Original Investigation

Genetic Susceptibility for Alzheimer Disease Neuritic Plaque Pathology

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IMPORTANCE While numerous genetic susceptibility loci have been identified for clinical Alzheimer disease (AD), it is important to establish whether these variants are risk factors for the underlying disease pathology, including neuritic plaques.

OBJECTIVES To investigate whether AD susceptibility loci from genome-wide association studies affect neuritic plaque pathology and to additionally identify novel risk loci for this trait.

DESIGN, SETTING, AND PARTICIPANTS Candidate analysis of single-nucleotide polymorphisms and genome-wide association study in a joint clinicopathologic cohort, including 725 deceased subjects from the Religious Orders Study and the Rush Memory and Aging Project (2 prospective, community-based studies), followed by targeted validation in an independent neuroimaging cohort, including 114 subjects from multiple clinical and research centers.

MAIN OUTCOMES AND MEASURES A quantitative measure of neuritic plaque pathologic burden, based on assessments of silver-stained tissue averaged from multiple brain regions. Validation based on β-amyloid load by immunocytochemistry, and replication with fibrillar β-amyloid positron emission tomographic imaging with Pittsburgh Compound B or florbetapir.

RESULTS Besides the previously reported *APOE* and *CR1* loci, we found that the *ABCA7* (rs3764650; $P = .02$) and *CD2AP* (rs9349407; $P = .03$) AD susceptibility loci are associated with neuritic plaque burden. In addition, among the top results of our genome-wide association study, we discovered a novel variant near the amyloid precursor protein gene (*APP*, rs2829887) that is associated with neuritic plaques ($P = 3.3 \times 10^{-6}$). This polymorphism was associated with postmortem β-amyloid load as well as fibrillar β-amyloid in 2 independent cohorts of adults with normal cognition.

CONCLUSIONS AND RELEVANCE These findings enhance understanding of AD risk factors by relating validated susceptibility alleles to increased neuritic plaque pathology and implicate common genetic variation at the *APP* locus in the earliest, presymptomatic stages of AD.

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Alzheimer disease (AD) is the most common cause of age-related cognitive impairment and dementia. At autopsy, AD neuritic plaque pathology consists of extracellular aggregates of the β-amyloid (Aβ) peptide, which is derived from proteolysis of the amyloid precursor protein (APP). Rare gene mutations in *APP* along with the presenilin 1 gene (*PSEN1*) and presenilin 2 gene (*PSEN2*), encoding components of the γ-secretase enzyme involved in APP processing, cause early-onset familial AD.[1] Further, an uncommon *APP* coding variant (frequency <1%) was recently discovered to be protective against AD in the Icelandic population.[2] However, common genetic variation at these loci has not been definitively linked to the later-onset form of disease that accounts for the majority of AD in the population.[3-6] Common polymorphisms in the apolipoprotein E gene (*APOE*) are well-established risk factors for late-onset AD, and recent investigations suggest that *APOE* participates in the aggregation and/or clearance of Aβ within the central nervous system.[7] Genome-wide association studies (GWAS) have identified several additional AD susceptibility loci,[8-12] and emerging evidence suggests that alterations in Aβ dynamics may explain some of these associations. One susceptibility locus, clusterin (*CLU*), encodes an apolipoprotein that binds Aβ, similar to APOE, and these proteins may jointly regulate Aβ accumulation.[13] In addition, we previously reported that the effects of both *APOE* and complement receptor 1 (*CR1*) on memory decline are mediated in part by an association with neuritic plaque burden,[14,15] and the complement pathway has also been implicated in brain Aβ deposition in murine models.[16] Lastly, the phosphatidylinositol-binding clathrin assembly protein gene (*PICALM*) has been shown to modulate Aβ toxicity in a yeast model system and in rat cortical neuron culture.[17] These studies reinforce the broader hypothesis that genetic networks affecting Aβ accumulation and/or aggregation in the brain may be important determinants of AD susceptibility. However, it is possible that not all AD risk variants will similarly affect the development of neuritic plaque pathology, as many other cellular processes likely impinge on the clinical manifestation of disease. Notably, genome-wide meta-analyses have identified a number of additional susceptibility loci with still undefined roles in AD pathogenesis.[9,10]

Although gene discovery in AD has largely focused on clinically diagnosed disease in case-control studies, it is now recognized that the AD pathophysiological process, including Aβ deposition in the brain, begins years before the development of dementia. In prospective, community-based autopsy studies, about 90% of persons with clinically diagnosed AD have the disease proven at autopsy.[18,19] However, AD is also the predominant brain lesion accounting for mild cognitive impairment, now recognized as a prodromal form of AD. Further, in older autopsy cohorts of subjects with normal cognition, a substantial minority of brains meet pathologic diagnostic criteria for AD.[18,19] More recently, positron emission tomographic (PET) imaging ligands, including Pittsburgh Compound B (PiB), have been developed to detect fibrillar Aβ pathology in living subjects.[20-22] Similar to postmortem studies, positive PiB imaging is detected in a substantial minority of subjects with normal cognition, and this proportion increases with age. The realization that Aβ pathology may represent the earliest changes of AD informed the recent revision of diagnostic criteria, including the development of research guidelines for preclinical AD for individuals with biomarker changes, such as increased PiB amyloid, but with preserved cognition.[23] Many genetic and environmental risk factors might be expected to accelerate preclinical AD brain changes and ultimately promote susceptibility for clinical disease. Indeed, the *APOE* ε4 risk allele is associated with increased fibrillar Aβ deposition, based on PiB-PET, as well concomitant changes in cerebrospinal fluid Aβ, even in individuals with normal cognition.[22,24]

The presence of substantial amounts of AD pathology in older subjects with little or no cognitive impairment might be expected to degrade the power of AD case-control GWAS to discover variants affecting the earliest brain pathologic correlates of AD. We and others have promoted using disease endophenotypes, including AD pathology, as a complementary approach.[25,26] Herein, extending our prior work on *APOE*, *CR1*, *CLU*, and *PICALM*, we investigate whether newly reported AD risk alleles[9,10,12] are also associated with a quantitative measure of neuritic plaque burden in a large autopsy cohort. We identify associations at 2 other loci, CD2-associated protein (*CD2AP*) and the adenosine triphosphate–binding cassette, subfamily A, member 7 (*ABCA7*). We then perform a GWAS to discover additional susceptibility loci for neuritic plaque pathology. Among our top results, we discover a variant near the *APP* locus that is also associated with fibrillar Aβ in 2 independent cohorts of cognitively normal subjects with PET imaging. Our findings relate validated AD susceptibility alleles to the development of AD neuritic plaques and begin to reveal the genetic architecture underlying the earliest known brain pathologic changes of AD.

Methods

Additional detailed methods (eMethods), eTables, eFigures, and additional acknowledgments and references are provided at http://dejager_lab.bwh.harvard.edu/wp-content/uploads/2013/02/Shulman-et-al-2013_-Online-1.pdf, http://dejager_lab.bwh.harvard.edu/wp-content/uploads/2013/02/Shulman-et-al.-2013_OnlineTables-21.xls, https://www.radc.rush.edu/publications/2013/Shulman%20et%20al%202013_%20Online.pdf, and https://www.radc.rush.edu/publications/2013/Shulman%20et%20al.%202013_OnlineTables.xls.

Subjects

Religious Orders Study (ROS) and Rush Memory and Aging Project (MAP) participants were free of known dementia at enrollment, agreed to annual clinical evaluations, and signed an informed consent and Anatomic Gift Act donating their brains at death.[27,28] The studies were approved by the institutional review board of Rush University Medical Center. For analyses of candidate single-nucleotide polymorphisms (SNPs), the joint ROS/MAP cohort included 725 subjects (408 ROS and 317 MAP) with genotyping and completed autopsies. The GWAS was based on a previously published subset of 651 subjects.[29] For replication, we relied on 2 additional study cohorts with brain

PET imaging of fibrillar Aβ (eTable 5): the Arizona *APOE* cohort (n = 56) and the Alzheimer's Disease Neuroimaging Initiative (ADNI) (n = 58). The Arizona *APOE* cohort is a longitudinal study of cognitively normal subjects with PiB-PET imaging, including *APOE* ε4 homozygotes, heterozygotes, and noncarriers.[22] The cognitively normal subsample of ADNI (www.adni-info.org) included subjects with genotyping and PET imaging using the florbetapir ligand Avid-45.

Clinical and Postmortem Evaluation

The clinical diagnoses of dementia and AD were made following National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association recommendations.[30] Level of cognition was based on 17 cognitive tests performed at annual evaluations proximate to death.[14] Modified Bielschowsky silver stain was used to visualize neuritic plaques, diffuse plaques, and neurofibrillary tangles in tissue sections from the midfrontal, middle temporal, inferior parietal, and entorhinal cortices and the hippocampal CA1 sector. As in prior work,[14,25] a quantitative composite score for neuritic plaque pathologic burden was created by dividing the raw counts in each region by the population standard deviation of the region-specific counts and then averaging the scaled counts over the 5 brain regions to create a single standardized summary measure. The Aβ load was additionally measured based on anti-Aβ immunohistochemistry. Neuropathologic diagnosis of AD was made based on intermediate or high likelihood of AD by criteria from the National Institute on Aging and the Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease.[31]

Statistical Analysis

Genome-wide genotyping, quality-control procedures, and imputation have been previously reported.[29] Dosage values for each of 2 465 581 imputed SNPs were coded additively in terms of the minor allele. Linear regression was used to relate each SNP to the square root-transformed summary measure of neuritic plaques, adjusting for age at death, study membership (ROS vs MAP), and the first 3 principal components from our population structure analysis. In the candidate analyses of AD SNPs, $P < .05$ was considered statistically significant. For the genome-wide association analyses, statistical significance was set at $P < 5 \times 10^{-8}$, and $P < 1 \times 10^{-4}$ was considered suggestive evidence of association. Secondary analyses included adjustment for cognitive status proximate to death (eTable 7) and *APOE* ε4 genotype (0, 1, or 2 copies) (eTable 3 and eTable 8), and we also tested for associations with AD susceptibility (eTable 9). Analyses of SNP effects on global cognitive and episodic memory decline and incident AD were performed as in prior work.[14]

For the analysis of SNP associations with Aβ PET measurements, cerebral to cerebellar florbetapir standard uptake value ratio (SUVR) images were generated in cognitively normal ADNI subjects and PiB SUVR images were generated in Arizona *APOE* cohort subjects. Statistical brain maps reflect the additive association of the minor allele of interest and SUVR measurements in the aggregate subject group, adjusting for age, *APOE* ε4 allele dose, and cohort membership. A Monte Carlo simulation procedure involving 1000 iterations was used to demonstrate that the number of voxels with increased SUVRs ($P < .05$) was significantly increased in the implicated direction. We also performed region of interest analysis on individual PET images, based on previously defined mean cortical and whole-cerebellar templates.

Results

We studied 725 deceased subjects for whom complete neuropathologic evaluation and genotype data were available (eTable 1). Proximate to death, 42.2% had clinically diagnosed AD, 24.6% had mild cognitive impairment, and 31.7% retained normal cognition. The remaining subjects (n = 21) had dementia due to other causes, as they did not meet clinical criteria for AD.[30] On postmortem examination, 62.5% met National Institute on Aging-Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease pathologic criteria for AD, consistent with the advanced age of this cohort (mean, 88.1 years). We used a quantitative measure of neuritic plaque burden to assess genetic susceptibility for AD neuritic plaque pathology.

We first evaluated published AD susceptibility loci from recent clinical case-control GWAS.[9,10] As previously reported,[14] the *CR1* locus was associated with neuritic plaque burden (rs6701713; $P = .03$), and we additionally found evidence that variants in *ABCA7* (rs3764650; $P = .03$) and *CD2AP* (rs9349407; $P = .03$) were associated with neuritic plaque pathology (**Table 1**). For all 3 SNPs, AD risk alleles showed consistent direction of effects for increased neuropathology. By contrast, other recently validated susceptibility loci, including *CLU*, *PICALM*, *BIN1*, *MS4A*, *CD33*, and *EPHA1*, were not associated with neuritic plaques in this cohort. These results suggest that, at least for 3 of the 9 loci discovered in GWAS, effects on neuritic plaque pathology may mediate the association of these polymorphisms with clinical AD risk.

To identify novel variants associated with neuritic plaque burden, we performed a genome-wide scan in ROS and MAP using the quantitative pathologic trait. The genomic inflation factor did not reveal any evidence of systematic inflation in our test statistic ($\lambda_{GC} = 1.009$) (eFigure 1). The only genome-wide significant SNP associations ($P < 5 \times 10^{-8}$) were detected on chromosome 19 near *APOE* (rs4420638; $P = 1.5 \times 10^{-17}$). The top independent loci ($P < 10^{-5}$) associated with neuritic plaque burden are shown in **Table 2** (see eTable 2 for detailed results). Among our strongest associations, we identified variants near candidate genes with roles in inflammation and immunity, including prostaglandin-endoperoxide synthase 1 (*PTGS1*, rs12551233; $P = 4.8 \times 10^{-7}$) and the human leukocyte antigen (HLA) class II region of the major histocompatibility complex (*HLA-DQA2*, rs3892710; $P = 2.3 \times 10^{-6}$). Notably, we also identified a common chromosome 21 SNP (rs2829887; frequency = 0.43; $P = 3.3 \times 10^{-6}$), which is located 149.2 kilobases (kb) from the 3′ end of *APP* (**Figure 1**) and is found in an intron of *ATP5J*. In addition, our GWAS detected evidence of associations at *KCNIP4* (rs6817475;

Table 1. Association of Alzheimer Disease Susceptibility Loci With Neuritic Plaque Pathologic Burden

Gene	SNP[a]	A1	A2	MAF	Beta (SE)[b]	95% CI	*P* Value
CR1	rs6701713	A	G	0.20	0.077 (0.03508)	0.0082 to 0.1457	.03
CLU	rs1532278	T	C	0.39	0.000 (0.03058)	−0.0595 to 0.0603	.99
PICALM	rs561655	G	A	0.35	−0.045 (0.02883)	−0.1017 to 0.0113	.12
BIN1	rs7561528	A	G	0.34	−0.009 (0.02801)	−0.0635 to 0.0463	.76
ABCA7	rs3764650	G	T	0.09	0.180 (0.08262)	0.0183 to 0.3422	.03
MS4A	rs4938933	C	T	0.43	−0.004 (0.02772)	−0.0583 to 0.0503	.89
CD33	rs3865444	A	C	0.30	−0.040 (0.02954)	−0.0981 to 0.0177	.17
CD2AP	rs9349407	C	G	0.25	0.071 (0.03223)	0.0075 to 0.1338	.03
EPHA1	rs11767557	C	T	0.18	0.024 (0.04087)	−0.0558 to 0.1044	.56

Abbreviations: A1, minor allele; A2, major allele; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

[a] Based on published Alzheimer disease susceptibility loci.[9,10]

[b] The beta value is an estimate based on the effect of increasing dosage of the SNP minor allele, adjusted for age at death, study membership, and 3 principal components.

Table 2. Top Results of the Neuritic Plaque Genome-Wide Association Studies[a]

Chromosome	SNP	Position[b]	A1	A2	MAF	Beta (SE)[c]	*P* Value	Genes
19	rs4420638	50 114 786	G	A	0.18	0.3463 (0.0395)	1.49×10^{-17}	*APOE*
4	rs6817475	20 370 609	G	T	0.33	0.1672 (0.0326)	3.80×10^{-7}	*KCNIP4*
9	rs12551233	124 190 709	G	A	0.06	−0.3216 (0.0632)	4.79×10^{-7}	*PTGS1*
6	rs3892710	32 790 840	T	C	0.15	−0.2007 (0.0421)	2.32×10^{-6}	*HLA-DQA2*
21	rs2829887	26 025 485	T	C	0.43	−0.1384 (0.0295)	3.33×10^{-6}	*ATP5J-APP*
9	rs9407730	16 221 985	G	A	0.13	−0.2118 (0.0453)	3.61×10^{-6}	
6	rs4642480	96 321 020	G	A	0.48	0.1432 (0.0307)	3.65×10^{-6}	
3	rs4564921	140 761 248	C	G	0.37	0.1388 (0.0304)	6.12×10^{-6}	*NMNAT3*
14	rs187911	57 084 834	G	A	0.41	−0.1434 (0.0315)	6.23×10^{-6}	*SLC35F4*
14	rs10149826	33 380 164	T	C	0.12	0.2210 (0.0494)	9.15×10^{-6}	*NPAS3*
2	rs12613305	205 054 912	A	G	0.47	0.1311 (0.0293)	9.36×10^{-6}	*PARD3B*

Abbreviations: A1, minor allele; A2, major allele; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

[a] Based on analysis of a subsample consisting of 651 autopsy cases from the Religious Orders Study and Rush Memory and Aging Project.

[b] Based on hg18 coordinates.

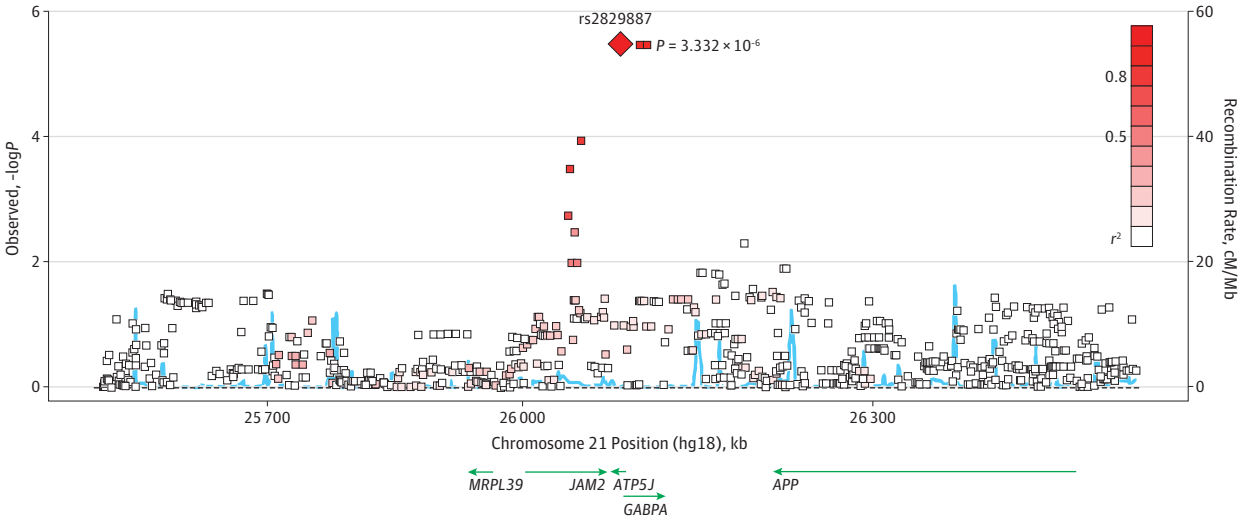
[c] The beta value is an estimate based on the effect of increasing dosage of the SNP minor allele, adjusted for age at death, study membership, and 3 principal components.

$P = 3.8 \times 10^{-7}$) and *NMNAT3* (rs4564921; $P = 6.1 \times 10^{-6}$), 2 additional genes previously implicated in AD.[32,33]

Given the proximity of rs2829887 to *APP*, a gene known to be associated with familial AD, we investigated this locus in more detail (**Table 3**). Based on the absence of a statistical interaction ($P = .77$), the effect of rs2829887 did not vary by cohort, demonstrating consistent associations with neuritic plaque burden in both the ROS sample ($\beta = -0.124$; $P = 4.0 \times 10^{-4}$) and the smaller MAP sample ($\beta = -0.106$; $P = .01$). Further, the *APP* locus variant was strongly associated with postmortem Aβ load ($P = 9.5 \times 10^{-5}$), which is measured in the same subjects using anti-Aβ immunohistochemistry. The associations between rs2829887 and these neuropathologic traits were robust to adjustment for *APOE* genotype (eTable 3). Rare mutations in *PSEN1* and *PSEN2* are also associated with early-onset familial AD[1]; however, common genetic variation at either locus (±500 kb) was not found in association with amyloid neuritic plaques in our GWAS data ($P > .01$).

There are few existing prospective, community-based cohorts such as ROS and MAP with large numbers of brain autopsies and assessments of quantitative neuropathology and genome-wide data with which to replicate our findings. To further validate our results, we leveraged data from subjects with PET imaging of fibrillar Aβ (eTable 5). The Arizona *APOE* cohort consists of 56 subjects with normal cognition and assessment with PiB-PET. Subjects carrying the *APOE* ε4 risk allele were previously shown to have increased levels of PiB amyloid, consistent with early AD pathologic changes in this genetically susceptible subgroup.[22] Compared with ROS and MAP, the Arizona *APOE* cohort is significantly younger (mean age, 64.6 years), and subjects uniformly have preserved cognition (mean Mini-Mental State Examination score, 29.7). We additionally used data from 58 cognitively normal older adults (mean age, 80.4 years; mean Mini-Mental State Examination score, 29.4) from the ADNI cohort with florbetapir-PET. In a voxel-based joint analysis of the Arizona *APOE* and ADNI cohorts, rs2829887 was associated with extensive fibrillar Aβ deposition across numerous brain regions, including the cingulate, frontal, temporal, and parietal cortices ($P < .005$) (**Figure 2**). These results were robust to adjustment for age and *APOE* ε4 genotype. Using a Monte Carlo simulation to address multiple regional comparisons, we found 64 743 voxels in which rs2829887 dosage was associated with increased fibrillar Aβ

Figure 1. rs2829887 at *APP* Locus in Association With Neuritic Plaque Pathology



Plot showing rs2829887 at the *APP* locus in association with neuritic plaque pathology. cM indicates centimorgans; kb, kilobases; and Mb, megabases.

Table 3. Association of rs2829887 With Alzheimer Disease Traits in the Religious Orders Study and Rush Memory and Aging Project

Trait[a]	Subjects, No.[b]	Estimate (95% CI)[c]	*P* Value
Neuritic plaques	725	−0.115 (−0.170 to −0.063)	1.54×10^{-5}
Amyloid load	716	−0.216 (−0.324 to −0.108)	9.52×10^{-5}
AD			
Clinical	328/470	1.050 (0.86 to 1.29)	.63
Clinicopathologic	244/212	0.643 (0.48 to 0.85)	.002

Abbreviation: AD, Alzheimer disease.

[a] Neuritic plaque burden was based on silver-stained tissue, and amyloid load was determined from β-amyloid immunohistochemistry. Clinicopathologic AD was based on clinical diagnosis and pathologic confirmation of cases vs nondemented controls with no or low likelihood of AD on pathologic examination.

[b] Expressed as total subjects or cases/controls.

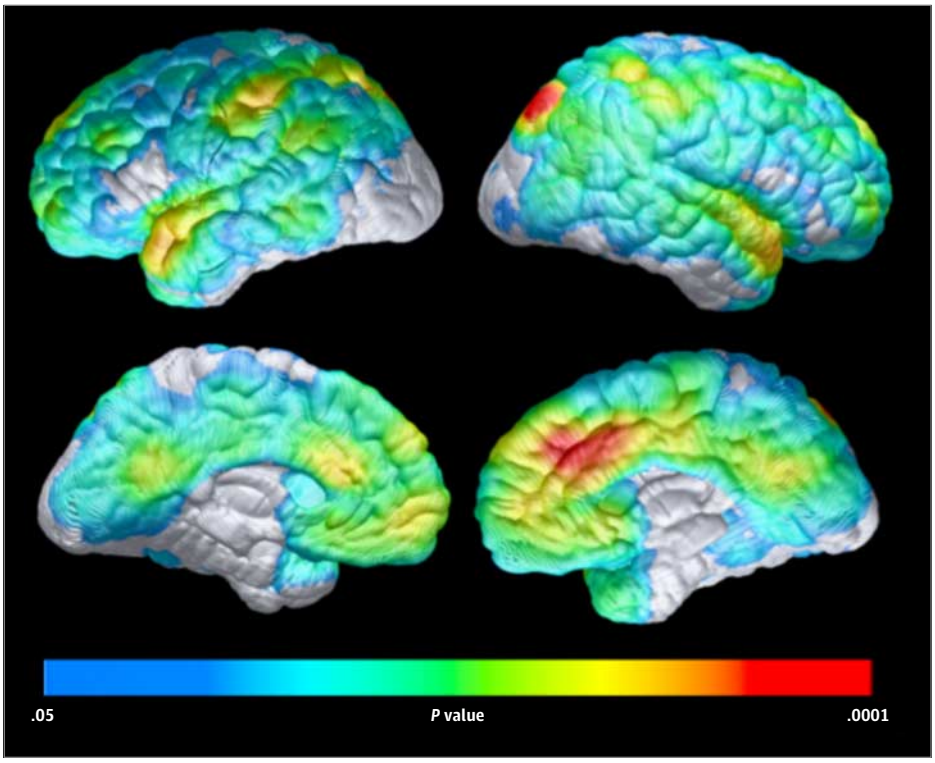
[c] Estimate represents beta values for quantitative pathologic traits or odds ratios for AD diagnosis, reflecting increasing dosage of the rs2829887 minor allele (T), adjusted for age at death, study membership, and 3 principal components.

compared with 1313 voxels—in smaller clusters—in which this allele was associated with decreased signal ($P < .001$). In a complementary analysis based on predefined brain regions of interest, rs2829887 was associated with level of mean cortical fibrillar Aβ ($P = .03$; see eTable 6 for full results). However, the direction of the association between rs2829887 and fibrillar Aβ in the PET cohorts was opposite to the association with neuritic plaque pathology in ROS and MAP. Specifically, the minor allele, $rs2829887^T$ (ROS and MAP, frequency = 0.43; Arizona *APOE* and ADNI cohorts, frequency = 0.45) was associated with reduced postmortem neuritic plaques in ROS/MAP but increased PiB/florbetapir SUVRs in the Arizona *APOE* and ADNI cohorts. Separate analyses of the independent ADNI and Arizona *APOE* cohorts revealed associations between $rs2829887^T$ and increased Aβ across numerous brain regions ($P < .005$) (eFigure 2), consistent with the joint analysis.

Subjects enrolled in ROS and MAP also receive annual assessment with a comprehensive neuropsychiatric battery, allowing genetic analyses of cognitive decline, as in prior studies.[14] We found that many, but not all, of the SNPs discovered for associations with neuritic plaque pathology also showed associations with longitudinal decline in global cognition and episodic memory (eTable 4); pathology-increasing alleles showed consistent direction of effects on the cognitive outcomes, accelerating rate of decline. We further developed an aggregate genetic risk score model incorporating the top 53 independently associated SNPs ($P < 10^{-4}$) from the neuritic plaque GWAS. This model was strongly associated with global cognitive ($P = 1.1 \times 10^{-16}$) and episodic memory ($P = 7.9 \times 10^{-21}$) decline and remained highly significant after excluding *APOE* SNPs from the model ($P_{global} = 4.8 \times 10^{-14}$; $P_{memory} = 1.2 \times 10^{-17}$). Thus, the top of the results distribution from our AD neuritic plaque pathology GWAS is significantly enriched for loci that are also pertinent for decline in cognitive function.

Notably, the *APP* locus SNP, rs2829887, was not related to clinical AD diagnosis ($P = .63$) (Table 3), change in global cognition ($P = .58$), change in episodic memory ($P = .41$), or incident AD ($P = .38$). These results may be explained by the presence of substantial AD pathology in individuals with mild or

Figure 2. Association of rs2829887 With Fibrillar Amyloid in Cognitively Normal Subjects From the Alzheimer's Disease Neuroimaging Initiative and Arizona *APOE* Cohorts



Statistical map of rs2829887[T] association with fibrillar amyloid projected onto the medial and lateral surfaces of a standardized brain, based on joint analysis of Pittsburgh Compound B or Avid-45 standard uptake value ratio images from 114 cognitively normal subjects in the Arizona *APOE* and Alzheimer's Disease Neuroimaging Initiative cohorts. Analyses were adjusted for subject age, *APOE* ε4 genotype, and cohort membership.

subclinical disease (classified as controls in a standard AD case-control analysis). Despite smaller sample sizes, the association with clinical AD was significant (odds ratio = 0.64; $P = .002$) in an analysis limited to clinically diagnosed and pathologically confirmed cases (n = 244) and controls without substantial AD pathology (n = 212). We further found that the association of rs2829887 with neuritic plaque pathology does not vary by clinical diagnosis proximate to death, demonstrating consistent effects (β values) in subjects with (β = −0.109; $P = .008$) or without (β = −0.108; $P = 6.1 \times 10^{-4}$) dementia. In an analysis restricted to the relatively few subjects with no cognitive impairment similar to our PET samples (n = 229), rs2829887 remained associated with Aβ load (β = −0.173; $P = .046$) and showed a trend toward an association with neuritic plaque burden (β = −0.068; $P = .09$). These results suggest that rs2829887 affects susceptibility for the earliest brain amyloid changes preceding overt cognitive impairment.

Discussion

Besides *APOE* and *CR1*, we found that AD susceptibility alleles at the *ABCA7* and *CD2AP* loci were associated with increased neuritic plaque pathology. The results suggest a potential mechanism for the effect of these loci on the clinical manifestations of AD. While our study was not sufficiently powered to definitively exclude associations for the other AD susceptibility variants (see later), it is possible that some of these loci predominantly affect pathogenic steps downstream of neuritic plaque deposition, such as synaptic loss, neuronal death, or the manifestation of cognitive changes. It is also plausible that some AD susceptibility loci affect other, non-AD related mechanisms that contribute to cognitive changes and the development of the clinical AD phenotype, which is known to be pathologically heterogeneous.[19]

The strongest support for a genetic link between Aβ pathology and AD risk comes from the established connection between rare mutations in *APP* and autosomal dominant, early-onset familial AD.[1] Further, an uncommon *APP* variant, A673T (frequency <1%), was recently discovered to confer protection against AD.[2] Although common *APP* polymorphisms have been evaluated in numerous association studies of late-onset AD, a definitive link has yet to be established,[3-6] and *APP* has not emerged from AD GWAS.[9,10] It is intriguing that our study discovered common variants near *APP* among the top loci associated with neuritic plaque burden. The strongest variant, rs2829887, fell within 150 kb of the 3′ end of the *APP* transcription unit, within an intron of *ATP5J*. No other adjacent genes (*JAM2*, *GABPA*, *MRPL39)* are known to be involved in amyloidogenesis. While *APP* is a compelling candidate, the causal variant(s) and responsible gene(s) require confirmation. rs2829887 may tag a haplotype associated with altered APP protein processing or gene expression, similar to other rare disease-causing mutations or duplications.[34] We found additional support for the *APP* locus based on evaluation of 2 independent cohorts of cognitively normal, older adults with PET imaging. While intriguing, this association was in the opposite direction for PET Aβ compared with directly measured neuritic

plaques or Aβ load, which may be due to differences in the cohorts (eg, participant age) or the outcome phenotypes (postmortem Aβ load vs PET Aβ).

Our GWAS identified other candidate susceptibility genes that will require replication. rs6817475 falls within an intron of *KCNIP4*, encoding a potassium channel–interacting protein. Notably, KCNIP4 physically interacts with PSEN2[35] and alters Aβ dynamics in cultured cells[32]; further, insertion-deletion polymorphisms in the *KCNIP4* promoter were associated with AD in a small case-control autopsy cohort. Also, rs12551233 is within an intron of *PTGS1*, also known as cyclooxygenase 1 (*COX1*), which encodes a key regulator of inflammation. Increased COX1 along with other inflammatory markers have been previously described in association with neuritic plaque pathology.[36] We also detected the SNP rs3892710 within the HLA locus (*HLA-DQA2*), which encodes the class II major histocompatibility complex on antigen-presenting cells and regulates adaptive immune responses. Our finding of associations at *COX1* and the *MHC* locus, along with the established roles of *CR1*, *CD33*, and *MS4A*, supports immune responses and neuroinflammation as important determinants of AD pathogenesis. Finally, we note an association between an SNP in the nicotinamide nucleotide adenylyltransferase 3 gene (*NMNAT3*, rs4564921) and neuritic plaque burden. This enzyme family has demonstrated neuroprotective activities in experimental models,[37] and the *NMNAT3* locus was previously implicated in an AD genome-wide scan.[33]

The main strength of our study comes from its use of 2 prospective, community-based, autopsy cohorts with uniform, quantitative assessment of neuritic plaque pathology along with thorough clinical assessment proximate to death. However, we ultimately had insufficient power to discover novel genome-wide significant susceptibility loci for neuritic plaque pathology. Importantly, both cohorts are ongoing, which will allow better-powered studies in the future, including genetic analyses of other informative pathologic traits (eg, neurofibrillary tangles, neurons, dendritic spines, and synaptic protein markers).[38] Given our current sample size, a polymorphism would need to explain at least 6.7% of the variance in pathologic burden for us to have 90% power for discovery at genome-wide significance. Based on the effect size of rs2829887 in the *APP* locus, which explains 3.5% of the variance in neuritic plaque burden, we estimate that a sample size of 1300 will be needed to have 90% power to establish a significant association.

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REFERENCES

1. Ertekin-Taner N. Genetics of Alzheimer's disease: a centennial review. *Neurol Clin*. 2007;25(3):611-667; v.

2. Jónsson T, Atwal JK, Steinberg S, et al. A mutation in *APP* protects against Alzheimer's disease and age-related cognitive decline. *Nature*. 2012;488(7409):96-99.

3. Hooli BV, Mohapatra G, Mattheisen M, et al. Role of common and rare *APP* DNA sequence variants in Alzheimer disease. *Neurology*. 2012;78(16):1250-1257.

4. Nowotny P, Simcock X, Bertelsen S, et al. Association studies testing for risk for late-onset Alzheimer's disease with common variants in the beta-amyloid precursor protein (APP). *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B(4):469-474.

5. Guyant-Maréchal L, Rovelet-Lecrux A, Goumidi L, et al. Variations in the *APP* gene promoter region and risk of Alzheimer disease. *Neurology*. 2007;68(9):684-687.

6. Athan ES, Lee JH, Arriaga A, Mayeux RP, Tycko B. Polymorphisms in the promoter of the human *APP* gene: functional evaluation and allele frequencies in Alzheimer disease. *Arch Neurol*. 2002;59(11):1793-1799.

7. Castellano JM, Kim J, Stewart FR, et al. Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. *Sci Transl Med*. 2011;3(89):89ra57.

8. 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.

9. Hollingworth P, Harold D, Sims R, et al; Alzheimer's Disease Neuroimaging Initiative; CHARGE Consortium; EADI1 Consortium. Common variants at *ABCA7*, *MS4A6A/MS4A4E*, *EPHA1*, *CD33* and *CD2AP* are associated with Alzheimer's disease. *Nat Genet*. 2011;43(5):429-435.

10. 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(5):436-441.

11. 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.

12. Seshadri S, Fitzpatrick AL, Ikram MA, et al; CHARGE Consortium; GERAD1 Consortium; EADI1 Consortium. Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA*. 2010;303(18):1832-1840.

13. DeMattos RB, Cirrito JR, Parsadanian M, et al. ApoE and clusterin cooperatively suppress Abeta levels and deposition: evidence that ApoE regulates extracellular Abeta metabolism in vivo. *Neuron*. 2004;41(2):193-202.

14. Chibnik LB, Shulman JM, Leurgans SE, et al. *CR1* is associated with amyloid plaque burden and age-related cognitive decline. *Ann Neurol*. 2011;69(3):560-569.

15. Bennett DA, Schneider JA, Wilson RS, Bienias JL, Berry-Kravis E, Arnold SE. Amyloid mediates the association of apolipoprotein E e4 allele to cognitive function in older people. *J Neurol Neurosurg Psychiatry*. 2005;76(9):1194-1199.

16. Wyss-Coray T, Yan F, Lin AH-T, et al. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc Natl Acad Sci U S A*. 2002;99(16):10837-10842.

17. Treusch S, Hamamichi S, Goodman JL, et al. Functional links between Aβ toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast. *Science*. 2011;334(6060):1241-1245.

18. Sonnen JA, Larson EB, Crane PK, et al. Pathological correlates of dementia in a longitudinal, population-based sample of aging. *Ann Neurol*. 2007;62(4):406-413.

19. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The neuropathology of probable Alzheimer disease and mild cognitive impairment. *Ann Neurol*. 2009;66(2):200-208.

20. Aizenstein HJ, Nebes RD, Saxton JA, et al. Frequent amyloid deposition without significant cognitive impairment among the elderly. *Arch Neurol*. 2008;65(11):1509-1517.

21. Jack CR Jr, Lowe VJ, Senjem ML, et al. 11C PiB and structural MRI provide complementary information in imaging of Alzheimer's disease and amnestic mild cognitive impairment. *Brain*. 2008;131(pt 3):665-680.

22. Reiman EM, Chen K, Liu X, et al. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2009;106(16):6820-6825.

23. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7(3):280-292.

24. 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(1):122-131.

25. Bennett DA, De Jager PL, Leurgans SE, Schneider JA. Neuropathologic intermediate phenotypes enhance association to Alzheimer susceptibility alleles. *Neurology*. 2009;72(17):1495-1503.

26. McQueen MB, Bertram L, Lange C, et al. Exploring candidate gene associations with neuropsychological performance. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B(8):987-991.

27. Bennett DA, Schneider JA, Arvanitakis Z, Wilson RS. Overview and findings from the Religious Orders Study. *Curr Alzheimer Res*. 2012;9(6):628-645.

28. Bennett DAD, Schneider JAJ, Buchman ASA, Barnes LLL, Boyle PAP, Wilson RSR. Overview and findings from the Rush Memory and Aging Project. *Curr Alzheimer Res*. 2012;9(6):646-663.

29. Keenan BT, Shulman JM, Chibnik LB, et al; Alzheimer's Disease Neuroimaging Initiative. A coding variant in *CR1* interacts with *APOE*-ε4 to influence cognitive decline. *Hum Mol Genet*. 2012;21(10):2377-2388.

30. 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.

31. National Institute on Aging; Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. *Neurobiol Aging*. 1997;18(4)(suppl):S1-S2.

32. Massone S, Vassallo I, Castelnuovo M, et al. RNA polymerase III drives alternative splicing of the potassium channel-interacting protein contributing to brain complexity and neurodegeneration. *J Cell Biol*. 2011;193(5):851-866.

33. Liu F, Arias-Vásquez A, Sleegers K, et al. A genomewide screen for late-onset Alzheimer disease in a genetically isolated Dutch population. *Am J Hum Genet*. 2007;81(1):17-31.

34. Rovelet-Lecrux A, Hannequin D, Raux G, et al. *APP* locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat Genet*. 2006;38(1):24-26.

35. Morohashi Y, Hatano N, Ohya S, et al. Molecular cloning and characterization of CALP/KChIP4, a novel EF-hand protein interacting with presenilin 2 and voltage-gated potassium channel subunit Kv4. *J Biol Chem*. 2002;277(17):14965-14975.

36. Hoozemans JJM, Rozemuller JM, van Haastert ES, Veerhuis R, Eikelenboom P. Cyclooxygenase-1 and -2 in the different stages of Alzheimer's disease pathology. *Curr Pharm Des*. 2008;14(14):1419-1427.

37. Zhai RG, Zhang F, Hiesinger PR, Cao Y, Haueter CM, Bellen HJ. NAD synthase NMNAT acts as a chaperone to protect against neurodegeneration. *Nature*. 2008;452(7189):887-891.

38. Arnold SE, Louneva N, Cao K, et al. Cellular, synaptic, and biochemical features of resilient cognition in Alzheimer's disease. *Neurobiol Aging*. 2013;34(1):157-168.