in numerous regions in the human brain including the hippocampus[37] and it was reported that neurotrophin-mediated synaptic plasticity played a role in learning and memory.[38]

Overexpression of *SREBF2* in cortical neurons of transgenic mice was associated with mitochondrial cholesterol accumulation, increasing susceptibility to $A\beta_{1-42}$ induced oxidative stress and release of apoptogenic proteins[39] and this gene was previously hypothesized as a genetic factor involved in the pathogenesis of vascular dementia.[40]

Although relatively large for a CSF study, a limitation of this report is the modest sample size for a GWAS, which precluded stratified analyses for each diagnostic group or as a function of biomarker results. Hopefully, larger studies in the future will be able to incorporate such analyses. In ADNI-2, all subjects will undergo lumbar punctures for CSF data collection, which will increase statistical power, and participants with early MCI will be included to broaden the sampling of prodromal stages of disease. In addition, RNA will be collected from peripheral blood so that a more dynamic picture of the longitudinal relationship of CSF abnormalities and gene expression should become available. We applied a more stringent MAF threshold (MAF $>$ 0.20) and a Bonferroni-corrected p value < 0.01. Due to this, we

may have excluded less common SNPs associated with CSF biomarker levels. Another limitation is that although we applied a stringent correction of individual p values for each CSF phenotype, we did not apply a global or family-wise p value correction for all 5 association tests. Since the CSF markers and derived ratios are not independent and the genetic markers are also not independent, we determined that additional Bonferroni corrections would be overly stringent[21] and likely to result in false-negative errors.

Replication studies with independent, larger samples will be important to confirm these findings. ADNI plans to substantially expand the available sample and to include CSF and DNA collection on all new participants. Future directions also include looking at the interaction of SNP and diagnosis and gene or pathway-based analyses to further investigate associations with CSF biomarker levels. Longitudinal GWAS are also planned with regard to CSF changes and clinical progression. It will be important in future studies to assess if a panel of genetic markers can be combined with CSF analytes to better predict longitudinal outcomes or response to emergent therapeutics.

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