Genetic Comparison of Symptomatic and Asymptomatic Persons With Alzheimer Disease Neuropathology

Sarah E. Monsell, MS, Charles Mock, MD, PhD,† David W. Fardo, PhD,‡ Sarah Bertelsen, MA, JD,§ Nigel J. Cairns, PhD, FRCPath,‖ Catherine M. Roe, PhD,‖ Sally R. Ellingson, PhD,‡ John C. Morris, MD,‖ Alison M. Goate, D.Phil,§ and Walter A. Kukull, PhD†*

Objective: The objective was to determine whether symptomatic and asymptomatic persons with Alzheimer disease (AD) neuropathology have different allele counts for single-nucleotide polymorphisms that have been associated with clinical late-onset AD.

Methods: Data came from the National Alzheimer's Coordinating Center Uniform Data Set and Neuropathology Data Set, and the Alzheimer's Disease Genetics Consortium (ADGC). Participants had low to high AD neuropathologic change. The 22 known/suspected genes associated with late-onset AD were considered. "Symptomatic" was defined as Clinical Dementia Rating global score >0.

Results: Sixty-eight asymptomatic and 521 symptomatic participants met inclusion criteria. Single-nucleotide polymorphisms associated with *ABCA7* [odds ratio (OR) = 1.66; 95% confidence interval (CI), 1.03-2.85] and *MAPT* (OR = 2.18; CI, 1.26-3.77) were associated with symptomatic status. In stratified analyses, loci containing *CD2AP* (OR = 0.35; 95% CI, 0.16-0.74), *ZCWPW1* (OR = 2.98; 95% CI, 1.34-6.86), and *MAPT* (OR = 3.73, 95% CI, 1.30-11.76) were associated with symptomatic status in *APOE* e4 carriers.

Conclusions: These findings potentially explain some of the variation in whether a person with AD neuropathology expresses symptoms. Understanding why some people remain cognitively normal despite having AD neuropathology could identify pathways to disease heterogeneity and guide treatment trials.

Key Words: Alzheimer disease, Alzheimer disease genetics, APOE, genetic risk score, preclinical Alzheimer disease

(*Alzheimer Dis Assoc Disord* 2017;31:232–238)

The association between apolipoprotein E (*APOE*) and late-onset AD (LOAD) is well documented.[1,2] Associations between LOAD and other genetic loci are increasingly recognized: to date, 22 loci have been associated with risk of LOAD.[3–5] Most studies documenting these associations compared genetic profiles of clinically-diagnosed cases of dementia or mild cognitive impairment (MCI) with cognitively normal controls.[3,4,6–8] Recently, several studies assessed the association between the above-noted loci and AD neuropathology determined at autopsy, finding associations between 14 of the 22 genes and extent of neuropathologic change.[9–12]

In addition to increasing the extent of neuropathology, other genetic pathways might exist, including varying degrees of expression of the same extent of AD neuropathology.

Received for publication April 27, 2016; accepted October 9, 2016.

From the *Department of Biostatistics; †National Alzheimer's Coordinating Center, University of Washington, Seattle, WA; ‡Department of Biostatistics, University of Kentucky, Lexington, KY; §Dept. of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY; and ‖Department of Neurology, Knight Alzheimer's Disease Research Center, Washington University School of Medicine, St. Louis, MI.

The NACC database is funded by NIA/NIH Grant U01 AG016976. NACC data are contributed by the NIA-funded ADCs: P30 AG019610 (PI Eric Reiman, MD), P30 AG013846 (PI Neil Kowall, MD), P50 AG008702 (PI Scott Small, MD), P50 AG025688 (PI Allan Levey, MD, PhD), P30 AG010133 (PI Andrew Saykin, PsyD), P50 AG005146 (PI Marilyn Albert, PhD), P50 AG005134 (PI Bradley Hyman, MD, PhD), P50 AG016574 (PI Ronald Petersen, MD, PhD), P50 AG005138 (PI Mary Sano, PhD), P30 AG008051 (PI Steven Ferris, PhD), P30 AG013854 (PI M. Marsel Mesulam, MD), P30 AG008017 (PI Jeffrey Kaye, MD), P30 AG010161 (PI David Bennett, MD), P30 AG010129 (PI Charles DeCarli, MD), P50 AG016573 (PI Frank LaFerla, PhD), P50 AG016570 (PI David Teplow, PhD), P50 AG005131 (PI Douglas Galasko, MD), P50 AG023501 (PI Bruce Miller, MD), P30 AG035982 (PI Russell Swerdlow, MD), P30 AG028383 (PI Linda Van Eldik, PhD), P30 AG010124 (PI John Trojanowski, MD, PhD), P50 AG005133 (PI Oscar Lopez, MD), P50 AG005142 (PI Helena Chui, MD), P30 AG012300 (PI Roger Rosenberg, MD), P50 AG005136 (PI Thomas Montine, MD, PhD), P50 AG033514 (PI Sanjay Asthana, MD, FRCP), and P50 AG005681 (PI John Morris, MD).The Alzheimer's Disease Genetics Consortium (ADGC) supported the collection of samples used in this study through National Institute on Aging (NIA) grants U01AG032984 and RC2AG036528. The ADGC also generated and kindly provided genotype data. Samples from the National Cell Repository for Alzheimer's Disease (NCRAD), which receives government support under a cooperative agreement grant (U24 AG21886) awarded by the NIA, were used in this study.Support was also provided by *the NIA Genetics of Alzheimer's Disease Data Storage Site (NIAGADS) at the University of Pennsylvania* (U24 AG041689).

D.W.F. is supported by NIH grant K25-AG043546. S.E. reports support from the National Institutes of Health (NIH). National Center for Advancing Translational Science grant KL2TR000116. J.C.M.: Neither J.C.M. nor his family owns stock or has equity interest (outside of mutual funds or other externally directed accounts) in any pharmaceutical or biotechnology company. J.C.M. is currently participating in clinical trials of antidementia drugs (A4 trial: The Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease), funded by the National Institute on Aging, Eli Lilly and Company, and several philanthropic organizations. J.C.M. has served as a consultant for Lilly, USA and Takeda Pharmaceuticals. He receives research support from Eli Lilly/Avid Radiopharmaceuticals and is funded by NIH grants # P50AG005681; P01AG003991; P01AG026276; and UF01AG032438. A.M.G. has served as a consultant for Cognition Therapeutics, Denali Therapeutics, AbbVie, and Amgen. She also was a speaker at Eli Lilly. She is funded by NIH grants R01 AG035083 and U01AG049508. The remaining authors declare no conflicts of interest.

Reprints: Charles Mock, MD, PhD, University of Washington, National Alzheimer's Coordinating Center, 4311 11th Ave NE #300, Campus Box 354983, Seattle, WA 98105 (e-mail: cmock@uw.edu).

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Website, www.alzheimerjournal.com.

APOE (ε4 carrier vs. noncarrier) has been shown to be associated with increased risk of dementia in people with underlying AD neuropathology, even after adjustment for extent of pathology. The presence of the *APOE* ε4 allele might thus be associated with pathologic processes not assessed at autopsy, such as greater levels of toxic Aβ oligomers.[13–15]

Potential relationships between clinical expression of AD neuropathology and other (non-*APOE*) loci associated with LOAD have not been adequately explored. We thus sought to determine whether symptomatic and asymptomatic persons with AD neuropathology have different allele counts for loci that have been associated with clinical AD.

METHODS

Study Sample

Individuals in this study were research participants assessed with the Uniform Data Set (UDS) evaluation at one of 34 past and present National Institute on Aging/NIH Alzheimer's Disease Centers (NIA ADCs) and whose data were submitted to the National Alzheimer's Coordinating Center (NACC) between 2005 and 2014. The UDS comprises data on demographics, health history, neuropsychological tests, and a complete neurological exam, for participants with normal cognition, MCI, and dementia. Participants with normal cognition are volunteers who are followed with the same study procedures as symptomatic participants.[16] Neuropathology data are available for a subset of UDS participants who consent to autopsy. Genetic data were obtained from the Alzheimer's Disease Genetics Consortium (ADGC), which houses genotype data for UDS participants and other cohorts (https://alios.med.upenn.edu/adgc).

Standard Protocol Approvals, Registrations, and Patient Consents

All participants provided written informed consent. The University of Washington Institutional Review Board-approved research using the NACC database.

Defining Neuropathologic AD

A low, medium, or high level of AD neuropathologic change according to modified NIA-Alzheimer's Association ABC criteria[17,18] was required of all participants. The ABC score comprises 3 criteria. Two of these (B score for Braak stage for neurofibrillary tangles[19] and C score for CERAD neuritic plaque frequency[20]) are recorded on all versions of the NACC Neuropathology Form. However, A score (Thal phase[21] for Aβ plaques) was not included on the Form before version 10 (implemented 2014), and thus is not available for most NACC participants. To include participants with an early form of Aβ plaque formation, we included those with "diffuse plaque," defined as plaques with no apparent dystrophic neurites, as detected by silver impregnation methods, ubiquitin, tau immunohistochemistry, or Aβ immunohistochemistry.

Participants with sparse, moderate, or frequent diffuse plaques were considered to have a Thal Aβ plaque phase of 1 or higher and thus met inclusion criteria for this study. Similarly, participants with sparse, moderate, or frequent neuritic plaques had a neuritic plaque C score of 1 or higher and met inclusion criteria. Limiting the sample to participants with either neuritic or diffuse plaques approximates to inclusion of all participants who meet NIA-Alzheimer's Association criteria for low to high AD neuropathologic change. The resulting study sample included only participants with Aβ plaques, regardless of clinical diagnosis.[13,22]

We excluded participants with a primary neuropathologic diagnosis of dementia with Lewy bodies. This data element is available in versions 1-9 of the Neuropathology Form. For the few participants assessed with version 10, we performed a conservative exclusion, removing those for whom any Lewy bodies were reported.

Defining Asymptomatic AD

Symptoms were defined using the Clinical Dementia Rating global score (CDR), an instrument that grades cognitive and functional abilities.[23] Participants with CDR global score of 0 at their last clinical assessment were defined as having normal cognition, and formed the asymptomatic group. Participants with CDR global score of 0.5 or higher were defined as having clinical characteristics consistent with MCI or dementia and formed the symptomatic group.[13,22] To best correlate symptoms and neuropathologic features, we limited the analytic sample to participants who died within 1 year of the last UDS clinical assessment.

Genetic Data

Genetic data were obtained from ADGC. These data were drawn from blood or brain tissue samples sent by individual ADCs. Imputation was used to generate a common set of single-nucleotide polymorphisms (SNPs). Imputations with probability ≥ 0.90 were included; imputations below this threshold were considered missing data. Uniform stringent quality control measures were applied to remove low-quality and duplicate samples and problematic SNPs. Data were transferred from PLINK format to Excel for additional quality assurance and statistical analysis.

All participants had missing data for no >4 SNPs. Three SNPs (in loci containing *BIN1*, *CLU*, and *MAPT*) had missing data for $>10\%$ of participants meaning the SNP was not genotyped and there was no proxy with good quality data to advise imputation. Analyses were performed on available data, but interpreted with caution for these SNPs. For the SNP associated with *DSG2*, analysis performed in *APOE* ε4 carriers was not possible due to inadequate variation in allele frequency. Of the 593 participants meeting study inclusion criteria, the 589 of European decent were retained for analysis to decrease potential effects of population stratification.

Data for this study included allelic count for 22 genes known or suspected to be associated with LOAD.[3–5] All SNPs satisfied the Hardy-Weinberg equilibrium at the 0.001 α level.

Statistical Analysis

Descriptive statistics (frequencies, percentages, χ^2 tests, Fisher exact tests) were calculated for demographic and neuropathologic features for asymptomatic and symptomatic participants. For each SNP, the risk allele was that allele (major or minor) associated with higher odds of AD in the literature.[4,5] For robustness to model misspecification, we assume the conventional additive mode of inheritance and use the number of risk alleles (0, 1, or 2) as the genetic predictor variable.[24] We then applied a logistic model where symptomatic AD status was the dependent variable. This model was fit for each SNP individually. To account for potential differences between *APOE* ε4 carriers and noncarriers, we performed stratified analyses. All

TABLE 1. Participant Demographics, Clinical and Neuropathologic Characteristics

	n (%)		
	Asymptomatic AD (N = 68)	**Symptomatic AD (N = 521)**	***P* from χ^2 Test***
Age at death (y)			0.41
< 60	0 (0)	8 (1)	
60-69	3 (4)	53 (10)	
70-79	14 (21)	99 (19)	
80-89	27 (40)	217 (42)	
90 +	24 (35)	144 (28)	
Sex			0.11
Female	30 (44)	287 (55)	
Male	38 (56)	234 (45)	
Education†			0.20
No college	13 (19)	149 (29)	
1-4 y of college	31 (46)	221 (43)	
At least some graduate school	24 (35)	145 (28)	
APOE			< 0.0001
Noncarrier (0 ε4 alleles)	52 (76)	245 (47)	
Heterozygous (1 ε4 allele)	16 (24)	226 (43)	
Homozygous (2 ε4 alleles)	0 (0)	50 (10)	
Braak stage			< 0.0001
0	1 (1)	4 (1)	
I-II	34 (50)	44 (8)	
III-IV	27 (40)	126 (24)	
V-VI	6 (9)	347 (67)	
CERAD neuritic plaque frequency			< 0.0001
None	12 (18)	16 (3)	
Sparse	24 (35)	69 (13)	
Moderate	18 (26)	134 (26)	
Frequent	14 (21)	302 (58)	
Diffuse plaque frequency‡			< 0.0001
None	1 (2)	10 (2)	
Sparse	23 (41)	61 (13)	
Moderate	13 (23)	91 (19)	
Frequent	19 (34)	318 (66)	
Infarcts or lacunes§			0.91
Not present	50 (74)	390 (75)	
Present	18 (26)	130 (25)	
Hemorrhorages and microbleeds			> 0.999
Not present	64 (94)	490 (94)	
Present	4 (6)	31 (6)	
Arteriosclerosis‖			0.04
None	8 (14)	66 (15)	
Mild	31 (55)	165 (37)	
Moderate	14 (25)	141 (31)	
Severe	3 (6)	79 (17)	
Lewy body pathology¶			0.002
Not present	63 (93)	385 (75)	
Present	5 (7)	131 (25)	
Cerebral amyloid angiopathy#			0.0003
Not present	56 (85)	312 (61)	
Present	10 (15)	196 (39)	

*The Fisher exact test performed for age at death, *APOE* ε4, Braak stage, diffuse plaques, hemorrhages and microbleeds, and arteriosclerosis.

†Six symptomatic participants missing information on years of education.

‡Twelve asymptomatic and 41 symptomatic participants were missing information on diffuse plaque frequency.

§One symptomatic participant was missing information on infarcts and lacunes.

‖Twelve asymptomatic and 70 symptomatic participants were missing information on arteriosclerosis.

¶Five symptomatic participants were missing information on Lewy body pathology.

#Two asymptomatic and 13 symptomatic participants were missing information on cerebral amyloid angiopathy.

AD indicates Alzheimer's disease.

models were adjusted for sex and age at death (continuous). Additional adjustment for years of education returned nearly identical odds ratios (ORs) and confidence intervals (CIs) (results not shown) as those models adjusted for sex and age at death alone. However, including education in the models required dropping 6 subjects with missing data. Hence, the main results presented herein are for the analyses adjusted for sex and age at death.

A risk score was calculated by multiplying the number of risk alleles by the corresponding log-transformed odds ratio from the individual models and summed across all 22 SNPs,[6] across the 21 SNPs with <15% missing data

(*MAPT* excluded), and across the 19 SNPs with < 10% missing data (*BIN1, CLU,* and *MAPT* excluded). The risk score was calculated for the entire sample and calculated separately for *APOE* ε4 carriers and noncarriers. The risk score was then included in a logistic regression model adjusted for sex and age at death.

Each gene was tested separately for association with symptomatic status and an alpha level of 0.05 was considered as significant in all tests of statistical significance.

All analyses were performed in R version 3.1.1.

RESULTS

At the time of data abstraction, there were 1127 UDS participants with genotype data and neuropathology data available. Of these, 999 met criteria for AD neuropathologic changes. An additional 77 participants were excluded for Lewy body pathology and 329 were excluded for not having a clinical examination within 1 year of death. Excluding the 4 African American and multiracial participants resulted in a sample size of 589 participants, 68 asymptomatic and 521 symptomatic participants. Demographic and neuropathologic features are shown in Table 1 and Supplemental e-Table 1 (Supplemental Digital Content 1, http://links.lww.com/WAD/A149). Notably, symptomatic participants were slightly younger than asymptomatic participants and more often had at least 1 *APOE* ε4 allele. They also had more advanced AD pathology (neuritic plaques and Braak stage for neurofibrillary tangles), as well as more arteriolosclerosis, Lewy bodies, and cerebral amyloid angiopathy.

In the adjusted logistic regression model assuming an additive model of inheritance, the SNP associated with the gene *ABCA7* met statistical significance at the 0.05 α level (OR = 1.66; 95% CI, 1.03-2.85; $P = 0.049$, as did the SNP associated with gene *MAPT* (OR = 2.18; CI, 1.26-3.77; $P = 0.005$) for association with symptomatic status.

In analyses stratified by *APOE* ε4 carrier status, the SNPs linked to *CD2AP*, *ZCWPWI*, and *MAPT* were associated with odds of symptomatic status in ε4 carriers (OR = 0.35, 2.98, and 3.73; 95% CI, 0.16-0.74, 1.34-6.86, and 1.30-11.76; $P = 0.007$, 0.008, and 0.017, respectively). No significant findings were observed in noncarriers. Full results for each of the 22 SNPs are presented in Table 2. Each of the SNPs was tested for interactions with APOE. Only 2 of the SNPs had significant interactions: CD2AP (OR = 0.38; 95% CI, 0.16-0.92; $P = 0.03$) and ZCWPW1 (OR = 2.93; 95% CI, 1.17-7.57; $P = 0.02$).

The genetic risk score was associated with increased odds of being symptomatic in all participants (OR = 2.61; 95% CI, 1.61-4.36; $P = 0.0002$) and among *APOE* ε4 carriers and noncarriers when the score included the 19 SNPs with mostly complete data. When all SNPs, except MAPT, which was missing for ∼40% of participants, were considered, the risk score remained significant. For both contingencies (19 or 21 SNPs in risk score), the effect size was

TABLE 2. Odds Ratio (Adjusted for Age and Sex) for Symptomatic AD Versus Asymptomatic AD for Each SNP

Gene	SNP	Risk Allele	OR From IGAP	OR (95% CI)	OR (95% CI) for *APOE* ε4 Carriers (1 or 2 ε4 Alleles)	OR (95% CI) for *APOE* ε4 Noncarriers (0 ε4 Alleles)
ABCA7	rs4147929	A	1.15	**1.66 (1.03-2.85)**	1.25 (0.55-3.34)	1.81 (1.00-3.57)
BIN1	rs6733839	T	1.22	0.96 (0.63-1.47)	1.02 (0.42-2.67)	1.06 (0.65-1.76)
CASS4	rs7274581	T	0.88*	1.12 (0.59-1.97)	1.53 (0.46-3.98)	1.03 (0.46-2.09)
CD2AP	rs10948363	G	1.10	0.73 (0.50-1.09)	**0.35 (0.16-0.74)**	0.99 (0.62-1.62)
CD33	rs3865444	C	0.94*	1.26 (0.86-1.82)	1.28 (0.59-2.69)	1.44 (0.93-2.23)
CELF1	rs10838725	C	1.08	0.99 (0.67-1.47)	0.70 (0.34-1.50)	1.14 (0.71-1.87)
CLU	rs9331896	T	0.86*	0.83 (0.55-1.25)	1.18 (0.47-2.86)	0.73 (0.44-1.18)
CR1	rs6656401	A	1.18	1.46 (0.87-2.57)	1.56 (0.59-5.35)	1.44 (0.78-2.83)
DSG2	rs8093731	C	0.73*	0.96 (0.05-5.41)	NA	0.94 (0.05-5.98)
EPHA1	rs11771145	G	0.90*	0.96 (0.65-1.40)	1.29 (0.58-2.75)	0.88 (0.56-1.37)
FERMT2	rs17125944	C	1.14	1.69 (0.81-4.10)	3.05 (0.60-55.84)	1.41 (0.63-3.77)
HLA-DRB5/ HLA-DRB1	rs9271192	C	1.11	1.01 (0.68-1.52)	0.93 (0.42-2.19)	1.03 (0.65-1.68)
INPP5D	rs35349669	T	1.08	0.88 (0.61-1.28)	1.07 (0.49-2.28)	0.75 (0.48-1.15)
MAPT	rs393152	A	NA	**2.18 (1.26-3.77)**	**3.73 (1.30-11.76)**	1.77 (0.92-3.42)
MEF2C	rs190982	A	0.93*	1.24 (0.84-1.82)	1.03 (0.44-2.34)	1.15 (0.74-1.81)
MS4A4A	rs983392	A	0.90*	1.29 (0.88-1.90)	1.57 (0.72-3.43)	1.19 (0.75-1.90)
NME8	rs2718058	A	0.93*	0.77 (0.51-1.14)	0.89 (0.38-1.98)	0.74 (0.45-1.18)
PICALM	rs10792832	G	0.87*	1.16 (0.79-1.69)	1.03 (0.45-2.27)	1.15 (0.75-1.77)
PTK2B	rs28834970	C	1.10	1.22 (0.85-1.78)	1.38 (0.68-3.02)	1.10 (0.71-1.72)
SLC24A4/ RIN3	rs10498633	G	0.91*	1.24 (0.82-1.86)	1.50 (0.63-3.36)	1.15 (0.70-1.84)
SORL1	rs11218343	T	0.77*	1.72 (0.62-4.08)	2.99 (0.63-10.64)	1.45 (0.32-4.97)
ZCWPW1	rs1476679	T	0.91*	1.31 (0.87-1.95)	**2.98 (1.34-6.86)**	1.04 (0.62-1.69)

ORs should be interpreted as the odds of symptomatic versus asymptomatic for an increase of 1 risk allele. For example, the odds of symptomatic AD for rs6656401 is 18% higher for a participant with 1 risk allele versus no risk alleles. Because of missing values, the number of participants in each cell varies: all participants: max = 589, min = 473; *APOE* ε4 carriers: max = 292, min = 234; *APOE* ε4 noncarriers: max = 297, min = 239 for all SNPs except the 1 associated with *MAPT*. For *MAPT*, n = 345 for all participants, n = 174 for *APOE* ε4 carriers, and n = 171 for *APOE* ε4 noncarriers. SNPs in bold were significant at the 0.05 α level.

ORs for IGAP are for minor allele count. ORs for the current study are for risk allele count. ORs denoted by "" indicate when the major allele is the risk allele. Hence, ORs denoted by "*" would be expected to be in the opposite direction for this study compared with IGAP.

AD indicates Alzheimer disease; CI, confidence interval; IGAP, International Genomics of Alzheimer's Project; NA, not applicable; data too sparse for analysis; OR, odds ratio; SNP, single-nucleotide polymorphism.

TABLE 3. Genetic Risk Score for SNP Association With Symptomatic AD, Adjusted for Age at Death and Sex

	19 SNPs With <10% Missing Data*			21 SNPs With <15% Missing Data†			All 22 SNPs		
	n	OR	95% CI	n	OR	95% CI	n	OR	95% CI
All participants	419	2.61	(1.61-4.36)	300	2.15	(1.24-3.88)	184	1.87	(1.02-3.60)
APOE ε4 carriers	201	5.01	(1.65-18.54)	143	6.01	(1.20-44.37)	93	NA	
APOE ε4 noncarriers	218	2.27	(1.30-4.10)	157	1.97	(1.05-3.84)	91	1.77	(0.87-3.82)

APOE ε4 carriers are those subjects with 1 or 2 ε4 alleles, whereas noncarriers contain 0 ε4 alleles. Also note that the "n" for each model is the overall n for both symptomatic and asymptomatic subjects.

*The SNPs associated with *BIN1, CLU,* and *MAPT* were not included.

†The SNP associated with *MAPT* was not included.

CI indicates confidence interval; NA, not applicable; data too sparse for analysis (1 cell in the analysis had < 5 entries); OR, odds ratio.

higher among *APOE* ε4 carriers. When all 22 SNPs (including MAPT) were included, the risk score was still significant in all participants, but sample size was too small for meaningful conclusions on APOE ε4 strata (Table 3).

Sensitivity Analysis

The main analysis showed the association with outcome for each SNP, adjusted for age and sex. A sensitivity analysis added adjustment for the following neuropathologic features: vascular disease, Braak stage, Lewy bodies, and cerebral amyloid angiopathy. One of the SNPs that had been significantly associated with symptomatic status (MAPT) remained significantly associated. One of the SNPs (CD2AP) that was significantly associated with symptomatic status only in the *APOE* ε4-positive strata became significant in the entire group. We also undertook a principal component analysis. Both SNPs that were associated with symptomatic status in the main analysis (ABCA7 and MAPT) remained significant in the additional principal component analysis (Supplemental e-Table 2, Supplemental Digital Content 2, http://links.lww.com/WAD/A150). Finally, we created an additional genetic risk score based on published ORs of developing AD. This was also significantly associated with development of symptoms (Supplemental e-Table 3, Supplemental Digital Content 3, http://links.lww.com/WAD/A151). However, it should be emphasized that these published ORs were derived based on risk of developing AD, whereas the genetic risk score in Table 3 were derived based on risk of expressing symptoms once someone already has AD neuropathology, which is a different concept.

DISCUSSION

We sought to determine whether cognitively symptomatic persons with AD neuropathologic change and asymptomatic persons with AD neuropathologic change have different allele counts for loci that have been previously associated with clinical AD. This potential association has not been well addressed. We found that *ABCA7* and *MAPT* were significantly associated with expression of symptoms. The loci containing *CD2AP* and *ZCWPW1* were significantly associated with symptoms, but only in the *APOE* ε4 positive strata.

The association of *ABCA7* with altered risk of clinical AD has been well documented.[1,2,25] Similarly to *APOE*, *ABCA7* is involved with cholesterol and lipid metabolism.[26] It is also involved with immune function.[2] It is not certain whether *ABCA7*'s effect on altering AD risk is through its effects on immune function, lipid metabolism, or both.[2] *ABCA7* has also been postulated to influence AD risk by clearing Aβ aggregates.[27,28]

For *CD2AP* and *ZCWPW1*, there are multiple genes within the loci that have been associated with increased LOAD risk.[1,2,4,9] Hence, it would be premature to postulate on potential biologic mechanisms of action, except to note that alterations in *CD2AP* have been associated with increased neuritic plaque burden in brains already having AD.[1,9] It has also been postulated to be involved with modulating Aβ clearance and suppression of Aβ toxicity.[2]

MAPT, the gene encoding tau, was known to be associated with Parkinson's disease, progressive supranuclear palsy, and corticobasal degeneration[29] but has only recently been shown to be associated with AD. It is possible that there are different genes at this locus that account for separate effects on AD and Parkinson's disease.[5]

There are several possible pathways by which *ABCA7, CD2AP*, *MAPT,* and *ZCWPW1* might influence risk of expression of symptoms in people with underlying AD neuropathology. These pathways can be considered in the 3 categories that have been postulated as mechanisms by which LOAD susceptibility alleles might affect the risk of clinical AD: (1) Aβ deposition; (2) downstream pathologic effects such as synaptic loss and neuronal death; and (3) other mechanisms, not related to AD, that contribute to cognitive change.[9]

First, *ABCA7* and *CD2AP* have been associated with increased neuritic plaque burden.[9] In the current study, although all persons met a minimum threshold for AD neuropathology, the symptomatic group had more frequent neuritic plaques. Hence, the effect the loci identified in this study on symptoms might be through known existing pathways of increasing extent of AD neuropathology.

Second, the above-noted clearance functions of *ABCA7* and *CD2AP* might also involve toxic Aβ oligomers. Also, *CD2AP* has been noted to have a direct effect in suppressing Aβ toxicity.[2] Finally, *CD2AP* is involved in synapse formation and thus may have pathologic effects downstream to AD neuropathology.[1,30] These effects of *ABCA7* and *CD2AP* might work through increasing the extent of neurofibrillary tangles and thus the Braak NFT stage. *MAPT* has likewise been shown to be involved with several tauopathies and might exert similar effects.[5] Abnormal tau and amyloid deposits may interact synergistically to cause AD,[31] perhaps through the abnormal tau upregulating the neurotoxicity of Aβ or through intensifying the mitochondrial damage caused by Aβ.[32,33]

Third, *ABCA7* is involved with cholesterol and lipid metabolism. The symptomatic group had a higher proportion of cerebrovascular disease, which may have

contributed to their higher odds of expressing cognitive symptoms. In addition to *APOE* ε4's effect on AD neuropathology, *APOE* ε4 carriers have increased risk of vascular dementia.[34] Although similar effects have not been demonstrated in humans for *ABCA7*, alterations in risk of vascular disease associated with alterations in *ABCA7* function have been noted in mouse models.[35]

Finally, a logical pathway through which genes might influence risk of cognitive symptoms among persons who already have AD neuropathology is through immune response. *ABCA7* does have immune functions in addition to its effect on lipid metabolism.[1,2] *ZCWPW1* has been found to be related to expression of PILRB, a microglia expressed gene tied to neuroinflammation.[36] However, none of the other genes thought to affect AD risk through immune response (*CR1, CD33, MS4A, CLU*, *EPHA1*)[1] had significant associations in the current study.

Several findings from the current study do not have ready explanations. The loci containing *CD2AP* and *ZCWPW1* have significant effects on expression of symptoms, but only in the *APOE* ε4-positive strata. For *CD2AP* that effect is in the opposite direction of the effect found in the existing literature.[3,4,6–8] It is not surprising that some SNPs might have effects in the opposite direction from that reported in the literature. ORs reported from the literature assess risk of developing AD, whereas the current study assesses a different characteristic, expression of cognitive symptoms once someone already has underlying AD neuropathology. Similarly, the effect of the genetic risk score was more pronounced among people with *APOE* ε4-positive status. One prior study found a stronger association with AD for *CR1* and *CLU* in *APOE* ε4-positive strata and for the *MSA4* gene cluster in *APOE* ε4-negative strata.[37] Another study found an increased risk of clinical AD for carriers of MAPT H1 haplotype, but only in *APOE* ε4-negative strata.[38] The current study found changes in MAPT to be associated with altered risk of cognitive symptoms, primarily driven by altered risk in the *APOE* ε4-positive strata.

These findings can also be compared with the literature on genome-wide association studies of AD neuropathology. In a genome-wide association studies meta-analysis of demented participants with moderate to high AD neuropathology versus non-demented participants with no or low AD neuropathology, the authors found an association with *ABCA7*. The strength of that association (OR = 1.24 to 1.32) was higher than previously reported (OR = 1.15). The value from the current study (OR = 1.66) is higher, but has 95% CIs that encompass both of the prior estimates.[4,12]

Before drawing conclusions, the study limitations must be addressed. First, the sample size was limited, especially in the asymptomatic group. Although significant associations were detected in several loci, other associations might not have been detectable.

Second, 70% of participants were aged 80 years or older. Several loci associated with LOAD have been associated with earlier onset of AD, including *APOE*, *CR1, BIN1*, and *PICALM*.[3] The ability of our study to detect differences might be diminished by the narrow age range studied.

Third, many participants who met clinical and neuropathologic criteria did not have available genetic data. Since both clinical AD cases and controls were selected by ADGC for study, the authors are not aware of a mechanism of selecting participants that would have influenced our results.

Fourth, the sample of subjects used in calculating the risk score was the same as the set used to determine the ORs. Although this approach is not ideal, it is sufficient for a priori hypothesis testing. Future work building risk score prediction models should employ a different sample to validate and expand on these findings.

We found differences in several SNPs (rs4147929, rs10948363, rs1476679, and rs393152; corresponding to *ABCA7* and loci containing *CD2AP*, *ZCWPW1*, and *MAPT*, respectively) between symptomatic and asymptomatic persons, all of whom had AD neuropathology. These findings potentially explain some of the variation in whether a person with AD neuropathology expresses symptoms. Understanding why some people remain cognitively normal despite having AD neuropathology could identify pathways to disease heterogeneity and guide treatment trials.

ACKNOWLEDGMENTS

The authors would like to thank all of the Alzheimer's Disease Center participants who volunteered for this study.

REFERENCES

1. Karch CM, Goate AM. Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biol Psychiatry*. 2015;77:43–51.
2. Rosenthal SL, Kamboh MI. Late-onset Alzheimer's disease genes and the potentially implicated pathways. *Curr Genet Med Rep*. 2014;2:85–101.
3. Naj AC, Jun G, Beecham GW, et al. Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet*. 2011;43:436–441.
4. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet*. 2013;45:1452–1458.
5. Desikan RS, Schork AJ, Wang Y, et al. Genetic overlap between Alzheimer's disease and Parkinson's disease at the MAPT locus. *Mol Psychiatry*. 2015;20:1588–1595.
6. Verhaaren BFJ, Vernooij MW, Koudstaal PJ, et al. Alzheimer's disease genes and cognition in the nondemented general population. *Biol Psychiatry*. 2013;73:429–434.
7. Miyashita A, Koike A, Jun G, et al. SORL1 is genetically associated with late-onset Alzheimer's disease in Japanese, Koreans and Caucasians. *PloS One*. 2013;8:e58618.
8. Hollingworth P, Harold D, Sims R, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet*. 2011;43:429–435.
9. Shulman JM, Chen K, Keenan BT, et al. Genetic susceptibility for Alzheimer disease neuritic plaque pathology. *JAMA Neurol*. 2013;70:1150–1157.
10. Chibnik LB, Shulman JM, Leurgans SE, et al. CR1 is associated with amyloid plaque burden and age-related cognitive decline. *Ann Neurol*. 2011;69:560–569.
11. Kramer PL, Xu H, Woltjer RL, et al. Alzheimer disease pathology in cognitively healthy elderly: a genome-wide study. *Neurobiol Aging*. 2011;32:2113–2122.
12. Beecham GW, Hamilton K, Naj AC, et al. Genome-wide association meta-analysis of neuropathologic features of Alzheimer's disease and related dementias. *PLoS Genet*. 2014;10:e1004606. doi:10.1371/journal.pgen.1004606.
13. Monsell SE, Mock C, Roe CM, et al. Comparison of symptomatic and asymptomatic persons with Alzheimer disease neuropathology. *Neurology*. 2013;80:2121–2129.
14. Kim J, Basak JM, Holtzman DM. The role of apolipoprotein E in Alzheimer's disease. *Neuron*. 2009;63:287–303.
15. Koffie RM, Hashimoto T, Tai H-C, et al. Apolipoprotein E4 effects in Alzheimer's disease are mediated by synaptotoxic oligomeric amyloid-β. *Brain J Neurol*. 2012;135(pt 7):2155–2168.

16. Beekly DL, Ramos EM, Lee WW, et al. The National Alzheimer's Coordinating Center (NACC) database: the Uniform Data Set. *Alzheimer Dis Assoc Disord*. 2007;21:249–258.
17. Hyman BT, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimers Dement*. 2012;8:1–13.
18. Montine TJ, Phelps CH, Beach TG, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. *Acta Neuropathol (Berl)*. 2012;123:1–11.
19. Braak H, Alafuzoff I, Arzberger T, et al. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol (Berl)*. 2006;112:389–404.
20. Mirra SS, Heyman A, McKeel D, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology*. 1991;41:479–486.
21. Thal DR, Rüb U, Orantes M, et al. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology*. 2002;58:1791–1800.
22. Monsell SE, Mock C, Hassenstab J, et al. Neuropsychological changes in asymptomatic persons with Alzheimer disease neuropathology. *Neurology*. 2014;83:434–440.
23. Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology*. 1993;43:2412–2414.
24. McCarthy MI, Hirschhorn JN. Genome-wide association studies: potential next steps on a genetic journey. *Hum Mol Genet*. 2008;17(R2):R156–R165.
25. Steinberg S, Stefansson H, Jonsson T, et al. Loss-of-function variants in ABCA7 confer risk of Alzheimer's disease. *Nat Genet*. 2015;47:445–447.
26. Quazi F, Molday RS. Differential phospholipid substrates and directional transport by ATP-binding cassette proteins ABCA1, ABCA7, and ABCA4 and disease-causing mutants. *J Biol Chem*. 2013;288:34414–34426.
27. Chan SL, Kim WS, Kwok JB, et al. ATP-binding cassette transporter A7 regulates processing of amyloid precursor protein in vitro. *J Neurochem*. 2008;106:793–804.
28. Kim WS, Li H, Ruberu K, et al. Deletion of Abca7 increases cerebral amyloid-β accumulation in the J20 mouse model of Alzheimer's disease. *J Neurosci Off J Soc Neurosci*. 2013;33:4387–4394.
29. Simón-Sánchez J, Schulte C, Bras JM, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet*. 2009;41:1308–1312.
30. Cormont M, Metón I, Mari M, et al. CD2AP/CMS regulates endosome morphology and traffic to the degradative pathway through its interaction with Rab4 and c-Cbl. *Traffic Cph Den*. 2003;4:97–112.
31. Zhang C-C, Xing A, Tan M-S, et al. The role of MAPT in neurodegenerative diseases: genetics, mechanisms and therapy. *Mol Neurobiol*. 2016;53:4893–4904.
32. Bhaskar K, Yen S-H, Lee G. Disease-related modifications in tau affect the interaction between Fyn and Tau. *J Biol Chem*. 2005;280:35119–35125.
33. Quintanilla RA, von Bernhardi R, Godoy JA, et al. Phosphorylated tau potentiates Aβ-induced mitochondrial damage in mature neurons. *Neurobiol Dis*. 2014;71:260–269.
34. Sun J-H, Tan L, Wang H-F, et al. Genetics of vascular dementia: systematic review and meta-analysis. *J Alzheimers Dis JAD*. 2015;47:565–581.
35. Meurs I, Calpe-Berdiel L, Habets KLL, et al. Effects of deletion of macrophage ABCA7 on lipid metabolism and the development of atherosclerosis in the presence and absence of ABCA1. *PloS One*. 2012;7:e30984.
36. Karch CM, Ezerskiy LA, Bertelsen S. Alzheimer's Disease Genetics Consortium, Goate AM. Alzheimer's disease risk polymorphisms regulate gene expression in the ZCWPW1 and the CELF1 Loci. *PLoS One*. 2016;11:e0148717. doi: 10.1371/journal.pone.0148717. eCollection 2016.
37. Jun G, Ibrahim-Verbaas CA, Vronskaya M, et al. A novel Alzheimer disease locus located near the gene encoding tau protein. *Mol Psychiatry*. 2016;21:108–117.
38. Pastor P, Moreno F, Clarimón J, et al. MAPT H1 haplotype is associated with late-onset Alzheimer's disease risk in APOEε4 noncarriers: results from the Dementia Genetics Spanish Consortium. *J Alzheimers Dis*. 2015;49:343–352.