

# Association and Expression Analyses With Single-Nucleotide Polymorphisms in *TOMM40* in Alzheimer Disease

Carlos Cruchaga, PhD; Petra Nowotny, PhD; John S. K. Kauwe, PhD; Perry G. Ridge, MS; Kevin Mayo, BA; Sarah Bertelsen, MS; Anthony Hinrichs, PhD; Anne M. Fagan, PhD; David M. Holtzman, MD; John C. Morris, MD; Alison M. Goate, PhD; for the Alzheimer's Disease Neuroimaging Initiative

**Background:** Apolipoprotein E (*APOE*) is the most statistically significant genetic risk factor for late-onset Alzheimer disease (LOAD). The linkage disequilibrium pattern around the *APOE* gene has made it difficult to determine whether all the association signal is derived from *APOE* or whether there is an independent signal from a nearby gene.

**Objective:** To attempt to replicate a recently reported association of *APOE 3–TOMM40* haplotypes with risk and age at onset.

**Design:** We used standard techniques to genotype several polymorphisms in the *APOE–TOMM40* region in a large case-control series, in a series with cerebrospinal fluid biomarker data, and in brain tissue.

**Setting:** Alzheimer's Disease Research Center.

**Participants:** Research volunteers who were cognitively normal or had Alzheimer disease.

**Main Outcome Measures:** Disease status and age at onset.

**Results:** We did not replicate the previously reported association of the polyT polymorphism (rs10524523) with risk and age at onset. We found a significant association between rs10524523 and risk of LOAD in *APOE 33* homozygotes but in the opposite direction as the previously reported association (the very long allele was underrepresented in cases vs controls in this study ( $P = .004$ )). We found no association between rs10524523 and cerebrospinal fluid tau or  $\beta$ -amyloid 42 levels or *TOMM40* or *APOE* gene expression.

**Conclusions:** Although we did not replicate the earlier association between the *APOE 3–TOMM40* haplotypes and age at onset, we observed that the polyT polymorphism is associated with risk of LOAD in *APOE 33* homozygotes in a large case-control series but in the opposite direction as in the previous study.

*Arch Neurol.* 2011;68(8):1013-1019

**T**HE MOST STATISTICALLY SIGNIFICANT signals in genome-wide association studies for late-onset Alzheimer disease (LOAD) are detected with single-nucleotide polymorphisms (SNPs) in the region encoding apolipoprotein E (*APOE*) (OMIM 107741) and *TOMM40* (translocase of outer mitochondrial membrane 40 homologue [OMIM 608061]).<sup>1-10</sup> For several reasons, it is difficult to determine whether the signals in this area are due solely to the *APOE* genotype. First, the SNPs that code for the *APOE*  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  isoforms are not included in the most popular genome-wide SNP chips. Second, extensive linkage disequilibrium in this region of the genome makes it difficult to definitively determine the genetic variant(s) that drives the asso-

ciation. Third, several SNPs up to 50 kilobase from *APOE* exhibit significant associations with LOAD.

Previous studies<sup>11-13</sup> have explored this issue using case-control data sets, endophenotypes, and genome-wide pathway analyses. Roses et al<sup>12</sup> used DNA sequencing and an evolutionary network approach to demonstrate that a polyT polymorphism in intron 6 of *TOMM40* (rs10524523) is associated with age at onset (AAO) in *APOE 33* and *34* individuals. Roses et al found that the very long allele of rs10524523 is associated with increased risk and lower AAO of LOAD.

In this study, we attempted to replicate the findings from Roses et al.<sup>12</sup> We analyzed a large case-control sample to test whether *APOE 3–TOMM40* haplotypes or *TOMM40* alleles exhibit an *APOE*-independent effect on risk of disease, AAO

Author Affiliations are listed at the end of this article.

**Group Information:** A list of the Alzheimer's Disease Neuroimaging Initiative investigators appears on the website listed on page 1018.

**Table 1. Demographics of the Samples**

Sample	Participants, No.	Age, Mean (SD) [Range], y <sup>a</sup>	Male Sex, %	APOE ε4 Positive, %	CDR Score
WU-ADRC CC series					
Cases	759	73 (9) [44-102]	45	57	>0
Controls	345	77 (8) [60-103]	40	41	0
NIA-LOAD CC series					
Cases	835	71 (47) [48-98]	33	76	>0
Controls	845	75 (9) [42-101]	39	30	0
WU-ADRC brain series					
Cases	82	86 (7) [72-102]	45	41	>0
Controls	39	85 (9) [64-107]	41	23	0
WU-ADRC CSF series	474	68 (11) [45-94]	39	40	0 = 72%:>0 = 28%
ADNI CSF series	259	75 (6) [56-91]	56	47	0 = 40%:>0 = 60%

Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE, apolipoprotein E; CC, case-control; CDR, Clinical Dementia Rating Scale; CSF, cerebrospinal fluid; NIA-LOAD, National Institute on Aging Late-Onset Alzheimer Disease Family Study; WU-ADRC, Alzheimer Disease Research Center at Washington University.

<sup>a</sup>For cases, the age at the last assignment; for controls, the age at onset.

of LOAD, cerebrospinal fluid (CSF) biomarker levels, and expression of *TOMM40*/APOE in the brain.

## METHODS

### PARTICIPANTS

Risk of disease and AAO analyses were performed in 1594 LOAD cases (474 APOE 33 homozygotes) and 1190 cognitively normal controls (701 APOE 33 homozygotes) matched for age, sex, and ethnicity. These samples were obtained from the Knight Alzheimer Disease Research Center at Washington University (WU-ADRC) (759 cases and 345 controls) and from the National Institute on Aging (NIA) LOAD Family Study (835 cases and 845 controls). Each case received a diagnosis of dementia of the Alzheimer type using criteria equivalent to those of the National Institute of Neurological Disorders and Stroke-Alzheimer's Disease and Related Disorders Association for probable AD.<sup>14,15</sup> Individuals with a Clinical Dementia Rating Scale (CDR) score of 0.5 who did not meet the clinical criteria for probable AD were not included in the analyses. Controls received the same assessment as cases but were cognitively normal. All the individuals were of European descent, and written consent was obtained from all the participants.

Expression studies were conducted using complementary DNA (cDNA) obtained from the parietal lobes of 82 AD cases and 39 cognitively normal individuals (CDR score=0) obtained through the WU-ADRC Neuropathology Core. Association with CSF tau, tau phosphorylated at threonine 181, β-amyloid (Aβ) 42, and Aβ40 levels was tested in an independent series of 474 samples from the WU-ADRC and 259 samples from the Alzheimers Disease Neuroimaging Initiative (ADNI) (**Table 1**). Cerebrospinal fluid was collected and biomarker measurements were obtained as described previously.<sup>16-18</sup> A summary of the demographics of all the participants is given in Table 1.

### GENOTYPING

rs7412 and rs429358 (which define the APOE ε2, ε3, and ε4 isoforms), rs1160985, and rs4420638 (*TOMM40*) were genotyped using KASPar (KBioscience, Herts, United Kingdom) and TaqMan (Applied Biosystems, Foster City, California) genotyping technologies. The APOE genotype for the NIA-LOAD and ADNI series was provided by the NIA-LOAD or ADNI. The polyT re-

peat in intron 6 of *TOMM40* (rs10524523) was genotyped using fluorescence-based fragment size analysis (Supplemental Figure 1; <http://neuroscicerearch.wustl.edu/pages/cruchaga2011.aspx>).<sup>19</sup> A detailed explanation of the fluorescence-based fragment size genotyping, quality control steps, allele frequency, and linkage disequilibrium between the studied polymorphisms can be found at <http://neuroscicerearch.wustl.edu/Pages/cruchaga2011.aspx>. The linkage disequilibrium between the genotyped variants can be found in Supplemental Table 1

### GENOTYPE CALLS

The polyT repeat (rs10524523) genotypes were placed into categories modeled after those reported by Roses et al<sup>12</sup>: short (246-267 base pair [bp]), long (268-279 bp), and very long (280-289 bp) (Supplemental Figure 2; <http://neuroscicerearch.wustl.edu/Pages/cruchaga2011.aspx>). The base pair numbers do not correspond to those provided by Roses et al because the present numbers refer to the total length of the polymerase chain reaction product, not the number of polyT repeats (see <http://neuroscicerearch.wustl.edu/Pages/cruchaga2011.aspx> for quality control and call comparison between the present study and previous studies).

### GENE EXPRESSION

Quantification of gene expression was performed by real-time polymerase chain reaction<sup>20</sup> as explained previously.<sup>21</sup> We also used the GEO data set GSE15222 for replication (see <http://neuroscicerearch.wustl.edu/Pages/cruchaga2011.aspx> for a detailed explanation).

### PHYLOGENETIC ANALYSES

Because the polyT repeat is reported as the key variant to define *TOMM40* clades A and B,<sup>12</sup> we used this marker and APOE isoform information to perform analyses based on phylogenetic groups as described by Roses et al.<sup>12</sup> Haplotype phase was estimated using PHASE software.<sup>22</sup> The phylogeny, which represents the evolutionary relatedness of the haplotypes, was estimated using neighbor-joining with 10 000 bootstrapping replicates in the CLC DNA workbench (CLC bio, Aarhus, Denmark) (Supplemental Figure 3; <http://neuroscicerearch.wustl.edu/Pages/cruchaga2011.aspx>). We tested for differences in mean AAO

between *APOE 3-TOMM40* clade A and B haplotypes using a *t* test. Association of *APOE 3-TOMM40* clade A and B haplotypes with case-control status was performed using a Fisher exact test.

## STATISTICAL ANALYSES

Additional association tests between the polyT repeat and disease status, AAO, *TOMM40* and *APOE* brain expression, and CSF tau and A $\beta$ 42 levels were performed using UNPHASED v3.1.4 and SAS, version 9.2 (SAS Institute Inc, Cary, North Carolina). Several analyses were restricted to *APOE 33* homozygotes, thus removing uncertainty in haplotype phasing as a possible confounding factor. (See <http://neuroscienceresearch.wustl.edu/Pages/cruchaga2011.aspx> for a detailed description of the statistical analyses.)

### Multiple Test Correction

We tested 4 SNPs for association with 2 phenotypes. A conservative threshold for multiple test correction would be to set the significance at  $P < .006$ , which would be the Bonferroni correction for  $4 \times 2$  tests. The SNPs that were associated with risk of disease or AAO were tested for association with CSF biomarker levels and gene expression to investigate potential pathogenic mechanisms. In this case, no multiple test correction was applied because only 1 or 2 SNPs with specific hypotheses were tested for association.

### ADNI Material and Methods

Data used in the preparation of this article were obtained from the ADNI database (<http://www.loni.ucla.edu/ADNI>). The ADNI was launched in 2003 by the NIA, the National Institute of Biomedical Imaging and Bioengineering, the Food and Drug Administration, private pharmaceutical companies, and nonprofit organizations. The primary goal of the ADNI has been to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessments can be combined to measure the progression of mild cognitive impairment and early AD. The principal investigator of this initiative is Michael W. Weiner, MD. The ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and participants have been recruited from more than 50 sites across the United States and Canada. The initial goal of the ADNI was to recruit 800 adults to participate in the research. For up-to-date information, see <http://www.adni-info.org>.

## RESULTS

The main aim of this study was to attempt to replicate the association of the *APOE 3-TOMM40* polyT repeat (rs10524523) haplotype groups with AAO and risk reported by Roses et al.<sup>12</sup> Frequencies of *APOE 3* alleles and *APOE* genotypes in *TOMM40* clades A and B in the phased haplotype data were consistent with those reported (Supplemental Figure 2).<sup>12</sup> We also included rs4420638 and rs1160985 (*TOMM40*), which have been reported to be associated with risk of disease or AAO independent of *APOE* genotype.<sup>23</sup>

### ASSOCIATION WITH AAO

When using sex, but not *APOE* genotype, as a covariate, we found a significant association between the polyT

repeat (rs10524523) and AAO in the WU-ADRC + NIA-LOAD case-control series ( $P = 1.03 \times 10^{-19}$ ). To discern whether this association was driven by the polyT repeat or by *APOE* genotype, we performed 2 additional analyses: 1 including *APOE* genotype as a covariate in the model and 2 analyzing the polyT association in the *APOE 33* stratum. When *APOE* genotype was included as a covariate, the *P* value dropped to .11, indicating that the association with AAO was driven by the *APOE* polymorphisms (**Table 2**). In the present analyses, the very long allele carriers had a higher, but not statistically significantly different, AAO than did the short allele carriers.

When the analyses were restricted to individuals with an *APOE 33* genotype, the polyT repeat showed no association with AAO in the WU-ADRC + NIA-LOAD case-control series ( $P = .19$ ) (Table 2). The same result was found when controls were included as censored data in the Kaplan-Meier analyses: in the *APOE 33* stratum, the very long allele carriers had a higher, but not statistically significantly different, AAO than did the short allele carriers (**Figure**). We also found the same pattern in *APOE 34* carriers: carriers of the long and very long alleles had a slightly higher, but not statistically significantly different, AAO than did carriers of the short alleles (Supplemental Figure 4; <http://neuroscienceresearch.wustl.edu/Pages/cruchaga2011.aspx>). Haplotype analyses showed similar results. We found a trend toward association between *APOE 3-TOMM40* haplotypes and AAO ( $P = .057$ ). Individuals with *APOE 3-TOMM40* clade A haplotypes had a mean AAO of 73.31 years vs 72.93 years for *APOE 3-TOMM40* clade B. Thus, in this much larger study (total cases = 1594, total controls = 1190; total *APOE 33* cases = 474, total *APOE 33* controls = 701) than the original study ( $N = 34$ ), we found a trend toward association but in the opposite direction than previously reported. In the *APOE 33* stratum, rs4420638 showed the most significant association with AAO ( $P = .01$ ), but this association did not pass multiple test correction (Table 2).

### ASSOCIATION WITH RISK OF DISEASE

We also analyzed whether the *TOMM40* polyT repeat (rs10524523) was associated with risk of LOAD. We found an allelic association when sex and age, but not *APOE* genotype, were included as covariates ( $P = 4.14 \times 10^{-88}$ ). The polyT repeat showed a trend toward association with risk of LOAD in the WU-ADRC + NIA-LOAD case-control series when *APOE* genotype was included in the model ( $P = .08$ ) (Table 2). When we restricted this analysis to individuals with an *APOE 33* genotype and used age and sex as covariates, there was a significant association with risk ( $P = .004$ ) (Table 2). The frequency of the very long allele of the polyT repeat (rs10524523) was significantly lower in cases compared with controls in the WU-ADRC + NIA-LOAD series (0.41 vs 0.48,  $P = .004$ ; odds ratio (OR) = 0.78, 95% confidence interval (CI) = 0.65-0.95) (Table 2). In this case, the association passed the multiple test correction threshold ( $\alpha = .006$ ). No other studied SNP showed a significant association with risk.

**Table 2. MAF and P Values for Association With Risk and AAO in the Entire Series and in the APOE 33 Substratum**

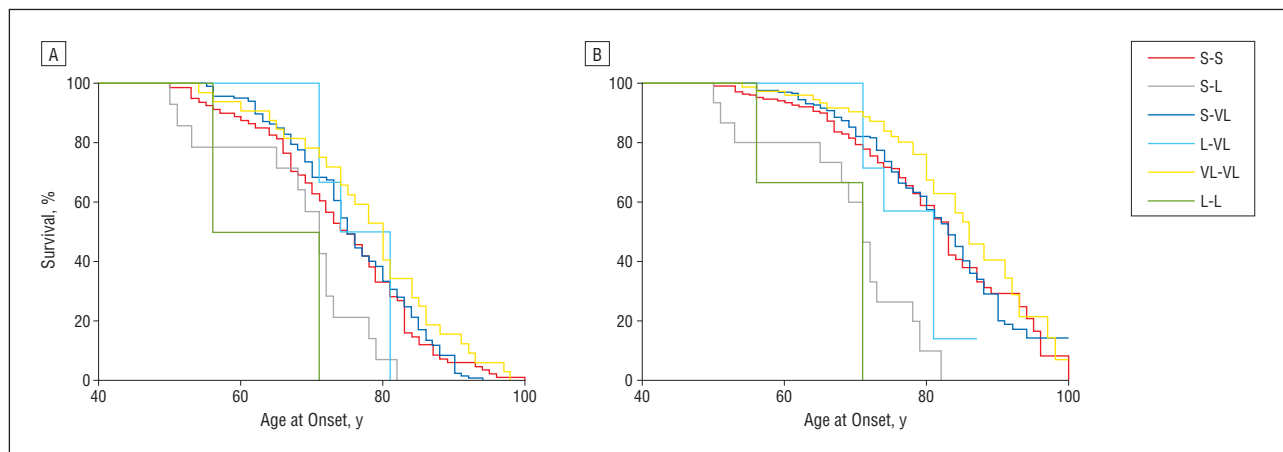
Variable	Entire Series					APOE 33 Substratum				
	Minor Allele	MAF		P Value		Minor Allele	MAF		P Value	
		Cases	Controls	Disease Status	AAO		Cases	Controls	Disease Status	AAO
WU-ADRC CC series <sup>a</sup>										
APOE 4 <sup>+</sup> (rs429358)	C	0.33	0.14	$1.69 \times 10^{-26}$	$9.78 \times 10^{-9}$	NA	NA	NA	NA	NA
rs10524523-polyT	L	0.32	0.14	.14	.04	VL	0.37	0.44	.10	.04
rs1160985	T	0.38	0.46	.32	.02	C	0.41	0.45	.14	.05
rs4420638	G	0.36	0.20	.29	.35	G	0.07	0.08	.84	.003
NIA-LOAD <sup>b</sup>										
APOE 4 <sup>+</sup> (rs429358)	C	0.46	0.16	$3.74 \times 10^{-68}$	$7.08 \times 10^{-12}$	NA	NA	NA	NA	NA
rs10524523-polyT	L	0.48	0.17	.55	.96	VL	0.44	0.49	.11	.69
rs4420638	G	0.51	0.19	.57	.87	G	0.08	0.05	.02	.94
WU-ADRC + NIA-LOAD <sup>c</sup>										
APOE 4 <sup>+</sup> (rs429358)	C	0.41	0.16	$6.07 \times 10^{-92}$	$6.28 \times 10^{-21}$	NA	NA	NA	NA	NA
rs10524523-polyT	L	0.41	0.16	.08	.11	VL	0.41	0.48	.004	.19
rs4420638	G	0.43	0.20	.82	.30	G	0.08	0.06	.08	.01

Abbreviations: AAO, age at onset; APOE, apolipoprotein E; C, cytosine; CC, case-control; G, guanine; L, long; MAF, minor allele frequency; NA, not applicable; NIA-LOAD, National Institute on Aging Late-Onset Alzheimer Disease Family Study; T, thymine; VL, very long; WU-ADRC, Alzheimer Disease Research Center at Washington University.

<sup>a</sup>For association with disease status, age, sex, and APOE genotype were included as covariates in the entire series and sex and age in the APOE 33 substratum. The APOE genotype was not included as a covariate when rs429358 was tested for association. For association with AAO, sex and APOE genotype were included as covariates in the entire series and sex in the APOE 33 substratum.

<sup>b</sup>For association with disease status, age, sex, APOE genotype, and the first to the third principal component factors were included as covariates in the entire series and sex, age, and PC1/3 (principal components first to third) in the APOE 33 substratum. The APOE genotype was not included as a covariate when rs429358 was tested for association. For association with AAO, sex, APOE genotype, and PC1/3 were included as covariates in the entire series and sex and PC1/3 in the APOE 33 substratum.

<sup>c</sup>For association with disease status, sex, age, and site were included as covariates in the APOE 33 substratum. For association with AAO, sex and site were included as covariates in the APOE 33 substratum.



**Figure.** rs10524523 is not associated with age at onset of late-onset Alzheimer disease (LOAD) in apolipoprotein E (APOE) 33 carriers. A, Age at onset was analyzed for association with rs10524523 in 282 APOE 33 LOAD cases from the Alzheimer Disease Research Center at Washington University (WU-ADRC) series using the Kaplan-Meier method and was tested for significant differences using the log-rank test. B, Age at onset was analyzed for association with rs10524523 in 282 LOAD cases and 213 controls from the WU-ADRC series with an APOE 33 genotype by the Kaplan-Meier method and was tested for significant differences using the log-rank test. In both analyses, the short-short (S-S), short-very long (S-VL), and very long-very long (VL-VL) genotypes are highlighted because they were the most frequent in these strata. No significant differences in the survival curves were found ( $P > .05$ ). L-L indicates long-long; L-VL, long-very long; and S-L, short-long.

### EVALUATION OF POSSIBLE MECHANISMS OF DISEASE RISK: ASSOCIATION WITH CSF BIOMARKER LEVELS AND GENE EXPRESSION

To determine a possible mechanism underlying the observed disease risk associated with the polyT repeat (rs10524523), we examined several endophenotypes, including CSF A $\beta$  and tau levels and APOE and TOMM40

gene expression in the brain. A very strong association was observed between rs10524523 and CSF A $\beta$ 42 levels when CDR score, age, and sex, but not APOE genotype, were included as covariates ( $P = 4.50 \times 10^{-8}$  for the WU-ADRC CSF series and  $P = 2.42 \times 10^{-12}$  for the WU-ADRC + ADNI CSF series). However, this association was driven by APOE genotype because inclusion of APOE genotype as a covariate in the model eliminated the association between

rs10524523 and CSF A $\beta$ 42 levels ( $P=.49$  for the WU-ADRC CSF series and  $P=.40$  for the WU-ADRC + ADNI CSF series) (Supplemental Table 2), suggesting that the rs10524523 association reflects linkage disequilibrium with the *APOE* genotype. We also did not detect an association between rs10524523 and CSF A $\beta$ 42, tau, or tau phosphorylated at threonine 181 levels in the entire series and in the *APOE* 33 stratum. For the WU-ADRC CSF samples, we also had CSF A $\beta$ 40 but found no association between the polyT repeat and this phenotype in the entire series or in the *APOE* 33 stratum (Supplemental Table 2).

Last, we tested whether these polymorphisms are associated with variability in *APOE* or *TOMM40* messenger RNA (mRNA) expression in the human parietal cortex. There was a marginal correlation between the cDNA levels of *APOE* and *TOMM40*, with  $P<.001$  and a Pearson correlation coefficient of  $-0.33$ . Because the brain samples are derived from cognitively normal (CDR score=0) and demented (CDR score $>0.5$ ) individuals, we first tested whether there was an association between mRNA levels and CDR score. We found no association between *APOE* cDNA levels and CDR score ( $P=.63$ ; age, sex, and postmortem interval as covariates). We found a significant association between *TOMM40* cDNA levels and CDR score ( $P=3.55 \times 10^{-3}$ ; age, sex, *APOE* genotype, and postmortem interval as covariates) in the WU-ADRC neuropathology series (82 AD cases and 39 cognitively normal individuals). However, we did not replicate this finding in the GEO data set GSE15222. In this data set, the *TOMM40* cDNA levels in cases ( $n=176$ ) and controls ( $n=188$ ) are not significantly different ( $P=.17$ ).

We found no association between any studied SNP and either *TOMM40* or *APOE* cDNA levels (Supplemental Table 3; <http://neuroscienceresearch.wustl.edu/Pages/cruchaga2011.aspx>). We also did not detect an association between *APOE* or *TOMM40* cDNA expression and *APOE* genotype ( $P=.45$  and  $P=.63$ , respectively). The association between *TOMM40* cDNA levels and CDR score led us to stratify the samples by CDR scores for further analyses, but we did not detect association between any SNP in either cases or controls (Supplemental Table 3). We also analyzed the *APOE* 33 stratum alone but found no associations (data not shown).

#### COMMENT

It is unclear whether all the association with risk of LOAD found in the *APOE*–*TOMM40* gene region in the genome-wide association studies<sup>1–10</sup> is driven by *APOE* genotype. Identification of new polymorphisms/genes that modify risk of LOAD could provide a better understanding of the pathways involved in LOAD and identify new drug targets for AD treatment. In this study, we attempted to replicate the recent study by Roses et al<sup>12</sup> that reported an association of *APOE* 3–*TOMM40* polyT polymorphism (rs10524523) haplotypes with AAO and risk of AD. We also performed extensive analyses in individuals with *APOE* 33 and analyzed several endophenotypes for LOAD to investigate different potential effects of the *TOMM40* polymorphisms. We did not find a significant association between the polyT polymorphism (rs10524523) and AAO despite the fact that this

large series of 2784 individuals (1175 *APOE* 33) provides high statistical power. Indeed, we found that in *APOE* 33 and 34 individuals, the longer alleles of the polyT polymorphism are associated with later onset and a protective effect in the opposite direction as the association in the original study.<sup>12</sup> We also studied 2 SNPs in *TOMM40* that have been suggested to modify risk of AD or AAO<sup>23</sup> but found no significant association when *APOE* genotype was included as a covariate or when the *APOE* 33 stratum was analyzed alone.

We found that the frequency of the very long alleles of the polyT polymorphism is significantly lower in cases than in controls in 2 independent series (7% and 5% lower for the WU-ADRC and NIA-LOAD case-control series, respectively). Only when we combined the 2 independent case-control series did we find a significant association after multiple test correction in the *APOE* 33 stratum. In the joint analyses, allele frequency for the very long rs10524523 allele was 0.41 in cases and 0.48 in controls ( $P=.004$ ; OR=0.78, 95% CI=0.65–0.95), which is opposite that reported by Roses et al.<sup>12</sup> Although the magnitude of the observed effect is stronger than that observed for the genome-wide association study significant SNP in *CLU*, rs11136000 (0.36 vs 0.40; OR=0.88, 95% CI=0.86–0.91), a 4-fold larger sample size would be required to detect a genome-wide significant association for rs10524523 in *APOE* 33 homozygous individuals (ie, 6376 unselected cases and 4760 unselected controls or 1896 *APOE* 33 cases and 2804 *APOE* 33 controls).

These results suggest that the *TOMM40* polyT repeat may be associated with risk of disease. *TOMM40* is in close proximity to *APOE*, but it is unknown whether the polyT repeat affects risk of AD through an *APOE*-dependent mechanism or a totally independent mechanism. We used CSF biomarker phenotypes to test whether the polyT repeat increases risk of LOAD through an A $\beta$ 42- (similar to *APOE*<sup>21,24–26</sup>) or a tau-dependent mechanism, but these results suggest that the polyT repeat may affect risk of AD through another mechanism. We also found no association of the polyT repeat with *APOE* or *TOMM40* mRNA expression in the parietal cortex. We did not find evidence of any obvious potential mechanism that could explain the association with risk, but the power of these analyses are limited, and studies in larger series should be performed to identify the potential disease mechanism.

In conclusion, these data do not support the findings reported by Roses et al<sup>12</sup> because we observed no association between *APOE* 3–*TOMM40* clade A and B haplotypes or the polyT repeat (rs10524523) and AAO. We observed an association between the polyT repeat and risk of disease but in the opposite direction as that reported previously.<sup>12</sup> It is unclear whether these results represent a type 1 error, but they highlight the importance of using large series, particularly when evaluating *APOE* subgroups, which require sample stratification—reducing power. Confirmation that rs10524523 is independently associated with AD risk will require a much larger sample. This could potentially be accomplished by imputation of rs1160985, a SNP that is in high linkage disequilibrium ( $r^2=0.93$ ) with the polyT variant in the *APOE* 33 carriers, in the large genome-wide association study data sets.<sup>9,10,27</sup>

TOMM40 codes for a mitochondrial protein, suggesting that mitochondrial integrity and energy metabolism could play an important role in LOAD. Mitochondrial morphologic features are altered in AD brains, and several studies have reported deficiencies in energy-related enzymes. The hypothesis that mitochondria may play an important role in LOAD is also supported by the fact that we did not find an association between the TOMM40 polymorphism and CSF A $\beta$ 42 and tau levels. However, more genetic and molecular studies are necessary to determine whether the reported genetic association with rs10524523 in TOMM40 is real.

**Accepted for Publication:** April 4, 2011.

**Author Affiliations:** Departments of Psychiatry (Drs Cruchaga, Nowotny, Hinrichs, and Goate; Mr Mayo; and Ms Bertelsen), Neurology (Drs Fagan, Holtzman, Morris, and Goate), Pathology and Immunology (Dr Morris), Genetics (Dr Goate), and Developmental Biology (Dr Holtzman), Knight Alzheimer's Disease Research Center (Drs Cruchaga, Fagan, Holtzman, Morris, and Goate), and Hope Center for Neurological Disorders (Drs Cruchaga, Fagan, Holtzman, Morris, and Goate), Washington University School of Medicine, St Louis, Missouri; Department of Biology, Brigham Young University, Provo, Utah (Dr Kauwe and Mr Ridge); and ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, Utah (Mr Ridge).

**Correspondence:** Alison M. Goate, PhD, Samuel & Mae S. Ludwig Professor of Genetics in Psychiatry, Department of Psychiatry, B8134 Washington University School of Medicine, 660 S Euclid Ave, St Louis, MO 63110 (goatea@psychiatry.wustl.edu).

**Author Contributions:** All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Cruchaga, Kauwe, Holtzman, and Goate. *Acquisition of data:* Nowotny, Mayo, Fagan, and Morris. *Analysis and interpretation of data:* Cruchaga, Kauwe, Ridge, Bertelsen, Hinrichs, Fagan, Holtzman, and Goate. *Drafting of the manuscript:* Cruchaga, Nowotny, Kauwe, Holtzman, and Goate. *Critical revision of the manuscript for important intellectual content:* Cruchaga, Nowotny, Kauwe, Ridge, Mayo, Bertelsen, Hinrichs, Fagan, Holtzman, Morris, and Goate. *Statistical analysis:* Cruchaga, Kauwe, Bertelsen, and Hinrichs. *Obtained funding:* Holtzman, Morris, and Goate. *Administrative, technical, and material support:* Nowotny, Ridge, Mayo, Bertelsen, Fagan, Holtzman, Morris, and Goate. *Study supervision:* Holtzman, Morris, and Goate.

**Financial Disclosure:** Dr Morris has served as a consultant to or has received speaking honoraria from AstraZeneca AB, Bristol-Myers Squibb, Eisai Inc, Elan/Janssen Alzheimer Immunotherapy Program, Genentech Inc, Eli Lilly & Co, Merck & Co Inc, Novartis International AG, Otsuka Pharmaceuticals, Pfizer/Wyeth, and Schering-Plough. Dr Goate has served as a consultant to AstraZeneca; has provided expert testimony to Howrey & Associates; has speaking honoraria for AstraZeneca AB and Pfizer; and has received royalties from Taconic.

**Funding/Support:** This work was supported by grants AG16208, P01AG03991, P50AG05681, P01AG026276,

AG23185, and AG05136 from the National Institutes of Health; the Barnes-Jewish Hospital Foundation; the Ford Foundation; and a fellowship from "Fundación Alfonso Martin Escudero" (Dr Cruchaga). Samples from the National Cell Repository for Alzheimer's Disease, which receives government support under a cooperative agreement grant (U24 AG21886) awarded by the NIA, were used in this study. Samples from the NIA-LOAD (National Institute on Aging Genetics Initiative for Late-Onset Alzheimer's Disease) family study were collected under a cooperative agreement awarded by the NIA (U24 AG026395). Data collection and sharing for this project was funded by the ADNI (National Institutes of Health grant U01 AG024904). The ADNI is funded by the NIA, the National Institute of Biomedical Imaging and Bioengineering, and generous contributions from Abbott Laboratories, AstraZeneca AB, Bayer Schering Pharma AG, Bristol-Myers Squibb, Eisai Global Clinical Development, Elan Corp, Genentech, GE Healthcare, GlaxoSmithKline, Innogenetics, Johnson & Johnson, Eli Lilly & Co, Medpace Inc, Merck & Co Inc, Novartis International AG, Pfizer Inc, F. Hoffman-La Roche, Schering-Plough, and Synarc Inc and from the nonprofit partners the Alzheimer's Association and the Alzheimer's Drug Discovery Foundation, with participation from the US Food and Drug Administration. Private sector contributions to the ADNI are facilitated by the Foundation for the National Institutes of Health (<http://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. The ADNI data are disseminated by the Laboratory for Neuroimaging at the University of California, Los Angeles. This research was also supported by grants P30 AG010129 and K01 AG030514 from the National Institutes of Health and by the Dana Foundation. **Role of the Sponsor:** The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

A complete list of ADNI investigators can be found at [http://adni.loni.ucla.edu/wp-content/uploads/how\\_to\\_apply/ADNI\\_Authorship\\_List.pdf](http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Authorship_List.pdf).

**Disclaimer:** Data used in the preparation of this article were obtained from the ADNI database (<http://www.loni.ucla.edu/ADNI>). As such, the investigators in the ADNI contributed to the design and implementation of the ADNI and provided data but did not participate in analysis or writing of this report.

**Previous Presentation:** This study is presented in part at the International Conference on Alzheimer Disease; July 16-21, 2011; Paris, France.

**Online-Only Materials:** The supplemental figures and table can be found at <http://neuroscienceresearch.wustl.edu/Pages/cruchaga2011.aspx>.

**Additional Contributions:** We thank the contributors, including the AD centers, who collected the samples used in this study; the patients and their families, whose help and participation made this work possible; the Clinical Core of the Knight ADRC for clinical and cognitive assessments of the participants; the Genetics Core of the Knight ADRC for APOE genotypes; and the Biomarker

## REFERENCES

1. Grupe A, Abraham R, Li Y, et al. Evidence for novel susceptibility genes for late-onset Alzheimer's disease from a genome-wide association study of putative functional variants. *Hum Mol Genet.* 2007;16(8):865-873.
2. Coon KD, Myers AJ, Craig DW, et al. A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. *J Clin Psychiatry.* 2007;68(4):613-618.
3. Reiman EM, Webster JA, Myers AJ, et al. GAB2 alleles modify Alzheimer's risk in APOE $\epsilon$ 4 carriers. *Neuron.* 2007;54(5):713-720.
4. Bertram L, Lange C, Mullin K, et al. Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *Am J Hum Genet.* 2008;83(5):623-632.
5. Li H, Wetten S, Li L, et al. Candidate single-nucleotide polymorphisms from a genome-wide association study of Alzheimer disease. *Arch Neurol.* 2008;65(1):45-53.
6. Carrasquillo MM, Zou F, Pankratz VS, et al. Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. *Nat Genet.* 2009;41(2):192-198.
7. Züchner S, Wendland JR, Ashley-Koch AE, et al. Multiple rare SAPAP3 missense variants in trichotillomania and OCD. *Mol Psychiatry.* 2009;14(1):6-9.
8. Beecham GW, Martin ER, Li YJ, et al. Genome-wide association study implicates a chromosome 12 risk locus for late-onset Alzheimer disease. *Am J Hum Genet.* 2009;84(1):35-43.
9. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat Genet.* 2009;41(10):1088-1093.
10. Lambert JC, Heath S, Even G, et al; European Alzheimer's Disease Initiative Investigators. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat Genet.* 2009;41(10):1094-1099.
11. 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(6):655-665.
12. 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.* 2010;10(5):375-384.
13. Hong MG, Alexeyenko A, Lambert JC, Amouyel P, Prince JA. Genome-wide pathway analysis implicates intracellular transmembrane protein transport in Alzheimer disease. *J Hum Genet.* 2010;55(10):707-709.
14. Berg L, McKeel DW Jr, Miller JP, et al. Clinicopathologic studies in cognitively healthy aging and Alzheimer's disease: relation of histologic markers to dementia severity, age, sex, and apolipoprotein E genotype. *Arch Neurol.* 1998;55(3):326-335.
15. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology.* 1984;34(7):939-944.
16. Fagan AM, Mintun MA, Mach RH, et al. Inverse relation between in vivo amyloid imaging load and cerebrospinal fluid A $\beta$ 42 in humans. *Ann Neurol.* 2006;59(3):512-519.
17. Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/ $\beta$ -amyloid<sub>(42)</sub> ratio as a prediction of cognitive decline in nondemented older adults. *Arch Neurol.* 2007;64(3):343-349.
18. Shaw LM, Vanderstichele H, Knapik-Czajka M, et al; Alzheimer's Disease Neuroimaging Initiative. Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol.* 2009;65(4):403-413.
19. Imle P. Fluorescence-based fragment size analysis. *Methods Mol Biol.* 2005;311:139-146.
20. Myers AJ, Gibbs JR, Webster JA, et al. A survey of genetic human cortical gene expression. *Nat Genet.* 2007;39(12):1494-1499.
21. Cruchaga C, Kauwe JS, Mayo K, et al; Alzheimer's Disease Neuroimaging Initiative. SNPs associated with cerebrospinal fluid phospho-tau levels influence rate of decline in Alzheimer's disease. *PLoS Genet.* 2010;6(9):pii: e1001101.
22. Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet.* 2003;73(5):1162-1169.
23. Huentelman MJ, Corneveaux J, Myers A, et al. Genome-wide association study for Alzheimer's disease risk in a large cohort of clinically characterized and neuropathologically verified subjects. In: *ICAD-2010*. Honolulu, Hawaii: Alzheimer's Association; 2010:16.
24. Kauwe JS, Cruchaga C, Mayo K, et al. Variation in MAPT is associated with cerebrospinal fluid tau levels in the presence of amyloid- $\beta$  deposition. *Proc Natl Acad Sci U S A.* 2008;105(23):8050-8054.
25. Kauwe JS, Jacquart S, Chakraverty S, et al. Extreme cerebrospinal fluid amyloid  $\beta$  levels identify family with late-onset Alzheimer's disease presenilin 1 mutation. *Ann Neurol.* 2007;61(5):446-453.
26. Kauwe JS, Cruchaga C, Bertelsen S, et al; Alzheimer's Disease Neuroimaging Initiative. Validating predicted biological effects of Alzheimer's disease associated SNPs using CSF biomarker levels. *J Alzheimers Dis.* 2010;21(3):833-842.
27. Jun G, Naj AC, Beecham GW, et al; Alzheimer's Disease Genetics Consortium. Meta-analysis confirms CR1, CLU, and PICALM as Alzheimer disease risk loci and reveals interactions with APOE genotypes. *Arch Neurol.* 2010;67(12):1473-1484.

### Announcement

Visit [www.archneuro.com](http://www.archneuro.com). As an individual subscriber, you may elect to be contacted when a specific article is cited. Receive an e-mail alert when the article you are viewing is cited by any of the journals hosted by HighWire. You will be asked to enter the volume, issue, and page number of the article you wish to track. Your e-mail address will be shared with other journals in this feature; other journals' privacy policies may differ from JAMA & Archives Journals. You may also sign up to receive an e-mail alert when articles on particular topics are published.