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

S. Kim, PhD*
S. Swaminathan, BTech*
L. Shen, PhD
S.L. Risacher, BS
K. Nho, PhD
T. Foroud, PhD
L.M. Shaw, PhD
J.Q. Trojanowski, MD, PhD
S.G. Potkin, MD
M.J. Huentelman, PhD
D.W. Craig, PhD
B.M. DeChairo, PhD
P.S. Aisen, MD
R.C. Petersen, MD
M.W. Weiner, MD
A.J. Saykin, PsyD
For the Alzheimer's Disease Neuroimaging Initiative

Address correspondence and reprint requests to Dr. Andrew J. Saykin, Center for Neuroimaging, Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 950 West Walnut Street, R2 E124, Indianapolis, IN 46202
asaykin@iupui.edu

Supplemental data at www.neurology.org

ABSTRACT

Objectives: CSF levels of $A\beta_{1-42}$, 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 ($A\beta_{1-42}$, t-tau, p-tau_{181p}, p-tau_{181p}/$A\beta_{1-42}$, and t-tau/$A\beta_{1-42}$).

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

$A\beta_{1-42}$ = 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 ($A\beta_{1-42}$), 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] $A\beta_{1-42}$ is decreased and t-tau and p-tau_{181p} are increased in the CSF of patients with AD.[4] Baseline $A\beta_{1-42}$ has been shown to be a good predictor of the 12-month change in

e-Pub ahead of print on December 1, 2010, at www.neurology.org.

*These authors contributed equally to this work.

From the Center for Neuroimaging, Department of Radiology and Imaging Sciences (S.K., S.S., L.S., S.L.R., K.N., T.F., A.J.S.), Center for Computational Biology and Bioinformatics (S.K., L.S., T.F., A.J.S.), and Department of Medical and Molecular Genetics (S.S., T.F., A.J.S.), Indiana University School of Medicine, Indianapolis; Regenstrief Institute (K.N.), Indianapolis, IN; Department of Laboratory Medicine (L.M.S., J.Q.T.), University of Pennsylvania School of Medicine, Philadelphia; Department of Psychiatry and Human Behavior (S.G.P.), University of California, Irvine; The Translational Genomics Research Institute (M.J.H., D.W.C.), Phoenix, AZ; Neuroscience (B.M.D.), Molecular Medicine, Pfizer Global R&D, New London, CT; Department of Neurosciences (P.S.A.), University of California, San Diego; Department of Neurology (R.C.P.), Mayo Clinic College of Medicine, Rochester, MN; Department of Radiology, Medicine and Psychiatry (M.W.W.), University of California, San Francisco; and Department of Veterans Affairs Medical Center (M.W.W.), San Francisco, CA.

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (www.loni.ucla.edu/ADNI). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. For a complete list of investigators involved in ADNI, see http://www.loni.ucla.edu/ADNI/Collaboration/ADNI_Authorship_list.pdf.

Study funding: Supported by the NIH (AG024904, AG010129, AG030514, and AG032984), the Dana Foundation, and the Alzheimer's Disease Genetics Consortium. Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (NIH U01 AG024904).

Disclosure: Author disclosures are provided at the end of the article.

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.

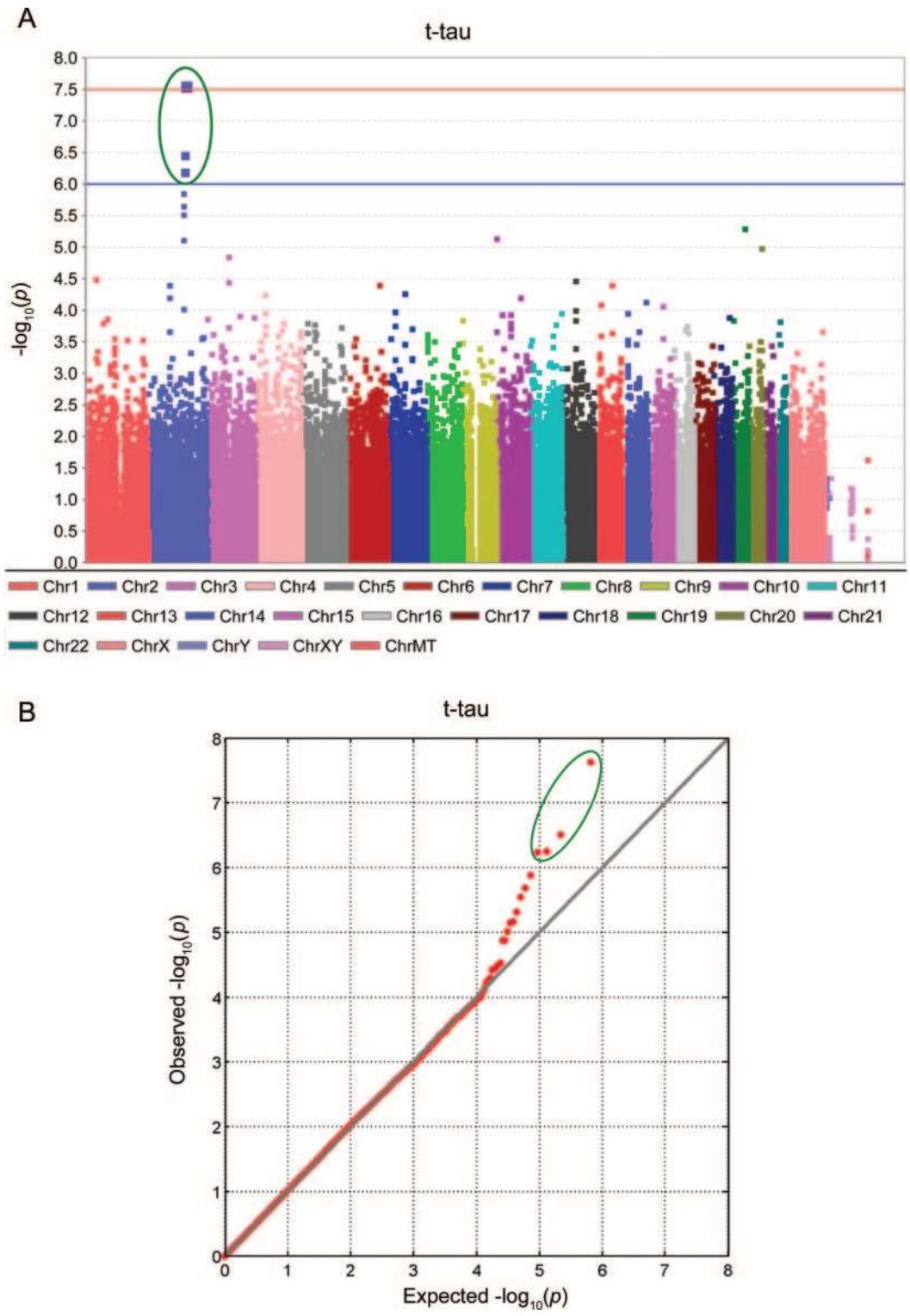
Standard QC assessment was performed on these 374 samples using the PLINK software package (http://pngu.mgh.harvard.edu/~purcell/plink/), release v 1.07,[15] as described previously.[12] Given the smaller size of the current sample (374) as compared to previous analyses, only SNPs with a minor allele 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/$A\beta_{1-42}$[c]
rs10171238	2	149285199	*EPC2*	23.6 kb downstream		t-tau[c]
						t-tau/$A\beta_{1-42}$[c]
rs157580	19	50087106	*TOMM40*	Intron	Translocase of outer mitochondrial membrane 40 homolog	$A\beta_{1-42}$[b]
rs2075650[a]	19	50087459	*TOMM40*	Intron		$A\beta_{1-42}$[a,b]
						p-tau_{181p}/ $A\beta_{1-42}$[a,b]
						t-tau/$A\beta_{1-42}$[a,b]
rs429358[a]	19	50103781	*APOE*	Exon	Apolipoprotein E	$A\beta_{1-42}$[a,b]
						p-tau_{181p}[b]
						p-tau_{181p}/$A\beta_{1-42}$[a,b]
						t-tau/$A\beta_{1-42}$[a,b]
rs439401[a]	19	50106291	*LOC100129500*	Intron		$A\beta_{1-42}$[a,b]
rs7364180	22	40548802	*CCDC134*	Intron	Coiled-coil domain containing 134	$A\beta_{1-42}$[b]

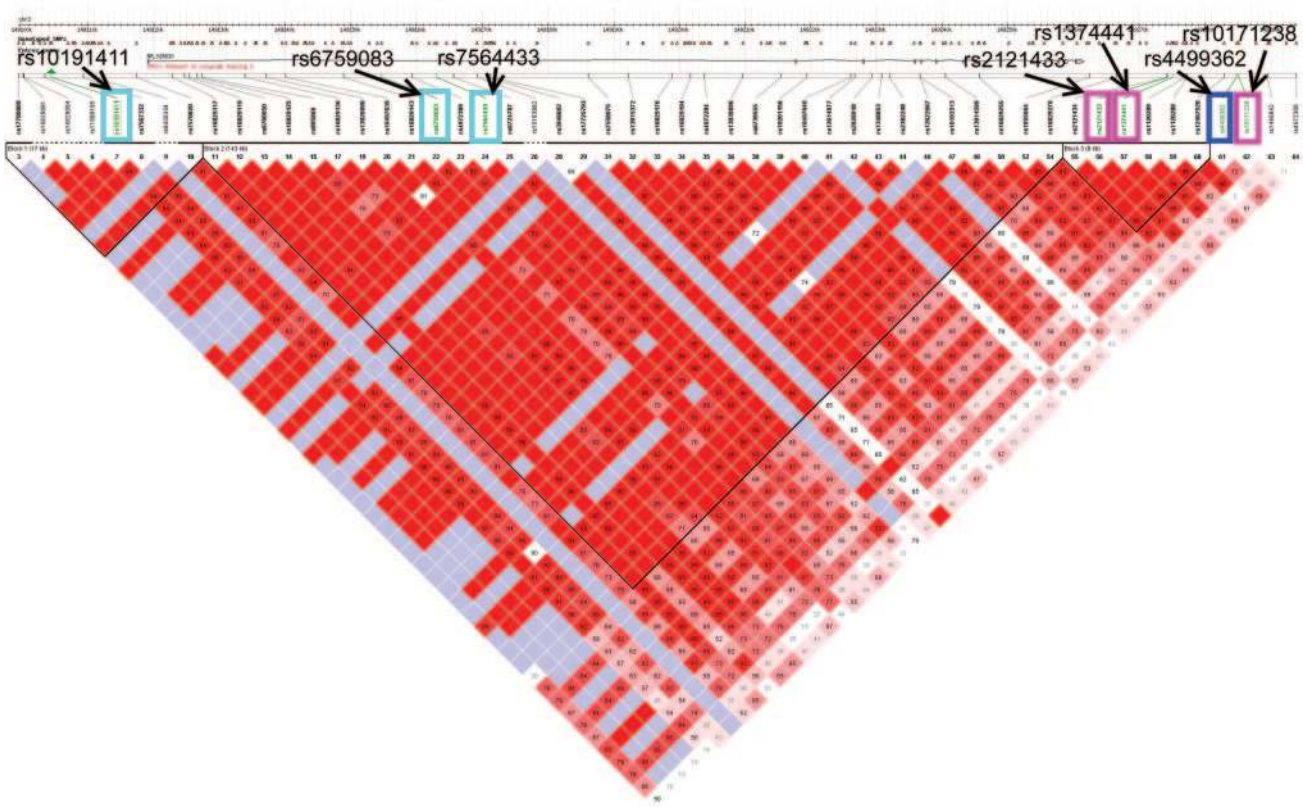
Abbreviations: $A\beta_{1-42}$ = amyloid-β 1–42 peptide; p-tau_{181p} = tau phosphorylated at the threonine 181; SNP = single nucleotide polymorphism; t-tau = total tau.
[a] SNPs and associated CSF phenotypes were at Bonferroni-corrected *p* threshold of 0.01.
[b] Significant without *APOE* adjustment.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

was associated (corrected $p < 0.01$) with $A\beta_{1-42}$, t-tau/$A\beta_{1-42}$, and p-tau_{181p}/$A\beta_{1-42}$. rs2075650 (*TOMM40*) was associated with $A\beta_{1-42}$, t-tau/$A\beta_{1-42}$, and p-tau_{181p}/$A\beta_{1-42}$. rs439401 (*LOC100129500*) was associated with $A\beta_{1-42}$. 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, $A\beta_{1-42}$, 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 $A\beta_{1-42}$ and t-tau/$A\beta_{1-42}$, but not with t-tau. The significant association of these SNPs with t-tau/$A\beta_{1-42}$ seems to have been driven by $A\beta_{1-42}$. *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 $A\beta_{1-42}$ in AD and healthy controls[10] and greater reduction in CSF $A\beta_{1-42}$ 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



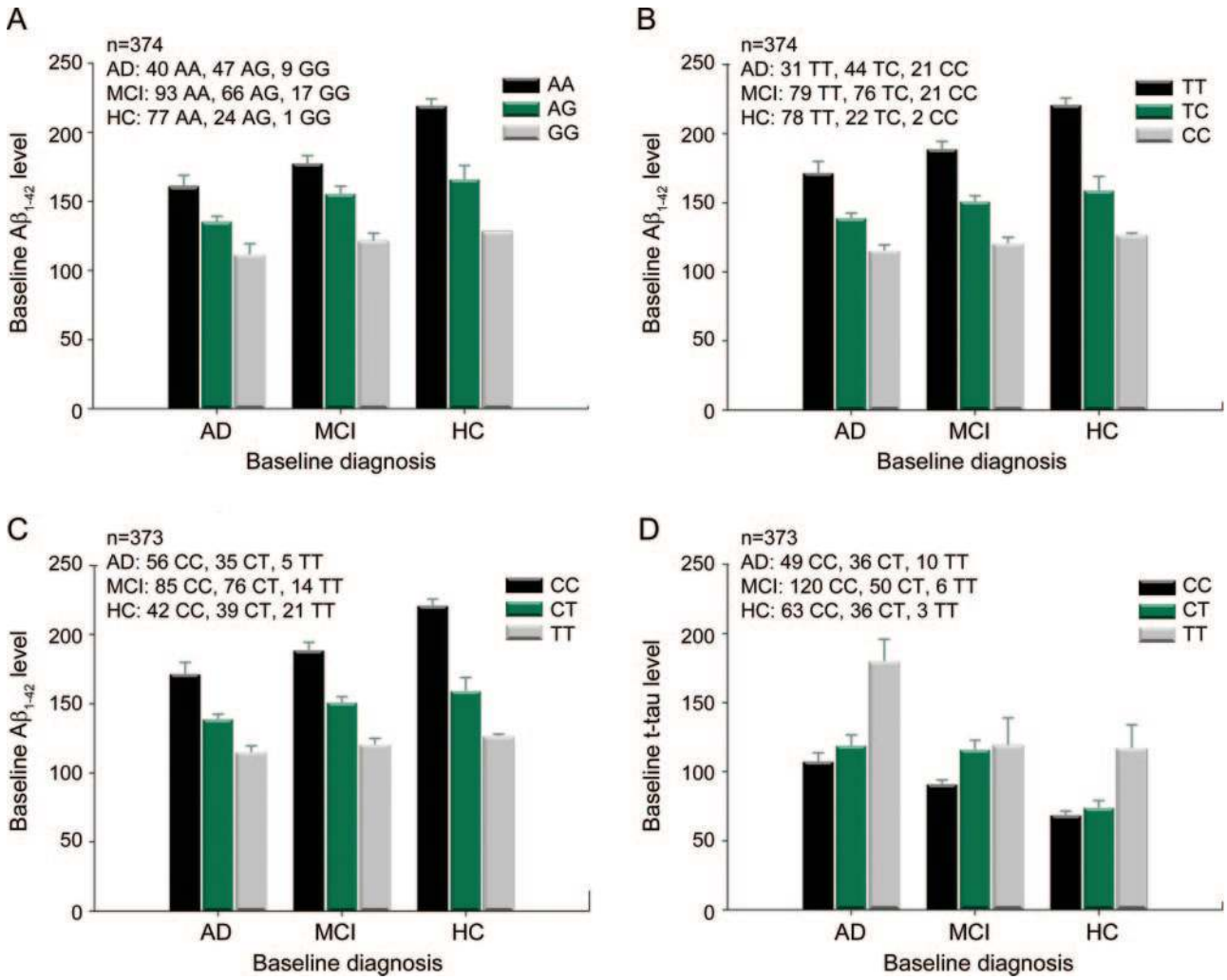
LD plot, showing D′, was created by Haploview v4.2 on chromosome 2 (149095–149295 kb, HapMap v3.0 release 27 panel CEU). SNPs, highlighted by blue, pink, and cyan rectangles, were at uncorrected *p* values less than 3.1×10^{-8}, 10^{-6}, 10^{-5}.

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.

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Sungeun Kim.

COINVESTIGATORS

Clifford R. Jack, Jr., MD (Mayo Clinic, Rochester, Core PI MRI, Executive Committee); William Jagust, MD (UC Berkeley, Core PI PET, Executive Committee); Arthur W. Toga, PhD (UCLA, Core PI Informatics, Executive Committee); Laurel Beckett, PhD (UC Davis, Core PI Biostatistics, Executive Committee); Anthony Gamst, PhD (UC San Diego, Clinical Informatics and Operations, Executive Committee); Holly Soares, PhD (Pfizer, Chair, Industry Scientific Advisory Board [iSAB]); Robert C. Green, MD, MPH (Boston University, Chair, Data and Publication Committee [DPC]); Tom Montine, MD, PhD (University of Washington, Chair, Resource Allocation Review Committee); Ronald G. Thomas, PhD (UC San Diego, Clinical Informatics and Operations); Michael Donohue, PhD (UC San Diego, Clinical Informatics and Operations); Sarah Walter, MSc (UC San Diego, Clinical Informatics and Operations); Anders Dale, PhD (UC San Diego, MRI); Matthew Bernstein, PhD (Mayo Clinic, Rochester, MRI); Joel Felmlee, PhD (Mayo Clinic, Rochester, MRI); Nick Fox, MD (University of London, MRI); Paul Thompson, PhD (UCLA School of Medicine, MRI); Norbert Schuff, PhD (UCSF MRI, MRI); Gene Alexander, PhD (Banner Alzheimer's Institute, MRI); Charles DeCarli, MD (UC Davis, MRI); Dan Bandy, MS, CNMT (Banner Alzheimer's Institute, PET); Kewei Chen, PhD (Banner Alzheimer's Institute, PET); Robert A. Koeppe, PhD (University of Michigan, PET); Norm Foster, MD (University of Utah, PET); Eric M. Reiman, MD (Banner Alzheimer's Institute, PET); Chet Mathis, MD (University of Pittsburgh, PET); John Morris, MD (Washington University, St. Louis, Neuropathology, Executive Committee); Nigel J. Cairns, PhD, MRCPath (Washington University, St. Louis, Neuropathology); Lisa Taylor-Reinwald, BA, HTL (ASCP) (Washington University, St. Louis, Neuropathology); Virginia M.-Y. Lee, PhD, MBA (UPenn School of Medicine, Biomarkers); Magdalena Korecka, PhD (UPenn School of Medicine, Biomarkers); Karen Crawford (UCLA, Informatics); Scott Neu, PhD (UCLA, Informatics); Danielle Harvey, PhD (UC Davis, Biostatistics); John Kornak, PhD (UC Davis, Biostatistics); Neil Buckholtz, PhD (National Institute on Aging/NIH); Jeffrey Kaye, MD (Oregon Health and Science University, site investigator); Sara Dolen, BS (Oregon Health and Science University, site investigator); Joseph Quinn, MD (Oregon Health and Science University, site investigator); Lon Schneider, MD (University of Southern California, site investigator); Sonia Pawluczyk, MD (University of Southern California, site investigator); Bryan M. Spann, DO, PhD (University of Southern California, site investigator); James Brewer, MD, PhD (University of California–San Diego, site investigator); Helen Vanderswag, RN (University of California–San Diego, site investigator); Judith L. Heidebrink, MD, MS (University of Michigan, site investigator); Joanne L. Lord, LPN, BA, CCRC (University of Michigan, site investigator); Ronald Petersen, MD, PhD (Mayo Clinic, Rochester, site investigator); Kris Johnson, RN (Mayo Clinic, Rochester, site investigator); Rachelle S. Doody, MD, PhD (Baylor College of Medicine, site investigator); Javier Villanueva-Meyer, MD (Baylor College of Medicine, site investigator); Munir Chowdhury, MS (Baylor College of Medicine, site investigator); Yaakov Stern, PhD (Columbia University Medical Center, site investigator); Lawrence S. Honig, MD, PhD (Columbia University Medical Center, site investigator); Karen L. Bell, MD (Columbia University Medical Center, site investigator); John C. Morris, MD (Washington University, St. Louis, site investigator); Mark A. Mintun, MD (Washington University, St. Louis, site investigator); Stacy Schneider, APRN, BC, GNP (Washington University, St. Louis, site investigator); Daniel Marson, JD, PhD (University of Alabama–Birmingham, site investigator); Randall Griffith, PhD, ABPP (University of Alabama–Birmingham, site investigator); David Clark, MD (University of Alabama–Birmingham, site investigator); Hillel Grossman, MD (Mount Sinai School of Medicine, site investigator); Cheuk Tang, PhD (Mount Sinai School of Medicine, site investigator); George Marzloff, BS (Mount Sinai School of Medicine, site investigator); Leyla deToledo-Morrell, PhD (Rush University Medical Center, site investigator); Raj C. Shah, MD (Rush University Medical Center, site investigator); Ranjan Duara, MD (Wein Center, site investigator); Daniel Varon, MD (Wein Center, site investigator); Peggy Roberts, CNA (Wein Center, site investigator); Marilyn S. Albert, PhD (Johns Hopkins University, site investigator); Julia Pedroso, MA (Johns Hopkins University, site investigator); Jaimie Toroney, BA (Johns Hopkins University, site investigator); Henry Rusinek, PhD (New York University, site investigator); Mony J. de Leon, EdD (New York University, site investigator); Susan M. De Santi, PhD (New York University, site investigator); P. Murali Doraiswamy, MD (Duke University Medical Center, site investigator); Jeffrey R. Petrella, MD (Duke University Medical Center, site investigator); Marilyn Aiello, BS (Duke University Medical Center, site investigator); Christopher M. Clark, MD (University of Pennsylvania, site investigator); Cassie Pham, BS (University of Pennsylvania, site investigator); Jessica Nunez (University of Pennsylvania, site investigator); Charles D. Smith, MD (University of Kentucky, site investigator); Curtis A. Given II, MD (University of Kentucky, site investigator); Peter Hardy, PhD (University of Kentucky, site investigator); Oscar L. Lopez, MD (University of Pittsburgh, site investigator); MaryAnn Oakley, MA (University of Pittsburgh, site investigator); Donna M. Simpson, CRNP, MPH (University of Pittsburgh, site investigator); M. Saleem Ismail, MD (University of Rochester Medical Center, site investigator); Connie Brand, RN (University of Rochester Medical Center, site investigator); Jennifer Richard, BA (University of Rochester Medical Center, site investigator); Ruth A. Mulnard, DNSc, RN, FAAN (University of California, Irvine, site investigator); Gaby Thai, MD (University of California, Irvine, site investigator); Catherine McAdams-Ortiz, MSN, RN, A/GNP (University of California, Irvine, site investigator); Ramon Diaz-Arrastia, MD, PhD (University of Texas Southwestern Medical School, site investigator); Kristen Martin-Cook, MA (University of Texas Southwestern Medical School, site investigator); Michael DeVous, PhD (University of Texas Southwestern Medical School, site investigator); Allan I. Levey, MD, PhD (Emory University, site investigator); James J. Lah, MD, PhD (Emory University, site investigator); Janet S. Cellar, RN, MSN (Emory University, site investigator); Jeffrey M. Burns, MD (University of Kansas, Medical Center, site investigator); Heather S. Anderson, MD (University of Kansas, Medical Center, site investigator); Mary M. Laubinger, MPA, BSN (University of Kansas, Medical Center, site investigator); George Bartzokis, MD (University of California, Los Angeles, site investi-

gator); Daniel H.S. Silverman, MD, PhD (University of California, Los Angeles, site investigator); Po H. Lu, PsyD (University of California, Los Angeles, site investigator); Neill R. Graff-Radford MBBCH, FRCP (London) (Mayo Clinic, Jacksonville, site investigator); Francine Parfitt, MSH, CCRC (Mayo Clinic, Jacksonville, site investigator); Heather Johnson, MLS, CCRP (Mayo Clinic, Jacksonville, site investigator); Martin Farlow, MD (Indiana University, site investigator); Scott Herring, RN (Indiana University, site investigator); Ann M. Hake, MD (Indiana University, site investigator); Christopher H. van Dyck, MD (Yale University School of Medicine, site investigator); Martha G. MacAvoy, PhD (Yale University School of Medicine, site investigator); Amanda L. Benincasa, BA (Yale University School of Medicine, site investigator); Howard Chertkow, MD (McGill University, Montreal-Jewish General Hospital, site investigator); Howard Bergman, MD (McGill University, Montreal-Jewish General Hospital, site investigator); Chris Hosein, MEd (McGill University, Montreal-Jewish General Hospital, site investigator); Sandra Black, MD FRCP(C) (Sunnybrook Health Sciences, Ontario, site investigator); Simon Graham, PhD (Sunnybrook Health Sciences, Ontario, site investigator); Curtis Caldwell, PhD (Sunnybrook Health Sciences, Ontario, site investigator); Ging-Yuek Robin Hsiung, MD, MHSc, FRCPC (U.B.C. Clinic for AD & Related, B.C., site investigator); Howard Feldman, MD FRCP(C) (U.B.C. Clinic for AD & Related, B.C., site investigator); Michele Assaly, MA (U.B.C. Clinic for AD & Related, B.C., site investigator); Andrew Kertesz, MD (Cognitive Neurology-St. Joseph's, Ontario, site investigator); John Rogers, MD (Cognitive Neurology-St. Joseph's, Ontario, site investigator); Dick Trost, PhD (Cognitive Neurology-St. Joseph's, Ontario, site investigator); Charles Bernick, MD (Cleveland Clinic Lou Ruvo Center for Brain Health, site investigator); Donna Munic, PhD (Cleveland Clinic Lou Ruvo Center for Brain Health, site investigator); Chuang-Kuo Wu, MD, PhD (Northwestern University, site investigator); Nancy Johnson, PhD (Northwestern University, site investigator); Marsel Mesulam, MD (Northwestern University, site investigator); Carl Sadowsky, MD (Premiere Research Inst [Palm Beach Neurology], site investigator); Walter Martinez, MD (Premiere Research Inst [Palm Beach Neurology], site investigator); Teresa Villena, MD (Premiere Research Inst [Palm Beach Neurology], site investigator); Raymond Scott Turner, MD, PhD (Georgetown University Medical Center, site investigator); Kathleen Johnson, NP (Georgetown University Medical Center, site investigator); Kelly E. Behan, BA (Georgetown University Medical Center, site investigator); Reisa A. Sperling, MD (Brigham and Women's Hospital, site investigator); Dorene M. Rentz, PsyD (Brigham and Women's Hospital, site investigator); Keith A. Johnson, MD (Brigham and Women's Hospital, site investigator); Allyson Rosen, PhD (Stanford University, site investigator); Jared Tinklenberg, MD (Stanford University, site investigator); Wes Ashford, MD, PhD (Stanford University, site investigator); Marwan Sabbagh, MD, FAAN, CCRI (Sun Health Research Institute, site investigator); Donald Connor, PhD, PhD (Sun Health Research Institute, site investigator); Sandra Jacobson, MD (Sun Health Research Institute, site investigator); Ronald Killiany, PhD (Boston University, site investigator); Alexander Norbash, MD (Boston University, site investigator); Anil Nair, MD (Boston University, site investigator); Thomas O. Obisesan, MD, MPH (Howard University, site investigator); Annapurni Jayam-Trouth, MD (Howard University, site investigator); Paul Wang, PhD (Howard University, site investigator); Alan Lerner, MD (Case Western Reserve University, site investigator); Leon Hudson, MPH (Case Western Reserve University, site investigator); Paula Ogrocki, PhD (Case Western Reserve University, site investigator); Charles DeCarli, MD (University of California, Davis-Sacramento, site investigator); Evan Fletcher, PhD (University of California, Davis-Sacramento, site investigator); Owen Carmichael, PhD (University of California, Davis-Sacramento, site investigator); Smita Kittur, MD (Neurological Care of CNY, site investigator); Seema Mirje, MBBS (Neurological Care of CNY, site investigator); Michael Borrie, MD (Parkwood Hospital, site investigator); T.-Y. Lee, PhD (Parkwood Hospital, site investigator); Dr. Rob Bartha, PhD (Parkwood Hospital, site investigator); Sterling Johnson, PhD (University of Wisconsin, site investigator); Sanjay Asthana, MD (University of Wisconsin, site investigator); Cynthia M. Carlsson, MD (University of Wisconsin, site investigator); Steven G. Potkin, MD (University of California, Irvine-BIC, site investigator); Adrian Preda, MD (University of California, Irvine-BIC, site investigator); Dana Nguyen, PhD (University of California, Irvine-BIC, site investigator); Pierre Tariot, MD (Banner Alzheimer's Institute, site investigator); Adam Fleisher, MD (Banner Alzheimer's Institute, site investigator); Stephanie Reeder, BA (Banner Alzheimer's Institute, site investigator); Vernice Bates, MD (Dent Neurologic Institute, site investigator); Horacio Capote, MD (Dent Neurologic Institute, site investigator); Michelle Rainka, PhD (Dent Neurologic Institute, site investigator); Barry A. Hendin, MD (Dent Neurologic Institute, site investigator); Douglas W. Scharre, MD (Ohio State University, site investigator); Maria Kataki, MD, PhD (Ohio State University, site investigator); Earl A. Zimmerman, MD (Albany Medical College, site investigator); Dzintra Celmins, MD (Albany Medical College, site investigator); Alice D. Brown, FNP (Albany Medical College, site investigator); Sam Gandy, MD, PhD, PhD (Thomas Jefferson University, site investigator); Marjorie E. Marenberg, MD (Thomas Jefferson University, site investigator); Barry W. Rovner, MD (Thomas Jefferson University, site investigator); Godfrey Pearlson, MD (Hartford Hosp, Olin Neuropsychiatry Research Center); Karen Blank, MD (Hartford Hosp, Olin Neuropsychiatry Research Center); Karen Anderson, RN (Hartford Hosp, Olin Neuropsychiatry Research Center); Andrew J. Saykin, PsyD (Dartmouth-Hitchcock Medical Center, site investigator); Robert B. Santulli, MD (Dartmouth-Hitchcock Medical Center, site investigator); Jessica Englert, PhD (Dartmouth-Hitchcock Medical Center, site investigator); Jeff D. Williamson, MD, MHS (Wake Forest University Health Sciences, site investigator); Kaycee M. Sink, MD, MS (Wake Forest University Health Sciences, site investigator); Franklin Watkins, MD (Wake Forest University Health Sciences, site investigator); Brian R. Ott, MD (Rhode Island Hospital, site investigator); Chuang-Kuo Wu, MD, PhD (Rhode Island Hospital, site investigator); Ronald Cohen, PhD (Rhode Island Hospital, site investigator); Stephen Salloway, MD, MS (Butler Hospital, site investigator); Paul Malloy, PhD (Butler Hospital, site investigator); Stephen Correia, PhD (Butler Hospital, site investigator); Howard J. Rosen, MD (UC San Francisco, site investigator); Bruce L. Miller, MD (UC San Francisco, site investigator); and Jacobo Mintzer, MD (Medical University South Carolina, site investigator).

ACKNOWLEDGMENT

Data collection and sharing for this project was funded by the Alzheimer's Disease Neuroimaging Initiative (ADNI) (NIH Grant U01 AG024904). ADNI is funded by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, and through contributions from the following: Abbott, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corporation, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson and Johnson, Eli Lilly and Co., Medpace, Inc., Merck and Co., Inc., Novartis AG, Pfizer Inc., F. Hoffman-La Roche, Schering-Plough, Synarc, Inc., and Wyeth, as well as nonprofit partners the Alzheimer's Association and Alzheimer's Drug Discovery Foundation, with participation from the US Food and Drug Administration. Private sector contributions to ADNI are facilitated by the Foundation for the NIH (www.fnih.org). The grantee organization is the Northern California Institute for Research and Education, and the study is coordinated by the Alzheimer's Disease Cooperative Study at the University of California, San Diego. ADNI data are disseminated by the Laboratory for Neuro Imaging at the University of California, Los Angeles. This research was also supported by NIH grants P30 AG010129, K01 AG030514, and the Dana Foundation. Samples from the National Cell Repository for AD (NCRAD), which receives government support under a cooperative agreement grant (U24 AG21886) awarded by the National Institute on Aging (NIA), were used in this study. Additional support for data analysis was provided by NIA R01 AG19771 and P30 AG10133. The authors thank contributors, including the Alzheimer's Disease Centers who collected samples used in this study, as well as patients and their families, whose help and participation made this work possible.

DISCLOSURE

Dr. Kim and S. Swaminathan reports no disclosures. Dr. Shen receives support from the NIH (NIBIB R03 EB008674 [PI], NIBIB R03 EB008674S1 [PI], and UL1 RR025761 [PI of an Indiana CTSI pilot project]). S.L. Risacher reports no disclosures. Dr. Nho receives support

from the NIH (NLM T15LM07117 [trainee]). Dr. Foroud serves on the editorial boards of *Psychiatric Genetics*, *Journal of Bone and Mineral Research*, *Behavior Genetics*, *Journal of Studies on Alcohol*, *Parkinsonism and Related Disorders*, and *Journal of Studies on Alcohol and Drugs*; has served as a consultant for the Institute for Behavioral Genetics at the University of Colorado; and receives research support from the NIH (NIA 5U24AG021886 [PI], R01AR047822 [PI], R01NS037167 [PI], N01NS32357 [PI], U01AA014809 [PI], R01CA141668 [PI], and P01AG018397 [PI]), Families of Spinal Muscular Atrophy, the Muscular Dystrophy Association, HP Therapeutics Foundation, and CHDI Foundation (previously High Q Foundation). Dr. Shaw has served on a scientific advisory board for Bristol-Myers Squibb; has received funding for travel and speaker honoraria from Pfizer Inc.; serves on the editorial board of *Therapeutic Drug Monitoring;* may potentially receive revenue for patent pending re: O-methylated rapamycin derivatives for alleviation and inhibition of lymphoproliferative disorders, licensed by the University of Pennsylvania to Novartis; receives royalties from publication of *Applied Pharmacokinetics and Pharmacodynamics: Principles of Therapeutic Drug Monitoring* (Wolters Kluwer/Lippincott Williams & Wilkins, 2005); receives research support from the NIH (AG024904 [Co-PI Biomarker Core Laboratory] and 1RC 2AG-036535 (ARRA) [PI Biomarker Core Laboratory]); and receives board of directors' compensation and holds stock options in Saladax Biomedical. Dr. Trojanowski has received funding for travel and honoraria from Takeda Pharmaceutical Company Ltd.; has received speaker honoraria from Pfizer Inc.; serves as an Associate Editor of *Alzheimer's & Dementia*; may accrue revenue on patents re: Modified avidin-biotin technique, Method of stabilizing microtubules to treat Alzheimer's disease, Method of detecting abnormally phosphorylated tau, Method of screening for Alzheimer's disease or disease associated with the accumulation of paired helical filaments, Compositions and methods for producing and using homogeneous neuronal cell transplants, Rat comprising straight filaments in its brain, Compositions and methods for producing and using homogeneous neuronal cell transplants to treat neurodegenerative disorders and brain and spinal cord injuries, Diagnostic methods for Alzheimer's disease by detection of multiple MRNAs, Methods and compositions for determining lipid peroxidation levels in oxidant stress syndromes and diseases, Compositions and methods for producing and using homogenous neuronal cell transplants, Method of identifying, diagnosing and treating alpha-synuclein positive neurodegenerative disorders, Mutation-specific functional impairments in distinct tau isoforms of hereditary frontotemporal dementia and parkinsonism linked to chromosome-17: genotype predicts phenotype, Microtubule stabilizing therapies for neurodegenerative disorders, and Treatment of Alzheimer's and related diseases with an antibody; and receives research support from the NIH (NIA P01 AG 09215-20 [PI], NIA P30 AG 10124-18 [PI], NIA PO1 AG 17586-10 [Project 4 Leader], NIA 1PO1 AG-19724-07 [Core C Leader], NIA 1 U01 AG 024904-05 [Co-PI Biomarker Core Laboratory], NINDS P50 NS053488-02 [PI], NIA UO1 AG029213-01 [Co-I]; RC2NS069368 [PI], RC1AG035427 [PI], and NIA P30AG036468 [PI]), and from the Marian S. Ware Alzheimer Program. Dr. Potkin serves on scientific advisory boards and speakers' bureaus for and has received funding for travel and speaker honoraria from AstraZeneca, Bioline, Bristol Myers-Squibb, Cortex Pharmaceuticals, Inc., Dainippon Sumitomo Pharma, Janssen, Novartis, Merck Serono, Pfizer Inc., Otsuka Pharmaceutical Co., Ltd., Roche, and Minster Pharmaceuticals plc; serves on the editorial board of *Brain Imaging and Behavior*; conducts clinical procedures/imaging studies at the UCI Brain Imaging Center and Neuropsychiatric Clinical Research Division (10% effort); and receives research support from the Transdisciplinary Imaging Genetics Center (TIGC) P20 RR020837-01, and the NIH (NCRR U24 RR021992 [PI], U01 AG032984 [PI], NCIRE AG036535 [coinvestigator]). Dr. Huentelman receives research support from the NIH/NINDS (R01 N5059873 [PI]). Dr. Craig has received research support from the Arizona Science Foundation, Arizona Alzheimer's Research Center, and the NIH (U01 HL086528 [PI] and R01 MH078151 [coinvestigator]). Dr. DeChairo was an employee of Pfizer Inc. and is presently an employee of Medco Health Solutions; has received research support from Medco Health Solutions and Pfizer Inc.; and holds/has held stock in Medco Health Solutions and Pfizer Inc. Dr. Aisen serves on a scientific advisory board for NeuroPhage; serves on the editorial advisory board of *Alzheimer's Research & Therapy*; serves as a consultant to Elan Corporation, Wyeth, Eisai Inc., Schering-Plough Corp., Bristol-Myers Squibb, Eli Lilly and Company, NeuroPhage, Merck & Co., Roche, Amgen, Genentech, Inc., Abbott, Pfizer Inc., Novartis, Bayer Schering Pharma, Medivation, Inc., Daiichi Sankyo, Astellas Pharma Inc., Dainippon Sumitomo Pharma Company Limited, BioMarin Pharmaceutical Inc., Solvay Pharmaceuticals, Inc., Otsuka Pharmaceutical Co., Ltd., AstraZeneca, and Janssen; receives research support from Pfizer Inc., Baxter International Inc., and the NIH (NIA U01-AG10483 [PI], NIA U01-AG024904 [Coordinating Center Director], NIA R01-AG030048 [PI], and R01-AG16381 [coinvestigator]); and has received stock options from Medivation, Inc. and NeuroPhage. Dr. Petersen serves on scientific advisory boards for Elan Corporation, Wyeth, and GE Healthcare; receives royalties from publishing *Mild Cognitive Impairment* (Oxford University Press, 2003); and receives research support from the NIH/NIA (P50-AG16574 [PI], U01-AG06786 [PI], R01-AG11378 [coinvestigator], and U01–24904 (coinvestigator)]. Dr. Weiner serves on scientific advisory boards for Bayer Schering Pharma, Eli Lilly and Company, Nestlé, CoMentis, Inc., Neurochem Inc., Eisai Inc., Avid Radiopharmaceuticals Inc., Aegis Therapies, Genentech, Inc., Allergan, Inc., Lippincott Williams & Wilkins, Bristol-Myers Squibb, Forest Laboratories, Inc., Pfizer Inc., McKinsey & Company, Mitsubishi Tanabe Pharma Corporation, and Novartis; has received funding for travel from Nestlé and Kenes International and to attend conferences not funded by industry; serves on the editorial board of *Alzheimer's & Dementia*; has received honoraria from the Rotman Research Institute and BOLT International; serves as a consultant for Elan Corporation; receives research support from Merck & Co., Avid Radiopharmaceuticals Inc., the NIH (U01AG024904 [PI], P41 RR023953 [PI], R01 AG10897 [PI], P01AG19724 [coinvestigator], P50AG23501 [coinvestigator], R24 RR021992 [coinvestigator], R01 NS031966 [coinvestigator], and P01AG012435 [coinvestigator]), the US Department of Defense, the Veterans Administration, and the State of California; and holds stock in Synarc and Elan Corporation. Dr. Saykin serves as Editor-in-Chief of *Brain Imaging and Behavior*; has served as a consultant to Baxter International Inc., Bristol-Myers Squibb, and Pfizer Inc.; and receives research support from Eli Lilly and Company, Siemens AG, Welch Allyn Inc., the NIH (R01 CA101318 [PI], R01 AG19771 [PI], RC2 AG036535 [Core Leader], P30 AG10133–18S1 [Core Leader], and U01 AG032984 [Site PI and Chair, Genetics Working Group]), the Indiana Economic Development Corporation (IEDC #87884), and the Foundation for the NIH.

Received April 12, 2010. Accepted in final form August 20, 2010.

REFERENCES

1. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. Ann Neurol 2009; 65:403–413.
2. Jagust WJ, Landau SM, Shaw LM, et al. Relationships between biomarkers in aging and dementia. Neurology 2009;73:1193–1199.
3. Hampel H, Blennow K, Shaw LM, Hoessler YC, Zetterberg H, Trojanowski JQ. Total and phosphorylated tau protein as biological markers of Alzheimer's disease. Exp Gerontol 2010;45:30–40.
4. Blennow K, Hampel H, Weiner M, Zetterberg H. Cerebrospinal fluid and plasma biomarkers in Alzheimer disease. Nat Rev Neurol 2010;6:131–144.
5. Trojanowski JQ, Vandeerstichele H, Korecka M, et al. Update on the biomarker core of the Alzheimer's Disease Neuroimaging Initiative subjects. Alzheimers Dement 2010;6:230–238.
6. Maddalena A, Papassotiropoulos A, Muller-Tillmanns B, et al. Biochemical diagnosis of Alzheimer disease by measuring the cerebrospinal fluid ratio of phosphorylated tau protein to β-amyloid peptide42. Arch Neurol 2003;60: 1202–1206.

7. Gatz M, Reynolds CA, Fratiglioni L, et al. Role of genes and environments for explaining Alzheimer disease. Arch Gen Psychiatry 2006;63:168–174.
8. Laws SM, Friedrich P, Diehl-Schmid J, et al. Fine mapping of the MAPT locus using quantitative trait analysis identifies possible causal variants in Alzheimer's disease. Mol Psychiatry 2006;12:510–517.
9. Kölsch H, Jessen F, Wiltfang J, et al. Influence of SORL1 gene variants: Association with CSF amyloid-beta products in probable Alzheimer's disease. Neurosci Lett 2008; 440:68–71.
10. Prince JA, Zetterberg H, Andreasen N, Marcusson J, Blennow K. APOE epsilon4 allele is associated with reduced cerebrospinal fluid levels of Abeta42. Neurology 2004;62: 2116–2118.
11. Petersen RC, Aisen PS, Beckett LA, et al. Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. Neurology 2010;74:201–209.
12. Shen L, Kim S, Risacher SL, et al. Whole genome association study of brain-wide imaging phenotypes for identifying quantitative trait loci in MCI and AD: a study of the ADNI cohort. NeuroImage Epub 2010 Jan 25.
13. Saykin AJ, Shen L, Foroud TM, et al. Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: genetics core aims, progress, and plans. Alzheimers Dement 2010;6:265–273.
14. Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. Hum Genet 1986;73:320–326.
15. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.
16. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. JAMA 1997;278:1349–1356.
17. Potkin SG, Guffanti G, Lakatos A, et al. Hippocampal atrophy as a quantitative trait in a genome-wide association study identifying novel susceptibility genes for Alzheimer's disease. PLoS ONE 2009;4:e6501.
18. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265.
19. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002; 296:2225–2229.
20. Sloan CD, Shen L, West JD, et al. Genetic pathway-based hierarchical clustering analysis of older adults with cognitive complaints and amnestic mild cognitive impairment using clinical and neuroimaging phenotypes. Am J Med Genet Part B Neuropsychiatric Genetics Epub 2010 Mar 31.
21. Potkin SG, Turner JA, Guffanti G, et al. Genome-wide strategies for discovering genetic influences on cognition and cognitive disorders: methodological considerations. Cogn Neuropsychiatry 2009;14:391–418.
22. Bertram L, Tanzi RE. Genome-wide association studies in Alzheimer's disease. Hum Mol Genet 2009;18: R137–R145.
23. Morris JC, Roe CM, Xiong C, et al. APOE predicts amyloid-beta but not tau Alzheimer pathology in cognitively normal aging. Ann Neurol 2010;67:122–131.
24. Abraham R, Moskvina V, Sims R, et al. A genome-wide association study for late-onset Alzheimer's disease using DNA pooling. BMC Medical Genomics 2008;1:44.
25. Roses AD, Lutz MW, Amrine-Madsen H, et al. A TOMM40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease. Pharmacogenomics J Epub 2009 Dec 22.
26. Yu CE, Seltman H, Peskind ER, et al. Comprehensive analysis of APOE and selected proximate markers for late-onset Alzheimer's disease: patterns of linkage disequilibrium and disease/marker association. Genomics 2007;89: 655–665.
27. Bekris LM, Millard SP, Galloway NM, et al. Multiple SNPs within and surrounding the apolipoprotein E gene influence cerebrospinal fluid apolipoprotein E protein levels. J Alzheimers Dis 2008;13:255–266.
28. Doyon Y, Selleck W, Lane WS, Tan S, Cote J. Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans. Mol Cell Biol 2004;24:1884–1896.
29. Gräff J, Mansuy IM. Epigenetic dysregulation in cognitive disorders. Eur J Neurosci 2009;30:1–8.
30. van Bon BW, Koolen DA, Brueton L, et al. The 2q23.1 microdeletion syndrome: clinical and behavioural phenotype. Eur J Hum Genet 2010;18:163–170.
31. Huang J, Shi T, Ma T, et al. CCDC134, a novel secretory protein, inhibits activation of ERK and JNK, but not p38 MAPK. Cell Mol Life Sci 2008;65:338–349.
32. Xiong H, Callaghan D, Jones A, et al. ABCG2 is upregulated in Alzheimer's brain with cerebral amyloid angiopathy and may act as a gatekeeper at the blood-brain barrier for Aβ(1–40) peptides. J Neurosci 2009;29:5463–5475.
33. Wu HY, Hudry E, Hashimoto T, et al. Amyloid beta induces the morphological neurodegenerative triad of spine loss, dendritic simplification, and neuritic dystrophies through calcineurin activation. J Neurosci 2010;30:2636–2649.
34. Fernandez A, Llacuna L, Fernandez-Checa JC, Colell A. Mitochondrial cholesterol loading exacerbates amyloid beta peptide-induced inflammation and neurotoxicity. J Neurosci 2009;29:6394–6405.
35. Zhang W, Mojsilovic-Petrovic J, Andrade MF, Zhang H, Ball M, Stanimirovic DB. The expression and functional characterization of ABCG2 in brain endothelial cells and vessels. FASEB J 2003;17:2085–2087.
36. Daood M, Tsai C, Ahdab-Barmada M, Watchko JF. ABC transporter (P-gp/ABCB1, MRP1/ABCC1, BCRP/ABCG2) expression in the developing human CNS Neuropediatrics 2008;39:211–218.
37. Vihma H, Pruunsild P, Timmusk T. Alternative splicing and expression of human and mouse NFAT genes. Genomics 2008;92:279–291.
38. Groth RD, Mermelstein PG. Brain-derived neurotrophic factor activation of NFAT (nuclear factor of activated T-cells)-dependent transcription: a role for the transcription factor NFATc4 in neurotrophin-mediated gene expression. J Neurosci 2003;23:8125–8134.
39. Barabash A, Marcos A, Ancin I, et al. APOE, ACT and CHRNA7 genes in the conversion from amnestic mild cognitive impairment to Alzheimer's disease Neurobiol Aging 2009;30:1254–1264.
40. Kim Y, Nam YJ, Lee C. Analysis of the SREBF2 gene as a genetic risk factor for vascular dementia. Am J Med Genet B Neuropsychiatr Genet 2005;139B:19–22.