Published in final edited form as:

Ann Neurol. 2015 March ; 77(3): 469–477. doi:10.1002/ana.24337.

A *TREM1* variant alters the accumulation of Alzheimer-related amyloid pathology

Joseph M. Replogle, BA^{1,2,3,4}, Gail Chan, PhD^{1,2,3,4}, Charles C. White, PhD^{1,2,3}, Towfique Raj, PhD^{1,2,3,4}, Phoebe A. Winn, BA^{1,2,3}, Denis A. Evans, MD⁵, Reisa A. Sperling, MD^{1,4}, Lori B. Chibnik, PhD^{1,2,3,4}, Elizabeth M. Bradshaw, PhD^{1,2,3,4}, Julie A. Schneider, MD⁵, David A. Bennett, MD⁵, and Philip L. De Jager, MD PhD^{1,2,3,4}

¹Program in Translational NeuroPsychiatric Genomics, Institute for the Neurosciences, Departments of Neurology and Psychiatry, Brigham and Women's Hospital, Boston, MA, 02115

²Center for Neurologic Diseases, Brigham and Women's Hospital, Massachusetts 02115

³Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, 02142

⁴Harvard Medical School, Boston, MA, 02115

⁵Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, 60612

Abstract

Objective—Genome-wide association studies (GWAS) have linked variants in *TREM2* and *TREML2* with Alzheimer's disease (AD) and AD endophenotypes. Here, we pursue a targeted analysis of the *TREM* locus in relation to cognitive decline and pathological features of AD.

Methods—Clinical, cognitive, and neuropathological phenotypes were collected in three prospective cohorts on aging (n=3421 subjects). Our primary analysis was an association with neuritic plaque pathology. To functionally characterize the associated variants, we used flow cytometry data to measure *TREM1* expression on monocytes.

Results—We provide evidence that an intronic variant, rs6910730^G, in Triggering Receptor Expressed on Myeloid cells 1 (*TREM1*), is associated with an increased burden of neuritic plaques ($p=3.7\times 10^{-4}$), diffuse plaques ($p=4.1\times 10^{-3}$), and A β density ($p=2.6\times 10^{-3}$) as well as an increased rate of cognitive decline ($p=5.3\times 10^{-3}$). A variant upstream of *TREM2*, rs7759295^C, is independently associated with an increased Tau tangle density ($p=4.9\times 10^{-4}$), an increased burden of neurofibrillary tangles ($p=9.1\times 10^{-3}$), and an increased rate of cognitive decline ($p=2.3\times 10^{-3}$). Finally, a cytometric analysis shows that the *TREM1* rs6910730^G allele is associated with decreased *TREM1* expression on the surface of myeloid cells ($p=1.7\times 10^{-3}$).

Interpretation—We provide evidence that two common variants within the *TREM* locus are associated with pathological features of AD and aging-related cognitive decline. Our evidence suggests that these variants are likely to be independent of known AD variants and that they may work through an alteration of myeloid cell function.

Introduction

Recent studies have revealed the strong deleterious effect of a rare missense variant, R47H (rs75932628^T), in the immunoglobulin (Ig) V-set domain of TREM2 on Alzheimer's disease (AD) susceptibility^{1–5}. Expressed by microglia and myeloid cells such as monocytes, dendritic cells, and osteoclasts, the transmembrane TREM2 receptor couples with TYROBP, an adaptor protein, to attenuate inflammatory activation and increase phagocytic clearance of cell debris^{6–9}. These known functions of TREM2 suggest that rs75932628^T may influence AD susceptibility via an alteration of microglial and/or infiltrating macrophage function. In this, it may be similar to the *CD33* AD susceptibility locus: the *CD33* risk allele leads to alternative splicing of the Ig V-set domain of CD33, altered myeloid cell function, and accumulation of AD neuropathology^{10–13}.

TREM2 lies on chromosome 6p21.1 in a genomic region containing six other genes, including *TREM1*, *TREML1*, *TREML2*, *TREML3*, *TREML4*, and *NCR2*, with significant homology and shared function in modulating immune processes^{14–16}. Many of the proteins encoded by these genes, including TREM1, TREM2, TREML4, and NCR2, interact with TYROBP, a protein that has been proposed as an important regulator of an AD-associated gene regulatory network¹⁷. In addition to the variant in *TREM2*, variants in other *TREM* family members have been associated with AD susceptibility and pathology. After an endophenotype association study of CSF Tau/ptau levels and a meta-analysis of AD susceptibility loci both suggested an association with rs9381040, 5.5 Kb downstream of *TREML2*, a recent exome sequencing study clarified that the association was independent of rs75932628 in *TREM2* and highlighted the missense variant S144G (rs3747742) in *TREML2* as the most likely causal variant^{18–20}. Given the convergence of genetic evidence and the homology among the *TREM* family members, it is likely that several different variants and genes in the *TREM* region influence different features of AD susceptibility.

Here, we examine the effect of common genetic variation within the region containing the *TREM* gene family on pathological features of AD and on aging-related cognitive decline. Using neuropathological (n=1001) and cognitive (n= 3421) assessments from three prospective cohort studies of aging, we first evaluated the locus in relation to the accumulation of neuritic plaques (NP) which we previously found to be associated with AD susceptibility variants in myeloid genes, such as *CRI*²¹ and *CD33*¹⁰. We provide evidence that an intronic genetic variant, rs6910730^G, found within *TREM1* increases the burden of NP, a key early pathologic feature of AD, and increases the rate of cognitive decline. Additionally, we find a second, independent variant upstream of *TREM2*, rs7759295^C, associated with an increased accumulation of Tau-related pathology and an increased rate of cognitive decline. These results highlight the complex role of the *TREM* region in influencing AD-related neuropathology susceptibility.

Methods

The Rush Religious Orders Study (ROS), Memory and Aging Project (MAP), and Chicago Health and Aging Project (CHAP)

Informed consent was obtained from all human subjects. All blood draws and data analyses were done in compliance with protocols approved by the Institutional Review Boards of each Institution.

For ROS, started in January 1994, participants were free of known dementia at enrollment, agreed to annual clinical evaluations, and signed an Anatomic Gift Act donating their brains at death. ROS enrolls Catholic priests, nuns and brothers, aged 53 or older from about 40 groups in 12 states. The follow-up rate of survivors and autopsy rate among the deceased both exceed 90%. A more detailed description of ROS can be found in previous publications²². For MAP, started in October 1997, participants were free of known dementia at baseline, agreed to annual clinical evaluations, and signed an Anatomic Gift Act donating their brains at death. MAP enrolls men and women aged 55 or older from retirement communities in Chicago. The follow-up rate of survivors exceeds 90% and the autopsy rate exceeds 80%. A more detailed description of MAP can be found in previous publications^{23, 24}. As ROS and MAP are similar in design and population, throughout this study, we analyzed data from ROS and MAP together. Subjects were genotyped in two batches on the Affymetrix SNP Array 6.0 and Illumina OmniExpress. Analyses were performed on the subset of subjects with various pathological and longitudinal cognitive data. The average age at enrollment is 78.8 years with 70% female subjects as detailed in Table 1.

CHAP, which began in 1993, is a biracial population study enrolling African American and European American residents from Chicago²⁵. Although CHAP resembles the prospective, community-based nature of ROS and MAP, clinical evaluation for AD is performed in a stratified random sample of CHAP subjects, so throughout our study, we analyzed CHAP separately and performed a fixed-effects meta-analysis between ROS/MAP and CHAP. Subjects were genotyped in two batches on the Affymetrix SNP Array 6.0 and Illumina OmniExpress. Analyses were performed on the subset of subjects with longitudinal cognitive data. The average age at enrollment is 72.7 years with 67% female subjects as detailed in Table 1.

Cognitive and Pathological Outcomes

In ROS, MAP, and CHAP, global cognition was computed as a composite score of 19, 17, and 4 cognitive tests performed at annual evaluations, respectively^{21, 24, 26, 27}. From these scores, we created normalized summary measures to limit the influence of outliers. First, for each cohort, we convert each test into a normalized Z-score using the mean and standard deviation from the baseline evaluation of all participants in that cohort. Next, each subject's score was calculated as the average of the non-missing scores, and the summary measure was considered missing if more than half of the composite scores were missing. For all subjects with at least 2 summary measures of cognition, we tested for association of SNPs

with the rate of cognitive decline using general linear mixed models within each cohort adjusted for age at enrollment, years of education, and sex.

Brain autopsies were performed as previously described^{27–30}. We visualized neuritic plaques, diffuse plaques (DP), and neurofibrillary tangles (NFT) by modified Bielschowsky silver stain in tissue sections from the hippocampus CA1 region, entorhinal cortex, inferior parietal cortex, mid-temporal cortex, and mid-frontal cortex. To compute standardized summary measures for each phenotype, we divided each subject's mean count by the population standard deviation and performed a square root transformation. Additionally, the A β and Tau tangle densities were measured by immunohistochemistry and square root transformed as previously described³¹. The diagnosis of Lewy Body disease was adapted from the recommendations of the Report of the Consortium on DLB International Workshop³². Cerebral amyloid angiopathy was assessed in the above five regions on a five point scale and averaged across all regions into a summary score³³. The density of terminally activated stage III microglia was averaged over the inferior temporal gyrus, the midfrontal cortex, the posterior putamen, and the ventral medial caudate based on immunohistochemistry and morphology¹⁰. Clinical AD diagnosis was based on the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria³⁴. Finally, microvascular and macrovascular cerebral infarcts were visualized as previously described³⁵ and were tested as a binary variable of presence or absence.

Statistical Analysis

To examine the association between genetic variants in the *TREM* region and neuropathological or cognitive phenotypes, we used linear regression, logistic regression, or ordinal regression where appropriate. We included cohort, age at death, sex, and the first three principal components of the genotype covariance matrix analyzed using EIGENSTRAT³⁶ as covariates unless otherwise noted. For meta-analysis of ROS/MAP and CHAP results, we conducted a fixed effects meta-analysis. All statistical analyses were conducted using R (www.r-project.org) and PLINK³⁷ (<http://pngu.mgh.harvard.edu/~purcell/plink/>).

We conducted mediation analyses to determine whether the relationship between genetic variants and global cognitive decline was mediated through intermediate pathological phenotypes. For mediation analysis, we used the mediation R package (<http://cran.r-project.org/web/packages/mediation/>) to perform model-based causal mediation analysis³⁸. We built two models using the 892 subjects with complete phenotypes: first, a linear regression model with the intermediate pathological phenotype as the response variable and cohort, age at death, sex, the first three principal components from EIGENSTRAT, and the SNP of interest as explanatory variables, and secondly, a linear regression model with the rate of global cognitive decline as the response variable and cohort, age at death, sex, the first three principal components from EIGENSTRAT, the intermediate pathological phenotype of interest, and the SNP of interest as explanatory variables. We estimated the average causal mediation effects by randomizing the SNP of interest using the default quasi-Bayesian Monte-Carlo method with 10,000 simulations. When the average causal mediation

effect (ACME) had a $p < 0.05$, we claim that the intermediate phenotype significantly mediates the effect of the SNP of interest on global cognitive decline.

To address the multiple hypothesis testing burden, we corrected for the number of independent haplotypes being tested in the *TREM* region. Therefore, we pruned the 1000 Genomes Pilot 1 CEU genotypes based on linkage disequilibrium (LD) in PLINK³⁷ using a window of 50 SNPs, a cutoff of $r^2 < 0.2$, and a step of 5 SNPs. Of the 596 SNPs with $MAF > 0.01$ in the *TREM* region, we identified 36 SNPs that captured the common haplotypic diversity of the *TREM* region, leaving a locus-wide Bonferroni-corrected significance threshold of $p < 1.4 \times 10^{-3}$ used throughout this study. Where we observed a genetic variant significantly associated with NP pathology, we then assessed the association between that variant and five secondary phenotypes, DP, amyloid, NFT, Tau, and global cognitive decline, using a secondary Bonferroni-corrected significance threshold of $p < 0.01$. Finally, we associated the variant with amyloid angiopathy, Lewy body disease, stage III microglial density, clinical AD diagnosis, microvascular pathology, and macrovascular pathology to control for the possibility that the observed association with pathology or cognitive decline was related to another known cause of dementia.

Flow Cytometry

For flow cytometric analysis of TREM1 expression, we used cryopreserved peripheral blood mononuclear cells (PBMCs) from 113 healthy European American subjects of the PhenoGenetic Project at Brigham and Women's Hospital that were profiled in the ImmVar project³⁹ as well as 31 European American healthy subjects from the Harvard Aging Brain Study. Frozen peripheral blood mononuclear cells (PBMCs) from each subject were thawed, washed in HL-1 medium (Lonza, Walkersville, MD), spun and resuspended in stain buffer (PBS containing 1% FBS (Lonza) and 0.1% sodium azide (Sigma-Aldrich, St. Louis, MO)). PBMCs were pre-treated with Fc receptor block (BioLegend, San Diego, CA) and co-stained with FITC-conjugated anti-human CD33 antibody (FITC-CD33)(Miltenyi Biotec, Auburn, CA) and APC-conjugated anti-human TREM1 antibody (APC-TREM1) (BioLegend) for 30 minutes on ice according to the manufacturers' recommendations. Cells were subsequently washed in stain buffer and fixed in 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA). Data was collected on a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (TreeStar Inc., Ashland, OR). For each subject, monocytes were gated based on FITC-CD33-positive staining; this population was then used to calculate the median fluorescence intensity (MFI) of APC-TREM1. For each cohort, we analyzed TREM1 surface expression in three steps. First, we applied an inverse normal transformation to the MFI of TREM1. Second, we corrected for remaining batch effects using ComBat⁴⁰. Third, we fit linear regression models to assess the association of rs6910730 and rs7759295 with TREM1 surface expression adjusting for age, gender, and monocyte quality. To combine the cohorts and obtain an overall p -value, we performed a meta-analysis using Fisher's Z-score method.

Results

Association of an intronic TREM1 variant with AD endophenotypes

To assess the effect of common variants in the *TREM* locus (chr6:41,111,998-41,323,625) on pathological features of AD, we examined deceased subjects of European ancestry with quantitative neuropathological measures; these subjects are part of ROS and MAP^{22, 23}. To follow-up these pathological associations, we used longitudinal cognitive data from ROS, MAP, and CHAP²⁵. The demographic characteristics of these cohorts are outlined in Table 1.

In our primary analysis, we evaluated the association between genetic variants in the *TREM* locus and quantitative measures of NP burden (n=1001). Secondly, we evaluated related measures of DP burden (n=1001) and directly measured A β density (n= 984)^{31, 41, 42}. Throughout this study, we used a linkage disequilibrium (LD) corrected primary significance threshold of $p < 1.4 \times 10^{-3}$ for this targeted analysis of a known susceptibility locus and a secondary significance threshold of $p < 0.01$, as detailed in our *Methods*. For these three phenotypes, we failed to detect significant associations with common variants within *TREM2* itself, and we were unable to assess the effect of rs75932628 due to its low minor allele frequency. Additionally, we did not observe significant or suggestive associations with the proposed AD-associated variants rs3747742 (MAF=0.29) and rs9381040 (MAF=0.28) in *TREML2*. Instead, we found an association between rs6910730^G (MAF=0.12), an intronic variant in *TREM1*, and increased accumulation of NP ($p = 3.7 \times 10^{-4}$, $\beta = 0.13$) (Fig 1A) (Table 2). This association did not change significantly when the *APOE* $\epsilon 4$ haplotype was added as a covariate ($p = 4.7 \times 10^{-3}$, $\beta = 0.10$). In secondary analyses, rs6910730^G was also associated with the other amyloid-related traits, A β density ($p = 2.6 \times 10^{-3}$, $\beta = 0.23$) and DP ($p = 4.1 \times 10^{-3}$, $\beta = 0.10$), but not with NFT or Tau tangle density (Table 2). 38 SNPs in the *TREM* region were in strong LD ($r^2 > 0.5$) with rs6910730, including rs2234237 (MAF=0.11) ($r^2 = 0.786$, $D' = 1.0$ in 1000 Genomes Pilot 1 CEU data) that encodes a missense T25S variant in the extracellular Ig V-set domain of TREM1⁴³; however, we found no significant association between rs2234237 (a genotyped SNP) and these pathological measures. The previously published *TREM* region AD susceptibility variants were not in LD with rs6910730 ($r^2 < 0.05$; $D' < 0.5$).

Intrigued by these initial associations, we evaluated whether rs6910730 also influenced global cognitive decline. Using data from 3421 ROS, MAP, and CHAP subjects, we found that rs6910730^G was associated with an increased rate of global cognitive decline ($p = 5.3 \times 10^{-3}$, $\beta = -0.011$) (Fig 2A). In the subset of 892 subjects with complete neuropathology and cognitive decline data, the effect size of the association of rs6910730 with cognitive decline was attenuated by 62% when NP was included as a covariate (before adding NP as a covariate: $p = 0.058$, $\beta = -0.02$; after adding NP as a covariate: $p = 0.44$, $\beta = -0.0076$). This result suggested that the increased rate of cognitive decline relative to rs6910730^G was mediated by increased accumulation of NP pathology, so we applied a formal model-based mediation analysis to test this hypothesis. We confirmed that the effect of rs6910730 on cognitive decline mediated by NP was significant (ACME = -0.0094 ; $p = 8 \times 10^{-4}$) and that the remaining averaged direct effect (ADE) of rs6910730 on cognitive

decline was not significant ($ADE=-0.0018$; $p=0.81$). Using this analysis, we estimated 75% mediated effect, which is similar to our original estimate of 62%.

To control for the possibility that the observed association with cognitive decline was related to another known cause of dementia, we explored the relationship between rs6910730 and amyloid angiopathy, Lewy body disease, stage III microglial density, clinical AD diagnosis, microscopic cerebral infarcts, and macroscopic cerebral infarcts but found no significant effects (data not shown).

Identification of an additional TREM variant associated with AD endophenotypes

To discover additional variants in the *TREM* region that influence AD-related pathology, we performed a conditional analysis to adjust for the effect of rs6910730. After regressing out the effect of rs6910730 on NP burden, we failed to detect a significant independent association with NP.

Evaluating Tau-related phenotypes, rs7759295^C (MAF=0.12), an intergenic SNP upstream of *TREM2*, was significantly associated with increased Tau tangle density, and this association was unaffected by rs6910730 (before conditioning on rs6910730: $p=4.9\times10^{-4}$, $\beta=0.33$; after conditioning on rs6910730: $p=7.7\times10^{-4}$, $\beta=0.32$) (Fig 1B). rs7759295^C was also associated with an increased rate of global cognitive decline ($p=2.3\times10^{-3}$, $\beta=-0.012$) (Fig 2B) and an increased burden of NFT ($p=9.1\times10^{-3}$, $\beta=0.076$) (Table 3). In the subset of 892 subjects with neuropathology and cognitive decline data, we found that the effect size of the association of rs7759295 with cognitive decline was attenuated by 48% when NFT was included as a covariate (before adding NFT as a covariate: $p=0.025$, $\beta=-0.025$; after adding NFT as a covariate: $p=0.1806$, $\beta=-0.013$). As in the mediation of the association of rs6910730 with cognitive decline by NP, this result suggested that the increased rate of cognitive decline relative to rs7759295^C was mediated by increased NFT pathology, so we applied a formal model-based mediation analysis to test this hypothesis. We confirmed that the effect of rs7759295 on cognitive decline mediated by NFT was significant (ACME= -0.012 ; $p=6\times10^{-4}$) and that the residual averaged direct effect of rs7759295 on cognitive decline was not significant ($ADE=-0.0065$; $p=0.33$). Using this complementary analysis, we estimated that NFT mediates 64% of the SNP's effect, which is similar to our original estimate of 48%.

When rs7759295 and rs6910730 were considered as covariates in a single model, both SNPs were still associated with the rate of global cognitive decline suggesting that they independently affect cognitive decline (rs7759295: $p=4.5\times10^{-3}$, $\beta=-0.012$; rs6910730: $p=0.010$, $\beta=-0.011$). rs7759295^C is on the same haplotype as the rare *TREM2* missense variant rs75932628^T ($r^2=0.003$, $D'=1.000$ in 1000 Genomes Pilot 1 CEU) that has been previously associated with AD¹⁻⁴ and CSF Tau levels¹⁸, and rs7759295^C is in weak LD with the *TREML2* SNP rs9381040 ($r^2=0.02$, $D'=0.602$ in 1000 Genomes Pilot 1 CEU). As rs7759295^C is in phase with rs75932628^T in this reference SNP data, it is possible that rs7759295^C simply tagged the effect of the rare rs75932628^T that was not assessed in our cohort because its MAF<0.01. We repeated the association between Tau tangle density and rs7759295 excluding the 10 subjects with the highest Tau tangle densities and showed that the association was not driven by a handful of outliers as would be expected if the effect was

caused by the rare rs75932628^T ($p=9.1\times10^{-4}$, $\beta=-0.29$). Therefore, the association may have been driven by a functional variant more common than rs75932628 or a haplotype containing multiple rare variants.

rs7759295 was not significantly associated with amyloid angiopathy, Lewy body disease, stage III microglial density, clinical AD diagnosis, microscopic cerebral infarcts, or macroscopic cerebral infarcts (data not shown) suggesting that the observed association with cognitive decline was not driven by another known cause of dementia.

Functional genetic effects of the neuropathology-associated TREM variants

To explore the possible mechanism by which the intronic rs6910730 variant influences the accumulation of amyloid-related pathologies and the intergenic rs7759295 variant influences Tau-related pathologies, we hypothesized that, like CD33 (another AD associated protein critical to monocyte function)^{10–13}, these variants may affect the function of monocytes. We measured TREM1 surface expression in CD33+ monocytes from young healthy patients of the PhenoGenetic Cohort at Brigham and Women's Hospital and older cognitively non-impaired subjects of the Harvard Aging Brain Study. While rs7759295 did not influence TREM1 expression ($p=0.429$), our cytometric data revealed an association of rs6910730^G with decreased TREM1 surface expression ($p=1.7\times10^{-3}$) (Fig 3). Future work will be necessary to establish whether the association of rs6910730 with neuritic plaque burden is mediated by the effect of rs6910730 on TREM1 surface expression.

Discussion

Given recent associations of TREM2 variants with AD and cerebrospinal fluid biomarkers and the emerging role of immune genes in AD susceptibility and the accumulation of AD pathology^{1–4}, we performed a detailed, targeted assessment of the 212 kb *TREM* region, which contains *TREM2* and five other members of the *TREM* gene family, to establish its role in the genetic architecture of neuropathological features of AD. We found that the *TREM* region harbors genetic variation that influences the accumulation of both amyloid and Tau related pathologies as well as the rate of cognitive decline. Thus, the region appears to have a complex role in modulating the accumulation of aging-related pathologies as well as its transition to measurable deficits in cognitive function in our aging population.

Previous studies allow us to propose a mechanism by which genetic variation in *TREM1* alters amyloid pathology and to generate hypotheses that can motivate future work. First, TREM1 is an activating receptor for myeloid cells, and increased *TREM1* expression has been noted in monocytes from patients with schizophrenia, bipolar disorder, and major depressive disorder⁴⁴. Second, an expression network-based study of brain tissues proposed TYROBP, an adaptor protein through which TREM1 signals, as a key regulator of AD expression networks that may modulate myeloid and microglial function¹⁷. Further, its role in mouse models of AD is not yet clear, with conflicting results emerging from current studies: increased TREM1 protein expression has been reported in cortical samples of htau mice, a murine model of Tau pathology⁴⁵, but not in APP23 transgenic mice, a murine model of amyloid pathology⁴⁶. Nonetheless, the myeloid literature is clear in reporting its role as an important receptor that has a role in augmenting pro-inflammatory responses, and

a null allele of *TREM1* in mice results in diminished inflammatory reactions⁴⁷. Here, we report that the risk-associated rs6910730^G allele decreases TREM1 surface expression on monocytes. Given its role as an activator of myeloid cells, we hypothesize that the reduced expression of TREM1 that is driven by the rs6910730^G susceptibility allele leads to a reduced capacity for activation in myeloid cells and a reduced ability to respond to AD-related pathologies. This is the same phenotype that we have described for the *CD33* susceptibility allele where increased expression of the full-length isoform of *CD33*, an inhibitor of myeloid cells, is associated with decreased A β and dextran uptake^{10, 13}. Future studies will be necessary to explore this hypothesis. The mechanism of the Tau-associated rs7759295 variant remains unclear at this time.

In both cases, our study is underpowered for fine-mapping with which to precisely identify the causal variant(s) in the *TREM* locus, so future studies are needed to robustly identify the causal SNPs for the associations with amyloid and Tau pathology. Nonetheless, from our analyses, it is clear that there are multiple different variants within the broader *TREM* locus that independently influence AD neuropathology. Our data expand the narrative of the *TREM* locus' role in AD by showing that it influences not just Tau-related pathology¹⁸ but also amyloid-related pathology and that these independent effects converge to influence cognitive decline.

These analyses reflect our continued dissection of the causal chain linking AD risk factors to a clinical syndrome of AD through a variety of important and medically relevant endophenotypes. The availability of these different endophenotypes enables the execution of mediation analyses, which begin to orient the directional component of the causal chain. The associations that we report further elaborate the role of immune processes and particularly the innate immune system in susceptibility to AD: they suggest that variation in innate immune genes affects not just amyloid pathology – already implicated by the *CD33* and *CRI* variants^{10–13, 21} – but also Tau-related pathology, consistent with the reported association of the *TREM* region with CSF measures of Tau¹⁸. The *TREM1* association with neuropathology is noteworthy as it is clearly not related to *TREM2* and does not affect Tau pathology. It suggests that the detailed examination of these *TREM* genes at different stages of the causal chain of AD is necessary and that the innate immune system may affect the impact of several different pathologies that relate to AD.

Acknowledgments

The authors are grateful to the participants in ROS, MAP, CHAP, the Brigham & Women's PhenoGenetic Project, and the Harvard Aging Brain Study. We thank Andrew Hong for his comments on the manuscript and assistance in figure design. The study was supported by NIH grants P30AG10161, R01AG15819, R01AG17917, R01AG30146, R01AG43617, R01AG11101, K25AG41906, P01AG036694, and U01AG46152.

References

1. Guerreiro R, Wojtas A, Bras J, et al. TREM2 variants in Alzheimer's disease. The New England journal of medicine. 2013 Jan 10; 368(2):117–27. [PubMed: 23150934]
2. Jonsson T, Stefansson H, Steinberg S, et al. Variant of TREM2 associated with the risk of Alzheimer's disease. The New England journal of medicine. 2013 Jan 10; 368(2):107–16. [PubMed: 23150908]

3. Benítez BA, Cooper B, Pastor P, et al. TREM2 is associated with the risk of Alzheimer's disease in Spanish population. *Neurobiology of aging*. 2013 Jun; 34(6):1711e15–7. [PubMed: 23391427]
4. Ruiz A, Dols-Icardo O, Bullido MJ, et al. Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia. *Neurobiology of aging*. 2013 Sep 13.
5. Jin SC, Benítez BA, Karch CM, et al. Coding variants in TREM2 increase risk for Alzheimer's disease. *Human molecular genetics*. 2014 Jun 4.
6. Turnbull IR, Gilfillan S, Cella M, et al. Cutting edge: TREM-2 attenuates macrophage activation. *J Immunol*. 2006 Sep 15; 177(6):3520–4. [PubMed: 16951310]
7. Hamerman JA, Jarjoura JR, Humphrey MB, Nakamura MC, Seaman WE, Lanier LL. Cutting edge: inhibition of TLR and FcR responses in macrophages by triggering receptor expressed on myeloid cells (TREM)-2 and DAP12. *J Immunol*. 2006 Aug 15; 177(4):2051–5. [PubMed: 16887962]
8. Jiang T, Yu JT, Zhu XC, Tan L. TREM2 in Alzheimer's disease. *Molecular neurobiology*. 2013 Aug; 48(1):180–5. [PubMed: 23407992]
9. Frank S, Burbach GJ, Bonin M, et al. TREM2 is upregulated in amyloid plaque-associated microglia in aged APP23 transgenic mice. *Glia*. 2008 Oct; 56(13):1438–47. [PubMed: 18551625]
10. Bradshaw EM, Chibnik LB, Keenan BT, et al. CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. *Nature neuroscience*. 2013 Jul; 16(7):848–50.
11. Griciuc A, Serrano-Pozo A, Parrado AR, et al. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. *Neuron*. 2013 May 22; 78(4):631–43. [PubMed: 23623698]
12. Malik M, Simpson JF, Parikh I, et al. CD33 Alzheimer's Risk-Altering Polymorphism, CD33 Expression, and Exon 2 Splicing. *J Neurosci*. 2013 Aug 14; 33(33):13320–5. [PubMed: 23946390]
13. Raj T, Ryan KJ, Replogle JM, et al. CD33: increased inclusion of exon 2 implicates the Ig V-set domain in Alzheimer's disease susceptibility. *Human molecular genetics*. 2013 Dec 30.
14. Allcock RJ, Barrow AD, Forbes S, Beck S, Trowsdale J. The human TREM gene cluster at 6p21.1 encodes both activating and inhibitory single IgV domain receptors and includes Nkp44. *European journal of immunology*. 2003 Feb; 33(2):567–77. [PubMed: 12645956]
15. Colonna M. TREMs in the immune system and beyond. *Nature reviews Immunology*. 2003 Jun; 3(6):445–53.
16. Ford JW, McVicar DW. TREM and TREM-like receptors in inflammation and disease. *Current opinion in immunology*. 2009 Feb; 21(1):38–46. [PubMed: 19230638]
17. Zhang B, Gaiteri C, Bodea LG, et al. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell*. 2013 Apr 25; 153(3):707–20. [PubMed: 23622250]
18. Cruchaga C, Kauwe JS, Harari O, et al. GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease. *Neuron*. 2013 Apr 24; 78(2):256–68. [PubMed: 23562540]
19. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nature genetics*. 2013 Oct 27.
20. Benítez BA, Jin SC, Guerreiro R, et al. Missense variant in TREML2 protects against Alzheimer's disease. *Neurobiology of aging*. 2013 Dec 21.
21. Chibnik LB, Shulman JM, Leurgans SE, et al. CR1 is associated with amyloid plaque burden and age-related cognitive decline. *Annals of neurology*. 2011 Mar; 69(3):560–9. [PubMed: 21391232]
22. Bennett DA, Schneider JA, Arvanitakis Z, Wilson RS. Overview and findings from the religious orders study. *Current Alzheimer research*. 2012 Jul; 9(6):628–45. [PubMed: 22471860]
23. Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS. Overview and findings from the rush Memory and Aging Project. *Current Alzheimer research*. 2012 Jul; 9(6): 646–63. [PubMed: 22471867]
24. Bennett DA, Schneider JA, Buchman AS, Mendes de Leon C, Bienias JL, Wilson RS. The Rush Memory and Aging Project: study design and baseline characteristics of the study cohort. *Neuroepidemiology*. 2005; 25(4):163–75. [PubMed: 16103727]
25. Bienias JL, Beckett LA, Bennett DA, Wilson RS, Evans DA. Design of the Chicago Health and Aging Project (CHAP). *Journal of Alzheimer's disease : JAD*. 2003 Oct; 5(5):349–55.

26. Keenan BT, Shulman JM, Chibnik LB, et al. A coding variant in CR1 interacts with APOE-epsilon4 to influence cognitive decline. *Human molecular genetics*. 2012 May 15; 21(10):2377–88. [PubMed: 22343410]
27. Bennett DA, Wilson RS, Schneider JA, et al. Natural history of mild cognitive impairment in older persons. *Neurology*. 2002 Jul 23; 59(2):198–205. [PubMed: 12136057]
28. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. *Neurology*. 2006 Jun 27; 66(12):1837–44. [PubMed: 16801647]
29. Bennett DA, Schneider JA, Bienias JL, Evans DA, Wilson RS. Mild cognitive impairment is related to Alzheimer disease pathology and cerebral infarctions. *Neurology*. 2005 Mar 8; 64(5):834–41. [PubMed: 15753419]
30. Schneider JA, Wilson RS, Bienias JL, Evans DA, Bennett DA. Cerebral infarctions and the likelihood of dementia from Alzheimer disease pathology. *Neurology*. 2004 Apr 13; 62(7):1148–55. [PubMed: 15079015]
31. Barnes LL, Schneider JA, Boyle PA, Bienias JL, Bennett DA. Memory complaints are related to Alzheimer disease pathology in older persons. *Neurology*. 2006 Nov 14; 67(9):1581–5. [PubMed: 17101887]
32. McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology*. 1996 Nov; 47(5):1113–24. [PubMed: 8909416]
33. Arvanitakis Z, Leurgans SE, Wang Z, Wilson RS, Bennett DA, Schneider JA. Cerebral amyloid angiopathy pathology and cognitive domains in older persons. *Annals of neurology*. 2011 Feb; 69(2):320–7. [PubMed: 21387377]
34. 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 Jul; 34(7):939–44. [PubMed: 6610841]
35. Buchman AS, Yu L, Boyle PA, et al. Microvascular brain pathology and late-life motor impairment. *Neurology*. 2013 Feb 19; 80(8):712–8. [PubMed: 23365057]
36. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nature genetics*. 2006 Aug; 38(8):904–9. [PubMed: 16862161]
37. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *American journal of human genetics*. 2007 Sep; 81(3):559–75. [PubMed: 17701901]
38. Imai K, Keele L, Tingley D. A general approach to causal mediation analysis. *Psychological methods*. 2010 Dec; 15(4):309–34. [PubMed: 20954780]
39. Raj T, Rothamel K, Mostafavi S, et al. Polarization of the effects of autoimmune and neurodegenerative risk alleles in leukocytes. *Science*. 2014 May 2; 344(6183):519–23. [PubMed: 24786080]
40. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics*. 2007 Jan; 8(1):118–27. [PubMed: 16632515]
41. Bell KF, Ducatenzeiler A, Ribeiro-da-Silva A, Duff K, Bennett DA, Cuellar AC. The amyloid pathology progresses in a neurotransmitter-specific manner. *Neurobiology of aging*. 2006 Nov; 27(11):1644–57. [PubMed: 16271419]
42. Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Education modifies the association of amyloid but not tangles with cognitive function. *Neurology*. 2005 Sep 27; 65(6):953–5. [PubMed: 16186546]
43. Su L, Liu C, Li C, et al. Dynamic changes in serum soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and its gene polymorphisms are associated with sepsis prognosis. *Inflammation*. 2012 Dec; 35(6):1833–43. [PubMed: 22798017]
44. Weigelt K, Carvalho LA, Drexhage RC, et al. TREM-1 and DAP12 expression in monocytes of patients with severe psychiatric disorders. EGR3, ATF3 and PU.1 as important transcription factors. *Brain, behavior, and immunity*. 2011 Aug; 25(6):1162–9.

45. Garwood CJ, Cooper JD, Hanger DP, Noble W. Anti-inflammatory impact of minocycline in a mouse model of tauopathy. *Frontiers in psychiatry*. 2010; 1:136. [PubMed: 21423446]
46. Melchior B, Garcia AE, Hsiung BK, et al. Dual induction of TREM2 and tolerance-related transcript, *Tmem176b*, in amyloid transgenic mice: implications for vaccine-based therapies for Alzheimer's disease. *ASN neuro*. 2010; 2(3):e00037. [PubMed: 20640189]
47. Weber B, Schuster S, Zysset D, et al. TREM-1 deficiency can attenuate disease severity without affecting pathogen clearance. *PLoS pathogens*. 2014 Jan.10(1):e1003900. [PubMed: 24453980]

FIGURE 1. rs6910730 and rs7759295 are independently associated with neuropathology in ROS and MAP

In post-mortem neuropathological assessments of ROS and MAP subjects, we visualized neuritic plaques by modified Bielschowsky silver stain and measured Tau tangle density by immunohistochemistry in tissue sections from five brain regions. Using linear regression, we assessed the association between common SNPs in the *TREM* locus and a normalized measure of NP and Tau tangle density. (A) rs6910730^G was associated with an increased accumulation of NP (n=1001)($p=3.7 \times 10^{-4}$, $\beta=0.13$). (B) rs7759295^C was associated with an increased Tau tangle density (n=976)($p=4.9 \times 10^{-4}$, $\beta=-0.33$), and this association was not

significantly diminished by conditioning on rs6910730 (after conditioning on rs6910730: $p=7.7\times 10^{-4}$, $\beta=0.32$). In both panels, the color of a SNP represents the strength of the linkage disequilibrium (measured by r^2 in the 1000 Genomes EUR March 2012 Release) between the SNP of interest and the lead SNP, rs6910730 or rs7759295, indicated in purple. Red corresponds to an $r^2 \geq 0.8$; orange corresponds to $0.6 \leq r^2 < 0.8$; green corresponds to $0.4 \leq r^2 < 0.6$; light blue corresponds to $0.2 \leq r^2 < 0.4$; dark blue corresponds to $r^2 < 0.2$.

FIGURE 2. rs6910730 and rs7759295 are associated with the rate of cognitive decline in ROS, MAP, and CHAP

Using annual cognitive assessments of ROS, MAP, and CHAP subjects, we computed normalized summary measures of global cognition and evaluated the relationship of rs6910730 and rs7759295 with the rate of cognitive decline using general linear mixed models. (A) rs6910730^G, which was associated with an increased accumulation of NP, was associated with an increased rate of cognitive decline (n=3421) ($p=5.3 \times 10^{-3}$, $\beta=-0.011$). (B) rs7759295^C, which was associated with an increased Tau tangle density, was associated with an increased rate of cognitive decline (n=3421) ($p=2.3 \times 10^{-3}$, $\beta=-0.012$).

FIGURE 3. rs6910730 is associated with TREM1 surface expression on monocytes

In CD33⁺ monocytes isolated from cryopreserved PBMCs of healthy subjects (n=139), we measured TREM1 surface expression by flow cytometric analysis. Panel (A) shows histograms of the fluorescence intensity of an isotype control (white), an rs6910730^{AG} subject with a median level of TREM1 expression (gray), and an rs6910730^{AA} subject with a median level of TREM1 expression (black). (B) After correcting the TREM1 MFI for batch effects and adding age, cohort, gender, and cell viability as covariates, rs6910730^G was associated with decreased TREM1 surface expression ($p=0.0028$, $\beta=-9.53$).

Table 1

Demographic characteristics of ROS, MAP, and CHAP.

Study	Number of subjects	Age at enrollment	Percent female	Genotyping Array
ROS/MAP (1)	1704	78.5 (± 7.5)	69%	Affymetrix SNP Array 6.0
ROS/MAP (2)	382	80.1 (± 7.3)	73%	Illumina OmniExpress
CHAP (1)	624	71.9 (± 5.2)	63%	Affymetrix SNP Array 6.0
CHAP (2)	711	73.4 (± 7.3)	60%	Illumina OmniExpress

Table 2

Association of rs6910730 with cognitive and pathological phenotypes in ROS and MAP.

Phenotype	Analysis	Sample Size	rs6910730 <i>p</i> -value	rs6910730 effect size
Neuritic Plaques	linear regression	1001	0.00037**	0.13 [†]
Aβ Density	linear regression	984	0.0026*	0.23 [†]
Diffuse Plaques	linear regression	1001	0.0041*	0.097 [†]
Tau Tangle Density	linear regression	976	0.22	0.11 [†]
Neurofibrillary Tangles	linear regression	1001	0.43	0.022 [†]
Global Cognition	general linear mixed model	3421	0.0053*	-0.011 [†]

** This association surpasses the locus-wide significance threshold of $p < 1.4 \times 10^{-3}$.

* This association surpasses the secondary association significance threshold of $p < 0.01$.

[†] The reported effect size is the regression coefficient, β , of rs6910730^G.

Table 3

Association of rs7759295 with cognitive and pathological phenotypes in ROS and MAP.

Phenotype	Analysis	Sample Size	rs7759295 <i>p</i> -value	rs7759295 effect size
Neuritic Plaques	linear regression	1001	0.0082 [*]	0.1 [†]
Aβ Density	linear regression	984	0.19	0.11 [†]
Diffuse Plaques	linear regression	1001	0.015	0.085 [†]
Tau Tangle Density	linear regression	976	0.00049 ^{**}	0.33 [†]
Neurofibrillary Tangles	linear regression	1001	0.0091 [*]	0.076 [†]
Global Cognition	general linear mixed model	3421	0.0023 [*]	−0.012 [†]

^{**} This association surpasses the locus-wide significance threshold of $p < 1.4 \times 10^{-3}$.

^{*} This association surpasses the secondary association significance threshold of $p < 0.01$.

[†] The reported effect size is the regression coefficient, β , of rs7759295^C.