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 × 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.
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. Further, an uncommon APP coding variant (frequency <1%) was recently discovered to be protective against AD in the Icelandic population. 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. 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. Genome-wide association studies (GWAS) have identified several additional AD susceptibility loci, 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. 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, and the complement pathway has also been implicated in brain Aβ deposition in murine models. 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. 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.

Although gene discovery in AD has largely focused on clinically diagnosed disease in case-control studies, it is now recognized that the AD pathophysiologic 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. 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. More recently, positron emission tomographic (PET) imaging ligands, including Pittsburgh Compound B (PiB), have been developed to detect fibrillar Aβ pathology in living subjects. 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. 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.

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. Herein, extending our prior work on APOE, CR1, CLU, and PICALM, we investigate whether newly reported AD risk alleles 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


### 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. 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. 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. 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. Level of cognition was based on 17 cognitive tests performed at annual evaluations proximate to death. Modified Bielschowsky silver stain was used to visualize neurotic 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, 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.

Statistical Analysis

Genome-wide genotyping, quality-control procedures, and imputation have been previously reported. 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 × 10−8, and P < 1 × 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.

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 neuropsychologic 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. 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. As previously reported, the CR1 locus was associated with neuritic plaque burden (rs7601713; 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, CD23, 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 (λGC = 1.009) (eFigure 1). The only genome-wide significant SNP associations (P < 5 × 10−8) were detected on chromosome 19 near APOE (rs4420638; P = 1.5 × 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, rs12551223; P = 4.8 × 10−7) and the human leukocyte antigen (HLA) class II region of the major histocompatibility complex (HLA-DQA2, rs3892710; P = 2.3 × 10−6). Notably, we also identified a common chromosome 21 SNP (rs2829887; frequency = 0.43; P = 3.3 × 10−5), 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

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>A1</th>
<th>A2</th>
<th>MAF</th>
<th>Beta (SE)</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR1</td>
<td>rs6701713</td>
<td>A</td>
<td>G</td>
<td>0.20</td>
<td>0.077 (0.03508)</td>
<td>0.0082 to 0.1457</td>
<td>.03</td>
</tr>
<tr>
<td>CLU</td>
<td>rs1512278</td>
<td>T</td>
<td>C</td>
<td>0.39</td>
<td>0.000 (0.03058)</td>
<td>-0.0595 to 0.0603</td>
<td>.99</td>
</tr>
<tr>
<td>PICALM</td>
<td>rs561655</td>
<td>G</td>
<td>A</td>
<td>0.35</td>
<td>-0.045 (0.02883)</td>
<td>-0.1017 to 0.0113</td>
<td>.12</td>
</tr>
<tr>
<td>BIN1</td>
<td>rs7561528</td>
<td>A</td>
<td>G</td>
<td>0.34</td>
<td>-0.009 (0.02801)</td>
<td>-0.0635 to 0.0463</td>
<td>.76</td>
</tr>
<tr>
<td>ABCA7</td>
<td>rs3764650</td>
<td>G</td>
<td>T</td>
<td>0.09</td>
<td>0.180 (0.08262)</td>
<td>0.0183 to 0.3422</td>
<td>.03</td>
</tr>
<tr>
<td>MS4A</td>
<td>rs4918939</td>
<td>C</td>
<td>T</td>
<td>0.43</td>
<td>-0.004 (0.02772)</td>
<td>-0.0583 to 0.0503</td>
<td>.89</td>
</tr>
<tr>
<td>CD33</td>
<td>rs3865444</td>
<td>A</td>
<td>C</td>
<td>0.30</td>
<td>-0.040 (0.02954)</td>
<td>-0.0981 to 0.0177</td>
<td>.17</td>
</tr>
<tr>
<td>CD2AP</td>
<td>rs9349407</td>
<td>C</td>
<td>G</td>
<td>0.25</td>
<td>0.071 (0.03223)</td>
<td>0.0075 to 0.1338</td>
<td>.03</td>
</tr>
<tr>
<td>EPHA1</td>
<td>rs11767557</td>
<td>C</td>
<td>T</td>
<td>0.18</td>
<td>0.024 (0.04087)</td>
<td>-0.0558 to 0.1044</td>
<td>.56</td>
</tr>
</tbody>
</table>

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

*Based on published Alzheimer disease susceptibility loci.  
**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

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>SNP</th>
<th>Position</th>
<th>A1</th>
<th>A2</th>
<th>MAF</th>
<th>Beta (SE)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>rs4420638</td>
<td>50 114 786</td>
<td>G</td>
<td>A</td>
<td>0.13</td>
<td>0.3463 (0.0395)</td>
<td>1.49 x 10^-17</td>
</tr>
<tr>
<td>4</td>
<td>rs5174775</td>
<td>20 370 609</td>
<td>G</td>
<td>T</td>
<td>0.33</td>
<td>-0.1672 (0.0326)</td>
<td>3.80 x 10^-7</td>
</tr>
<tr>
<td>9</td>
<td>rs12551233</td>
<td>124 190 709</td>
<td>G</td>
<td>A</td>
<td>0.06</td>
<td>-0.3216 (0.0632)</td>
<td>4.79 x 10^-7</td>
</tr>
<tr>
<td>6</td>
<td>rs3892710</td>
<td>32 790 840</td>
<td>T</td>
<td>C</td>
<td>0.15</td>
<td>-0.2007 (0.0421)</td>
<td>2.32 x 10^-6</td>
</tr>
<tr>
<td>21</td>
<td>rs2829887</td>
<td>26 025 485</td>
<td>T</td>
<td>C</td>
<td>0.43</td>
<td>-0.1384 (0.0295)</td>
<td>3.33 x 10^-6</td>
</tr>
<tr>
<td>9</td>
<td>rs907730</td>
<td>16 221 985</td>
<td>G</td>
<td>A</td>
<td>0.13</td>
<td>-0.2118 (0.0453)</td>
<td>3.61 x 10^-6</td>
</tr>
<tr>
<td>6</td>
<td>rs4642480</td>
<td>96 321 020</td>
<td>G</td>
<td>A</td>
<td>0.18</td>
<td>-0.1432 (0.0307)</td>
<td>3.65 x 10^-6</td>
</tr>
<tr>
<td>3</td>
<td>rs4564921</td>
<td>140 761 248</td>
<td>C</td>
<td>G</td>
<td>0.37</td>
<td>0.1388 (0.0304)</td>
<td>6.12 x 10^-6</td>
</tr>
<tr>
<td>14</td>
<td>rs187911</td>
<td>57 084 834</td>
<td>G</td>
<td>A</td>
<td>0.41</td>
<td>-0.1434 (0.0315)</td>
<td>6.23 x 10^-6</td>
</tr>
<tr>
<td>14</td>
<td>rs1014982</td>
<td>33 380 164</td>
<td>T</td>
<td>C</td>
<td>0.12</td>
<td>0.2210 (0.0494)</td>
<td>9.15 x 10^-6</td>
</tr>
<tr>
<td>2</td>
<td>rs1261305</td>
<td>205 054 912</td>
<td>A</td>
<td>G</td>
<td>0.47</td>
<td>0.1311 (0.0293)</td>
<td>9.36 x 10^-6</td>
</tr>
</tbody>
</table>

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

*Based on analysis of a subsample consisting of 651 autopsy cases from the Religious Orders Study and Rush Memory and Aging Project.  
**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 PiB amyloid load (\( P = 9.5 \times 10^{-5} \)), which is measured in the same subjects using anti-\( \beta \) 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;\(^{12} \) however, common genetic variation at either locus (\( \pm 500 \) 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 A\( \beta \) (eTable 5). The Arizona APOE cohort consists of 56 subjects with normal cognition and assessment with PiB-PET. Subjects carrying the APOE \( \varepsilon 4 \) risk allele were previously shown to have increased levels of PiB amyloid, consistent with early AD pathologic changes in this genetically susceptible subgroup.\(^{25}\) 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 cohort, using a Monte Carlo simulation to address multiple regional comparisons, we found 64 of 74 voxels in which rs2829887 dosage was associated with increased fibrillar A\( \beta \).
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, rs2829887T (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 rs2829887T 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 \( (P_{\text{global}} = 4.8 \times 10^{-14}; P_{\text{memory}} = 1.2 \times 10^{-17}) \). 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

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

<table>
<thead>
<tr>
<th>Trait</th>
<th>Subjects, No.b</th>
<th>Estimate (95% CI)c</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuritic plaques</td>
<td>725</td>
<td>−0.115 (−0.170 to −0.063)</td>
<td>1.54 \times 10^{-5}</td>
</tr>
<tr>
<td>Amyloid load</td>
<td>716</td>
<td>−0.216 (−0.324 to −0.108)</td>
<td>9.52 \times 10^{-5}</td>
</tr>
<tr>
<td>AD Clinical</td>
<td>328/470</td>
<td>1.050 (0.86 to 1.29)</td>
<td>.63</td>
</tr>
<tr>
<td>AD Clinicopathologic</td>
<td>244/212</td>
<td>0.643 (0.48 to 0.85)</td>
<td>.002</td>
</tr>
</tbody>
</table>

Abbreviation: AD, Alzheimer disease.

* 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.
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 (\(\beta\) values) in subjects with (\(\beta = -0.109; P = .008\)) or without (\(\beta = -0.108; P = 6.1 \times 10^{-4}\)) 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 (\(\beta = -0.173; P = .046\)) and showed a trend toward an association with neuritic plaque burden (\(\beta = -0.068; P = .09\)). 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 plaque deposition.
plagues or Aβ load, which may be due to differences in the cohorts (eg, participant age) or the outcome phenotypes (post-mortem 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 and alters Aβ dynamics in cultured cells; 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. 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, CD3, 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 adenyltransferase 3 gene (NMNAT3, rs4564921) and neuritic plaque burden. This enzyme family has demonstrated neuroprotective activities in experimental models, and the NMNAT3 locus was previously implicated in an AD genome-wide scan.

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).

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**


