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Examination of the Effect of Rare Variants in *TREM2*, *ABI3*, and *PLCG2* in LOAD Through Multiple Phenotypes

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Abstract

Background: Rare variants in *PLCG2* (p.P522R), *ABI3* (p.S209F), and *TREM2* (p.R47H, p.R62H) have been associated with late onset Alzheimer's disease (LOAD) risk in Caucasians. After the initial report, several studies have found positive results in cohorts of different ethnic background and with different phenotype.

Objective: In this study, we aim to evaluate the association of rare coding variants in *PLCG2*, *ABI3*, and *TREM2* with LOAD risk and their effect at different time points of the disease.

Methods: We used a European American cohort to assess the association of the variants prior onset (using CSF $A\beta_{42}$, tau, and pTau levels, and amyloid imaging as endophenotypes) and after onset (measured as rate of memory decline).

Results: We confirm the association with LOAD risk of *TREM2* p.R47H, p.R62H and *ABI3* p.S209F variants, and the protective effect of *PLCG2* p.P522R. In addition, *ABI3* and *TREM2* gene-sets showed significant association with LOAD risk. *TREM2* p.R47H and *PLCG2* p.P522R variants were also statistically associated with increase of amyloid imaging and AD progression, respectively. We did not observe any association of *ABI3* p.S209F with any of the other AD endophenotypes.

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SUPPLEMENTARY MATERIAL

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Conclusion: The results of this study highlight the importance of including biomarkers and alternative phenotypes to better understand the role of novel candidate genes with the disease.

Keywords

ABI3; endophenotypes; late onset Alzheimer's disease; *PLCG2*; progression; *TREM2*

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in the elderly and there is not a known effective method to treat or prevent it. About 95% of all AD cases are considered sporadic, of which approximately 90% have a late-onset (LOAD) of the disease [1]. People affected with AD start presenting symptoms at the age of 65–70, 15 to 20 years after the etiology of this disease starts [2]. Apolipoprotein E (*APOE*) *ε*4 allele was the first locus discovered to be significantly associated with LOAD risk [3, 4]. Up until today, genome-wide association studies (GWAs) have identified 29 independent risk loci for AD, most of them tagged by common variants (minor allele frequency (MAF) >5%) with very small risk effects [4–9]. Only ~31% of the heritability of this trait can be explained by the known common variants identified by GWAs [10]. Thus, this high missing heritability is currently being searched within rare variants that have moderate to high effect on disease risk [10–13]. Back in 2013, rare variants in *TREM2* (p.R47H, p.R62H among others) were associated with LOAD [14, 15] not only in European Americans but also in African Americans [16]. Recently, Sims et al. reported the genome-wide association with LOAD of four rare coding variants: two risk variants in *TREM2* (p.R62H, OR = 1.67; p.R47H, OR = 2.46), a protective variant in *PLCG2* (p.P522R, OR = 0.68), and a risk variant in *ABI3* (p.S209F, OR = 1.43) [17].

Recent studies have replicated the association of those variants in different ethnicities. Dalmasso et al. reported the significant association with AD of *TREM2* p.R47H ($p = 0.02$, OR = 2.29) and *PLCG2* p.P522R ($p = 0.05$, OR = 0.60) in 905 Argentinian individuals with European ancestry [18]. Similarly, Lancaster et al. reported the association of *TREM2* p.R62H, *PLCG2* p.P522R, and *ABI3* p.S209F with increased AD risk in early life of 766 young healthy European Americans [19]. Their results suggest that the association of *TREM2* p.R62H variant and *PLCG2* p.P522R variant with volumetric reductions in basal ganglia may be linked with LOAD susceptibility [19]. *PLCG2* p.P522R variant has also been associated with reduced AD risk and increased likelihood to reach the age of 90 years [20]. Other groups have replicated the association with AD of p.R47H and p.P522R variants in an Argentinian cohort [18], and p.P522R and p.S209F variants in African American subjects [16, 21]. Due to the heterogeneity of populations, replication of GWAs findings in independent cohorts with the same ancestry as the original finding is necessary to provide evidence for the impact that novel genetic components have in the same population for a complex trait like AD.

AD-endophenotypes can help to provide a biological interpretation of the genes identified in genetic studies. Levels of tau, phosphorylated tau (pTau), and amyloid-β_{42} ($A\beta_{42}$) biomarkers in cerebrospinal fluid (CSF) change before clinical symptoms of AD can be

observed [2]. CSF $A\beta_{42}$ and higher CSF tau and pTau, decrease with progression of the disease whereas CSF levels of tau and pTau, are associated with AD status. Amyloid imaging allows the measurement of *in vivo* $A\beta_{42}$ deposition in the brain, is one of the most reliable current biomarkers for AD at this moment, and increases as the disease progresses [22, 23]. Both measures complement each other and can be used as intermediate phenotypes that can link genetic variation caused by these variants, to disease-predisposing factors [2, 24, 25]. Finally, rate of dementia progression over time provides longitudinal data also useful to complete the understanding of the association of a genotype with AD [8].

Our study aims to replicate, in an independent European American cohort, the association with AD of the TREM2 variants (p.R62H, p.R47H [14, 15]) as well as the two novel genes/variants reported by Sims et al. (*PLCG2* p.P522R and *ABI3* p.S209F). In addition, here we aim to expand the knowledge on the effects these variants have on AD prior and after diagnosis. We study the effects of the variants on endophenotypes measurable prior to disease (amyloid imaging and CSF levels of tau, pTau, and $A\beta_{42}$ [2]) and rate memory decline after disease onset.

MATERIALS AND METHODS

Ethics statement

The Institutional Review Boards of all participating institutions approved the study and research was carried out in accordance with the approved protocols. Written informed consent was obtained from participants or their family members. The IRB approval number for this study is 201104178.

Cohorts descriptions

For this study, we aimed to generate a large dataset of European American unrelated individuals combining data from the Knight-Alzheimer's Disease Research Center (Knight ADRC) [26], the National Institute on Aging Genetics Initiative for Late-Onset Alzheimer's Disease (NIA-LOAD) [27], and the Alzheimer Disease Sequencing Project (ADSP - pht003392.v7.p4) (Table 1). A description of the Knight-ADRC and the NIA-LOAD datasets has been previously reported [26, 27]. The ADSP data is available to qualified researchers through the database of Genotypes and Phenotypes (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/dataset.cgi?studyid=phs000572.v8.p4&pht=3392).

We used GWAs and whole exome sequencing (WES) data from the previous studies as well as CSF ($A\beta_{42}$, tau, and pTau), amyloid imaging and AD progression data, to perform the analyses in this study (Supplementary Table 1). Neuropathology autopsy information and clinical diagnosis using Clinical Dementia Rating (CDR) [28] were used to define the phenotype status of the subjects included in our analyses. People with dementia caused by neurological diseases other than AD or younger than 65 years old were not included in the analyses.

Genetic data

The generation and quality control (QC) of the GWAs and WES data was performed as previously described [29, 30]. Principal Component Analysis (PCA) was performed using HapMap reference panel to select European ancestry subjects. We used Identity-by-Descendent (IBD) estimations to identify and remove duplicated samples across cohorts and to evaluate relatedness. Unrelated samples (Pihat <0.2) and one sample from every pair of related individuals were included in the analyses, prioritizing the selection of older controls and younger cases. From the NIA-LOAD dataset there was GWAs and WES data available for 928 and 279 unrelated European American samples, respectively. From the Knight-ADRC dataset, there was GWAs and WES data available for 1,218 and 416 unrelated European American samples, respectively. We also included 9,621 unrelated European American samples with WES data from the ADSP study (Supplementary Table 1). There were 90 overlapping samples between the NIA-LOAD and the ADSP dataset; we examined each pair and kept the sample with better genotyping rate. Our final dataset included 7,000 cases (5,791 with WES data and 1,209 with GWAs data) and 5,462 controls (4,525 with WES data and 937 with GWAs data; Supplementary Table 1).

We restricted our analyses to all variants with a 98% genotyping rate, in either the GWAs or WES datasets, for the *TREM2* (chr.6 : 41,126,244–41,130, 924), *PLCG2* (chr.16 : 81,772,702–81,991,899), and *ABI3* (chr.17 : 47,287,589–47300587) genes (chromosomic ranges refer to the GRCh37 reference genome). The genotyping rate for the previously reported variants (*TREM2* p.R47H and p.R62H, *PLCG2* p.P522R and *ABI3* p.S209F) within the GWAs dataset was higher than 98%. In the WES dataset *TREM2* and *PLCG2* variants had a genotyping rate higher than 99% but *ABI3* p.S209F had a 96% genotyping rate, so we had to force its incorporation in the dataset (Supplementary Table 2).

Statistical analyses

AD risk analyses: Single variant—Single variant analysis was conducted to test the association of *PLCG2* p.P522R, *ABI3* p.S209F, and *TREM2* p.R62H and p.R47H with AD risk. Genotypic information for these variants was obtained by combining all GWAs and WES data [30] from the previously mentioned projects. The final analysis was done with 12,462 subjects from the ADSP, Knight ADRC, and NIA-LOAD cohorts (Supplementary Table 1). Given the high and similar genotyping rate in both datasets, we decided to run joint analysis for *TREM2* and *PLCG2* variants using the logistic regression model from PLINKv1.9 [23] and correcting by the two first principal components (PCs) and sex. The analysis for *ABI3* variant also included whether the sample came from the GWAs or the WES data as covariate given the different genotyping rate for variants within this gene (Supplementary Table 2). Given that there is certain sample overlap between Sims et al. [17] and the cohorts used in this analysis, we also performed the single variant analysis association after removing the overlapping samples. Bonferroni correction was applied.

Cerebrospinal fluid—CSF levels of tau, pTau, and $A\beta_{42}$ were measured for individuals in the Knight-ADRC cohort. CSF $A\beta_{42}$ thresholds have been previously determined only for this cohort. Individuals with CSF $A\beta_{42}$ values ≥500 pg/mL were excluded as individuals below these thresholds have been shown to have fibrillary $A\beta_{42}$ deposits in brain [31, 32]

whereas those with higher levels are more likely to not have AD. CSF data normalization was done as previously described [24, 33].

We used the linear regression model in PLINKv1.9 [23] to assess the association of the SNPs of interest with the following AD endophenotypes, employed as the dependent variable assuming an additive model: CSF levels of tau (N = 559), pTau (N = 553), and $A\beta_{42}$ (N = 558) (Table 1). The two first PCs, sex and age at CSF collection were used as covariates for these analyses. Bonferroni correction was applied.

Imaging

Molecular imaging with positron emission tomography (PET) was used to detect $A\beta_{42}$ deposition during life. Detailed methods are described in previous reports for Knight-ADRC [34]. The analysis of these data was done using only one value of $A\beta_{42}$ scan for each individual, thus we only used the baseline visit for the analysis of individuals with more than one $A\beta_{42}$ scan. After this selection, we included a total of 420 individuals in this analysis (Table 1) that was performed using the linear regression model in PLINKv1.9 [23] and corrected by the two first PCs and sex. Bonferroni correction was applied.

Memory decline

Clinical Dementia Rating Scale Sum of Boxes (CDR-SB) was used to determine rate of memory decline; CDR-SB is a sum of the scores from six tests (boxes) that measure different AD dementia symptoms to provide a score in staging dementia severity from 0 (cognitively normal) to 18 (the most severe dementia). We included only samples with three or more clinical assessments and 1.5 years of follow-up and we removed samples with a constant 0 or 18 value of CDR-SB over time [8]. Finally, 446 samples from Knight-ADRC were included in this analysis (Table 1). The association of the SNPs of interest with AD progression was tested by longitudinal regression using the nlme package in R [35]. Change of CDR-SB was set as the independent variable with the following covariates:

$$\begin{aligned} &\textit{CDR Sum of Boxes} = \textit{SNP} * \textit{time interval} \\ &+ \textit{CDR baseline} * \textit{time interval} \\ &+ \textit{SNP} + \textit{time interval} \\ &+ \textit{CDR baseline} \\ &+ \textit{Sex} + \textit{Age baseline} \\ &+ \textit{Education} + \textit{PC}1 + \textit{PC}2 \end{aligned}$$

Gene based

We performed gene-based analysis for the following AD phenotypes: AD risk, CSF $A\beta_{42}$, CSF tau, CSF pTau, and amyloid imaging.

For the AD risk phenotype, we restricted our gene-based analysis to those samples with WES data (10,316 individuals from Knight-ADRC, NIA-LOAD, and ADSP). We used SnpEff [36] to predict the functional consequence of the observed polymorphisms in *TREM2*, *ABI3*, and *PLCG2* and we restricted our analysis to predicted nonsynonymous,

missense and loss-of-function (LOF) variants [37] with MAF<0.01 and we kept for analysis those variants with a genotyping rate over 90% across both datasets. The final dataset included 211 nonsynonymous variants, 39 variants in *TREM2*, 136 in *PLCG2*, and 36 in *ABI3* (Supplementary Table 3). We performed the gene-based analysis at three levels; one that included all non-synonymous variants, one that included all non-synonymous variants minus the top leading variant, and one in which only common variants across all cohorts were included. We also performed the gene-based analysis stratified per each one of the major cohorts to highlight the contribution of each one of these cohorts to the final value reported (Supplementary Table 4).

For the AD endophenotypes (CSF $A\beta_{42}$, tau, pTau, and amyloid imaging), we used the variants from the previous subset of rare non-synonymous variants that were present in the GWAs and WES samples that had data for these endophenotypes. That included three variants for *TREM2*, five variants for *PLCG2* and one variant for *ABI3*.

To perform gene-based analysis, we used the SKAT-O algorithm in the R-package Sequence Kernel Association Test (SKAT) which combines collapsing and variance component tests into one Statistical method [38]. We used the SKAT null model and adjust the analysis for the two first PCs and sex (for the AD Risk analysis) and we also corrected for age at what the CSF sample, or the PET Imaging was taken (for the endophenotype analysis). Bonferroni correction was applied.

RESULTS

We performed single variant analysis to assess the association with AD risk of the four variants of interest (*PLCG2* p.P522R, *ABI3* p.S209F, and *TREM2* p.R62H and p.R47H) followed by a gene-based analysis to test the burden of rare non-synonymous variants in each gene. In addition, we conducted AD progression and quantitative trait analyses to evaluate the association of the variants with other characteristics of the disease. Demographic summary statistics for the samples included in each analysis and the datasets used for each analysis are shown in Table 1.

After merging GWAs and WES data from NIA-LOAD, Knight ADRC, and ADSP, genotyping data for 12,372 individuals for the variants of interest passed QC. We confirmed the previously reported associations and in the same direction for all the SNPs of interest [14, 15, 17]; both variants in *TREM2* (p.R62H, $p = 1.73 \times 10^{-03}$, OR = 1.49; p.R47H, $p = 2.09 \times 10^{-11}$, OR = 4.38) and the variant in *ABI3* (p.S209F, $p = 1.03 \times 10^{-03}$, OR = 1.59) were associated with increased AD risk, while the variant in *PLCG2* (p.P522R, $p = 2.16 \times 10^{-03}$, OR=0.63) confirmed its protective effect in our cohort (Table 2). In order to evaluate whether the Knight-ADRC and NIA-LOAD datasets from WASHU are contributing to the already reported effect for these variants by the ADSP consortia [41], we performed the previous analysis stratified by major cohort (Supplementary Tables 4–6).

Some of the samples in this study were also included in the analysis performed by Sims et al. [17]. After excluding overlapping samples (final sample size = 5,972), *TREM2* (p.R47H)

and *PLCG2* (p.P522R) remained nominally significant with similar OR to the analysis including all samples (Supplementary Table 7).

For the gene-based analysis, only rare nonsynonymous variants were included. 39 variants in *TREM2* (including p.R62H and p.R47H), 36 variants in *ABI3* (including p.S209F), and 136 variants in *PLCG2* (including p.P522R) across 10,316 individuals from Knight-ADRC, NIA-LOAD, and ADSP cohorts went into analysis (Supplementary Table 3). We found a significant association with AD risk for *TREM2* ($p = 9.37 \times 10^{-14}$) and *ABI3* ($p = 0.001$). *TREM2* remained significant ($p = 1.75 \times 10^{-03}$) after removing p.R47H and p.R62H from analysis. We even still observed a nominal association in the *TREM2* gene-based analyses event after the p.R47H, p.R62H, p.D87N, and p.H157T were removed (Supplementary Table 8), indicating that additional variants in *TREM2* contribute to AD risk. *ABI3* and *PLCG2* were not significant after removing the p.S209F and the p.P522R variant respectively. The overall gene direction for *ABI3* (OR = 1.21) followed that of the key variants, even when these were removed from the analysis (Table 3), suggesting that additional risk variants in these genes confer risk for AD. Finally, since some variants were private for each of the cohorts included, we performed a gene-based analysis considering only the common variants across datasets, to confirm whether the association was driven by any particular cohort. All three genes resulted significant when all cohorts were analyzed together (Table 3); a closer analysis revealed that significance for *TREM2* was driven by the ADSP and Knight-ADRC cohorts, *PLCG2* was significant for the NIA-LOAD cohort, and *ABI3* was significant for the ADSP cohort.

After confirming that our study has power to identify significant association with AD risk, we analyzed if any of the variants and genes were associated with other AD-related phenotypes. *TREM2* p.R47H showed a trend toward association with CSF levels of $A\beta_{42}$ ($p = 0.041$, $\beta = -0.68$) and a significant association (after Bonferroni correction) for amyloid imaging ($p = 0.008$; Table 4). *TREM2* was also associated at gene-level with the levels of amyloid imaging ($p = 0.012$, Supplementary Table 9). No significant association with this phenotype was observed for *PLCG2* p.P522R, *TREM2* p.R62H, and *ABI3* p.S209F. None of the variants analyzed showed significant association with CSF levels of tau and pTau (Table 4). The *PLCG2* p.P522R variant showed a trend towards association with change in CDR-SB ($p = 0.028$) equivalent to a slower progression of AD dementia (Table 5). *TREM2* p.R62H, pR27H and *ABI3* p.S209F variants were not significant in this analysis.

DISCUSSION

GWAs have been instrumental in the field of AD to identify novel loci associated with the disease. However, the functional genetic variants are mainly unknown as so is their real effect on the pathology of the disease.

In this study, we analyzed the association of *TREM2* p.R62H, *TREM2* p.R47H, *PLCG2* p.P522R, and *ABI3* p.S209F variants previously associated with AD using GWAs and WES data of 12,462 individuals. Using single variant analysis, we confirmed the association with LOAD status and the direction of effect previously reported for the four variants of interest (Table 2). Additionally, we observed that other nonsynonymous rare variants in *TREM2*

contribute to AD risk (gene-based after removing p.R62H and p.R47H variants, $p = 1.75 \times 10^{-03}$), reinforcing the important role of rare variants in *TREM2* towards LOAD susceptibility [16, 40]. Particularly, we observe that variant p.D87N (6 : 41129133:C:T, rs142232675) is nominally significantly associated with AD risk (Supplementary Table 4). Even though this variant has been found in AD cases before [14, 41], this is the first study in which a significant association with AD risk is found even in the Sims et al. study [17]. The *TREM2* variant p.H157Y (6 : 41127543:G:A, rs2234255) also showed a trend to association with AD risk (Supplementary Table 4) in our study. This variant has been previously reported to be significantly associated with AD in the ADSP cohort [41], in a Chinese cohort and in a meta-analysis of Caucasian, Japanese, and African American cohorts [42].

TREM2 p.R47H has been associated with AD risk in multiple studies [14, 16, 18, 43–52]. This variant increases risk for AD almost three-fold and is the greatest genetic risk factor identified for AD after the *APOE ε*4 allele [14, 15, 53]. *TREM2* p.R47H has been previously associated with increasing levels of tau and pTau in CSF, without affecting $A\beta_{42}$ [43, 49]. In this cohort the *TREM2* p.R47H showed the same direction of association but not significant in our quantitative trait analysis for tau and pTau; however, we observe a significant association with decreasing levels of $A\beta_{42}$ in CSF ($p = 0.041$, $\beta = -0.68$). In addition, we observe a significant association for p.R47H with increased amyloid imaging ($p = 0.008$, $\beta = 0.07$). This is the first time, to our knowledge, that *TREM2* is associated with AD risk, $A\beta_{42}$ levels in CSF and amyloid imaging at the same time using the same cohort. *TREM2* p.R62H variant has been previously associated with a faster AD progression ($p = 0.027$, $\beta = 0.31$) in 1,499 samples from ADNI and Knight-ADRC datasets [8], but it does not show association with progression in our 446 samples from Knight-ADRC, probably due to the smaller size of our cohort.

In this study we replicate the association of *PLCG2* p.P522R with AD risk after removing the overlapping samples with Sims et al., which support the role of this variant and gene in AD and reinforces the importance of investigating the role of this variant in AD. In addition, we also observed that this variant has a “protective” effect on memory decline ($p = 0.028$, $\beta = -1.25$) which relates to previous observations that this variant is enriched in a healthy centenarians cohort [20]. *PLCG2* p.P522R has been seen to modify the generation of the PLCγ2 enzyme[36,3] [20, 54], opening the venues for novel pharmacological targets that may attenuate the progression of this disease.

This study faces some limitations. 48% of the samples used in this study for the AD risk analysis have been previously published (e.g., Sims et al. [17] and Kunkle et al. [7]). However, the main goal of this study is not to replicate the association of those rare variants with AD but to determine if those variants also influence other AD phenotypes such as CSF and imaging biomarkers. Another limitation is that we only have WES data for half of the samples with endophenotype information available at the Knight-ADRC. WES data is being generated for the entire Knight-ADRC cohort; hence, future studies should be able to better evaluate the role of rare variants on AD endophenotypes.

In conclusion, we confirmed the association of *TREM2* p.R47H, not only with AD risk but also with amyloid imaging. Also, other rare variants in this gene participate in disease

pathology which deserves further examination. Finally, we confirm the protective effect of *PLCG2* p.P522R variant, which could exert its role by slowing the rate of memory decline.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The ADGC cohorts include: Adult Changes in Thought (ACT), the Alzheimer's Disease Centers (ADC), the Chicago Health and Aging Project (CHAP), the Memory and Aging Project (MAP), Mayo Clinic (MAYO), Mayo Parkinson's Disease controls, University of Miami, the Multi-Institutional Research in Alzheimer's Genetic Epidemiology Study (MIRAGE), the National Cell Repository for Alzheimer's Disease (NCRAD), the National Institute on Aging Late Onset Alzheimer's Disease Family Study (NIA-LOAD), the Religious Orders Study (ROS), the Texas Alzheimer's Research and Care Consortium (TARC), Vanderbilt University/Case Western Reserve University (VAN/CWRU), the Washington Heights-Inwood Columbia Aging Project (WHICAP) and the Washington University Sequencing Project (WUSP), the Columbia University Hispanic- Estudio Familiar de Influencia Genetica de Alzheimer (EFIGA), the University of Toronto (UT), and Genetic Differences (GD).

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Table 1

Summary statistics of the samples included in each analysis

Phenotype	N	%CA	%Fe	Age (±SD)	%*APOE ε4*	Cohort	Dataset
$A\beta_{42}$	558	33.51	68.33	75.98 (6.47)	40.04	K	GWAs
Tau	560	33.63	51.34	76.06 (6.56)	40.22	K	GWAs
pTau	553	32.73	51.18	76.85 (7.65)	40.15	K	GWAs
Amyloid imaging	420	20.95	53.57	76.25 (6.52)	37.38	K	GWAs-WES
AD progression	446	100.00	57.17	75.85 (7.02)	62.50	K	GWAs-WES
AD risk single variant	12,372	55.95	54.84	80.79 (8.32)	30.84	K-N-A	GWAs-WES
AD risk gene based	10,226	55.87	54.46	81.64 (8.27)	28.56	K-N-A	WES
Total dataset	12,372	55.95	54.84	80.79 (8.32)	30.84	K-N-A	GWAs-WES

N, number of samples included; %CA, percentage of cases; %Fe, percentage of females; AGE, average age at onset for cases and average age at last assessment for controls; K, Knight-ADRC; N, NIA-LOAD; A, ADSP; SD, standard error.

Table 2

Single variant analysis for *TREM2*, *PLCG2*, and *ABI3* variants of interest using individuals with GWAs or WES data from ADSP, Knight-ADRC, and NIA-LOAD cohorts

Locus (variant)	OR	95% CI	*P*	MAF $_{cases}$	MAF $_{controls}$	N
TREM2 (p.R62H)	1.49	1.16, 1.93	$\mathbf{1.73 \times 10^{-3}}$	0.013	0.008	12,332
TREM2 (p.R47H)	4.38	2.84, 6.78	$\mathbf{2.09 \times 10^{-11}}$	0.010	0.002	12,337
PLCG2 (p.P522R)	0.63	0.47, 0.85	$\mathbf{2.16 \times 10^{-3}}$	0.006	0.009	12,360
ABI3 (p.S209F)*	1.59	1.20, 2.11	$\mathbf{1.03 \times 10^{-3}}$	0.011	0.007	11,887

OR, odds ratio; CI, confidence interval;*p*,*p*-value; MAF, minor allele frequency; N, number of samples included in the analysis. Significant *p*-values after Bonferroni correction in bold.

*The association analysis for this variant was run using WES/GWAs as covariate, in addition to SEX, PC1, PC2 and to account for the difference in genotyping rate between the GWAs data and the WES data.

Table 3

Gene based analysis for *TREM2*, *PLCG2*, and *ABI3* including rare (MAF < 1%) nonsynonymous variants, using 10,316 individuals with WES data from ADSP, Knight-ADRC, and NIA-LOAD cohorts

Locus	OR	95% CI	*P*	N
TREM2	1.99	1.52, 2.62	**9.37×10^{-14}**	39
*TREM2**	1.89	1.12, 3.20	**2.59×10^{-05}**	38
*TREM2***	1.69	1.50, 2.05	**3.91×10^{-12}**	3
PLCG2	0.91	0.73, 1.12	0.064	136
*PLCG2**	1.00	0.79, 1.28	0.683	135
*PLCG2***	0.51	0.41,0.54	0.049	6
ABI3	1.44	1.07, 1.95	**6.64×10^{-4}**	36
*ABI3**	1.21	0.76, 1.90	0.127	35
*ABI3***	2.49	2.07, 3.21	**5.02×10^{-5}**	2

OR, odds ratio; CI, confidence interval; *p*, *p*-value; N, number of variants included.

* gene-based analyses using the same variants but excluding variants of interest.

** gene-based analyses using only the common variants across all cohorts Significant *p*-values after Bonferroni correction in bold.

Table 4

Quantitative trait analyses for *TREM2*, *PLCG2*, and *ABI3* variants of interest using CSF levels of $A\beta_{42}$, tau, pTau, and amyloid imaging, using individuals with GWAs or WES data from Knight-ADRC and NIA-LOAD cohorts

Phenotype	Locus (variant)	β	95% CI	*P*	N
$A\beta_{42}$	*TREM2* (p.R62H)	−0.19	−1.32, 0.95	0.745	556
	TREM2 (p.R47H)	−0.68	−1.34, 0.03	0.041	557
	PLCG2 (p.P522R)	0.19	−0.47, 0.85	0.576	558
	ABI3 (p.S209F)	0.08	−0.49, 0.66	0.773	556
tau	*TREM2* (p.R62H)	0.23	−0.80, 1.26	0.666	557
	TREM2 (p.R47H)	0.12	−0.51, 0.75	0.710	558
	PLCG2 (p.P522R)	−0.05	−0.65, 0.55	0.860	559
	ABI3 (p.S209F)	0.01	−0.51, 0.53	0.973	557
pTau	*TREM2* (p.R62H)	0.21	−0.86, 1.29	0.695	551
	TREM2 (p.R47H)	0.23	−0.47, 0.93	0.526	552
	PLCG2 (p.P522R)	−0.02	−0.64, 0.61	0.957	553
	ABI3 (p.S209F)	0.05	−0.49, 0.60	0.854	551
Amyloid imaging	*TREM2* (p.R62H)	0.01	−0.13, 0.16	0.889	420
	TREM2 (p.R47H)	0.17	0.05, 0.30	**0.008**	420
	PLCG2 (p.P522R)	−0.01	−0.09, 0.08	0.904	420
	ABI3 (p.S209F)	0.06	−0.02, 0.15	0.143	418

β, beta; CI, confidence interval; *p*, *p*-value; N, number of samples included. Significant *p*-values after Bonferroni correction in bold.

Table 5

AD progression analysis for *TREM2, PLCG2,* and *ABI3* variants of interest, using individuals with GWAs or WES data from Knight-ADRC and NIA-LOAD cohorts

Phenotype	Locus (variant)	Beta	SE	*P*	MAF $_{cases}$	N
Progression	*TREM2* (p.R62H)	0.20	0.76	0.789	0.005	440
	TREM2 (p.R47H)	0.07	0.31	0.798	0.014	441
	PLCG2 (p.P522R)	−1.25	0.58	0.028	0.007	445
	ABI3 (p.S209F)	−0.24	0.57	0.677	0.006	438

SE, standard error; *p*, *p*-value; MAF, minor allele frequency; N, number of samples included. Significant *p*-values after Bonferroni correction in bold.