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Genetic Modification of the Relationship between Phosphorylated Tau and Neurodegeneration

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Abstract

Background—A subset of individuals present at autopsy with the pathological features of Alzheimer's disease (AD) having never manifest the clinical symptoms. We sought to identify genetic factors that modify the relationship between phosphorylated tau (PTau) and dilation of the lateral inferior ventricles.

Methods—We used data from 700 subjects enrolled in the AD Neuroimaging Initiative (ADNI). A genome-wide association study (GWAS) approach was used to identify PTau × single nucleotide polymorphism (SNP) interactions. Variance explained by these interactions was quantified using hierarchical linear regression.

Results—Five SNP×PTau interactions passed a Bonferroni correction, one of which (rs4728029, *POT1*, 2.6% of variance) was consistent across ADNI-1 and ADNI-2/GO subjects. This interaction also showed a trend level association with memory performance and levels of interleukin-6-receptor.

Conclusions—Our results suggest that rs4728029 modifies the relationship between PTau and both ventricular dilation and cognition, perhaps through an altered neuroinflammatory response.

Keywords

Alzheimer's Disease; Phosphorylated Tau; Ventricular Volume; MRI; CSF; Memory; GWAS; Genetic Interactions; Imaging Genetics; Quantitative Traits; Plasma

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1. INTRODUCTION

The pathological cascade in Alzheimer's disease (AD) has been widely debated. Some suggest that tauopathies play a causal role and initiate the disease process when they arise early in life;¹ whereas others posit that amyloid pathology plays a causal role arising through an independent disease process, perhaps after early tauopathies appear, and ultimately leading to an acceleration of the disease process.^{2,3} Emerging out of this literature is evidence that a subset of individuals present at autopsy with tauopathies and amyloid plaques having never shown clinical symptoms of AD during their lifetime.⁴⁻⁶ Some of this work has suggested that asymptomatic individuals show acute differences in brain activity,⁷ and longitudinal hypertrophy in hippocampal and cortical cell bodies that may act as a potential mechanism of resilience.⁶ Moreover, risk for AD pathology in asymptomatic individuals is much higher in those with a family history of AD than in those without a family history, suggesting that genetic factors may play an important role in the presence and response to AD pathology.⁸ Advances in biomarker detection have led to validated measures that can be used to approximate the pathological processes of AD *in vivo* including amyloid deposition imaged using positron emission tomography (PET) and levels of A β -42, of tau, and phosphorylated tau (PTau) in cerebrospinal fluid (CSF). PET and CSF biomarkers show a strong relationship to each other,⁹⁻¹¹ and both predict risk for AD.¹¹ The availability of genome wide association study (GWAS) data has led to the identification of a wide array of genetic risk factors for AD^{12,13} and associations with AD biomarkers.¹⁴⁻¹⁸ Yet, no study to date has leveraged the availability of these two rich data sources to investigate individual predictors of cognitive resilience seemingly present in asymptomatic individuals.

We sought to identify genetic variants that modify the relationship between biomarkers of tau pathology and a magnetic resonance imaging (MRI) measure of disease progression – lateral ventricle dilation. The lateral ventricles have shown a strong relationship to AD onset and progression,^{19,20} and measures of ventricular dilation have been successfully applied as quantitative endophenotypes in genetic interaction analyses previously.²¹ We approached this research by first characterizing the relationship between tau CSF measures and ventricular volume. Next we performed a tau-gene interaction analysis to test whether genetic variants modified the relationship between pathology and atrophy. Finally, in post-hoc analyses we tested whether observed tau-gene interactions were associated with cognitive performance or neuroinflammatory cytokine levels.

2. SUBJECTS AND METHODS

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a \$60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific

markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years and 200 people with early AD to be followed for 2 years. For up-to-date information, see www.adni-info.org.

2.1 Subjects

Participants were enrolled based on criteria outlined in the ADNI protocol (<http://www.adni-info.org/Scientists/AboutADNI.aspx>). Participants genotyped in both the ADNI-1 and ADNI-2/GO protocols were included. To avoid spurious genetic effects due to population stratification, only Caucasian participants were used in all analyses. Demographic data are presented in Table 1.

2.2 Genotyping

In ADNI-1, genotyping was performed using the Illumina Infinium Human-610-Quad BeadChip. In ADNI-2/GO, genotyping was performed on the Illumina OmniQuad array. After quality control (QC) procedures using PLINK,²² 256,790 SNPs remained for data analysis (Appendix A).

2.3 Quantification of Ventricular Dilation

All volumetric data from 1.5 Tesla MRI scans in ADNI were used in our analyses.^{23,24} We used the volume of the left inferior lateral ventricle as our primary outcome measurement given its previous association with neurofibrillary tangle pathology,²⁵ and included a measurement of intracranial volume (ICV) as a covariate in all volume analyses. Both were defined in Freesurfer.²⁶⁻³⁰ Slopes of change in left ventricular volume over time were calculated in SAS 9.3 (SAS Institute Inc., Cary, NC) using mixed model regression (PROC MIXED) to leverage the longitudinal data. In the mixed model regression, time was modeled based on days from baseline for each subject. This was then rescaled so that slopes would represent annual change (days from baseline/365.25). Details on the longitudinal data are presented in Table 1. For additional technical details see Appendix A.

2.4 Statistical Analyses: Relationship between Tau and Brain Volume

Biomarker quantification in ADNI was performed previously.^{11,31} To test the relationship between PTau and ventricular dilation we performed a univariate general linear model regression analysis using IBM SPSS v.20 (<http://www-01.ibm.com/software/analytics/spss/>) with slope of change in left ventricular volume set as the quantitative outcome measure. Predictors in the model included baseline age, baseline intracranial volume (ICV), gender, years of education, diagnosis at baseline, and PTau. In order to maximize our power to

identify genetic effects we chose to exclude *APOE* genotype as a covariate. However, we also wanted to ensure that observed effects were not simply carrying signal related to *APOE* genotype, so we implemented an approach taken in previous research,³² in which we performed posthoc hierarchical linear regression including *APOE* genotype to calculate variance explained by observed genetic interactions above and beyond variance explained by *APOE*. This approach aimed to balance statistical power with interpretability.

2.5 Statistical Analyses: Gene – PTau Interaction Analysis

Genetic interaction analyses were performed in PLINK using the `--linear` command with slopes of ventricular volume set as the outcome variable. An additive model was used for all genotypes (0, 1, 2) with the same covariates entered above and the addition of the SNP predictor ($Y = \beta_0 + \beta_1 \text{Baseline Age} + \beta_2 \text{BaselineICV} + \beta_3 \text{Gender} + \beta_4 \text{Education} + \beta_5 \text{Dx} + \beta_6 \text{PTau} + \beta_7 \text{SNP} + \beta_8 \text{SNPxPTau}$). Our term of interest was a PTau $\times$ SNP interaction term which we used to identify SNPs that modified the relationship between ventricular volume and CSF PTau. A correction for multiple comparisons using the Bonferroni procedure (256,790 total tests) was applied to the PTau $\times$ SNP interaction term. Finally, all significant interactions were stratified across the ADNI-1 and ADNI-2/GO data sources to verify the consistency of the observed effect.

2.6 Hierarchical Linear Regression to Identify Variance Explained

In order to place our observed SNP interaction effects in the context of known predictors of brain volume including *APOE*, we used hierarchical linear regression to calculate the change in R^2 when adding in the SNP main effect, and after adding in the SNP $\times$ PTau interaction term. We report the variance explained by the final block in this model, which is the variance explained by the SNP $\times$ PTau interaction term. Population covariates were calculated using Structure.³³ For additional details see Appendix A.

2.7 Post-hoc Statistical Analyses

Posthoc analyses were performed using cognitive^{34,35} and plasma based proteomic markers from ADNI (see Appendix A for technical details). Predictors included baseline age, gender, years of education, diagnostic group, *APOE*, PTau, SNP, and the SNP $\times$ PTau interaction term.

3. Results

3.1 Relationship between PTau and Brain Volume

As expected, we observed a relationship between levels of PTau in CSF and ventricular volume when controlling for known predictors ($t = 3.08$, $p = 0.002$). All predictors except gender and education were associated with ventricular volume. We chose to leave all of the covariates in our subsequent statistical models. The overall regression model explained 34% of variance in ventricular dilation.

3.2 PTau – Gene Interaction

Results are presented in Table 2. Five SNP-PTau interactions remained significant after correcting for multiple comparisons. SNPs were annotated using the Illumina annotation file. Two SNPs (rs10973683, rs7859035) were annotated to the *SHB* gene, one SNP (rs10514128) was annotated to the *TBCA* gene, one SNP (rs2273704) was annotated to the *EML1* gene, and one SNP (rs4728029) was annotated to the *POT1* gene. When stratifying across the datasets, only the rs4728029-PTau interaction result remained significant in both ADNI-1 and ADNI-2/GO. Therefore we focused all post-hoc analyses on this SNP-PTau interaction. For the rs4728029 SNP, approximately 20% of participants were homozygous carriers of the A allele (N = 141) and 30% were homozygous carriers of the G allele (N = 217). This distribution was reflected in both the ADNI-1 and ADNI-2/GO cohorts. PTau was related to ventricular dilation, and this effect was strongly amplified in homozygous carriers of the rs4728029 A allele (Figure 1).

3.3 Hierarchical Linear Regression Result

Results are presented in Table 3. Our statistical model without *APOE*, rs4728029, or the rs4728029 $\times$ PTau interaction term explained 34% of variance in ventricular dilation. *APOE* genotype explained an additional 0.9% of variance. A weak, though significant, relationship between *APOE* and longitudinal change in volume is consistent with previous findings in which *APOE* was not related to volume loss in normal controls or MCI and only showed a moderate effect in AD.³⁶ The main effect of rs4728029 explained an additional 0.1% of variance. Finally the rs4728029 $\times$ PTau interaction term explained an additional 2.5% of variance.

3.4 Post-hoc Statistical Analyses

3.4.1 Cognitive Performance—In the executive function analysis, the rs4728029 $\times$ PTau interaction term did not reach statistical significance. However, in the memory analysis the rs4728029 $\times$ PTau interaction term was statistically significant ($t = -2.22$, $p = 0.026$). This effect did not survive correction for multiple comparisons. PTau was related to poor memory performance, and the effect was amplified in homozygous carriers of the rs4728029 A allele (Figure 2).

3.4.2 Neuroinflammatory Cytokine Analysis—Proteomic data were only available from ADNI-1. There was no association between the rs4728029 $\times$ PTau interaction term and any of the neuroinflammatory cytokines. We also ran the model with rs4728029 recessively coded as A/A = 1 and all other genotypes = 0, and in this model, the interaction term approached nominal statistical significance ($t = 1.950$, $p = 0.052$). This effect did not survive a correction for multiple comparisons. PTau was related to lower levels of IL-6R, but only in A/A homozygotes (Figure 3).

4. DISCUSSION

The present project has replicated the relationship previously observed between CSF levels of phosphorylated tau and neurodegeneration.^{37,38} We have also identified a novel genetic-PTau interaction by which variation in a SNP annotated to *POT1* modified the observed

relationship between PTau and ventricular dilation. This interaction remained significant after correcting for multiple comparisons. Finally, we demonstrated that this SNP $\times$ PTau interaction was nominally associated with memory performance.

4.1 Genetic Modification of PTau-related Neurodegeneration

Across diagnostic categories, increased levels of PTau were related to increased ventricular dilation, although this effect was most pronounced in the MCI diagnostic group (the diagnosis by PTau interaction was not statistically significant, $p = 0.16$). The genetic modification of this effect highlighted a sub-group of individuals who showed the strongest relationship between PTau and neurodegeneration. This effect was not driven by a low frequency of homozygous minor allele carriers, as A/A allele carriers of rs4728029 made up 20% of the total sample (146 subjects) and the interaction effect remained present when stratifying across the two independent data sources. Importantly, although the rs4728029 SNP did not show a strong main effect on ventricular dilation, the PTau interaction explained 2.4% of variance above and beyond known predictors of neurodegeneration including the *APOE-ε4* genotype. This highlights how the inclusion of genetic interaction effects, particularly when focusing on common variants, can improve prediction models and provide some suggestions as to the mechanisms of risk and resilience as outlined below.

The correlative nature of the current analyses precludes our ability to make definitive statements about the mechanisms of the observed interaction; however, some possible hints can be garnered from the rich data sources now available on SNP function. Rs4728029 is an intergenic SNP upstream from Protection of Telomeres 1 (*POT1*) and downstream from the metabotropic glutamate receptor 8 (*GRM8*). This SNP modifies three regulatory motifs and has the strongest effect on the CEBPB_known2 motif (Transfac M00201).³⁹ In the case of this motif, the A allele of rs4728029 is associated with a strong decrease in regulatory action, suggesting that the A allele in the present analyses may be associated with lower levels of *POT1*. Previous research has demonstrated that *POT1* expression is related to telomere length, with lower levels of *POT1* expression showing a relationship to shorter telomeres,⁴⁰ and the gene product of *POT1* has demonstrated a similar relationship to telomere length.⁴¹ Interestingly, length of telomeres has also been associated with Alzheimer's disease status in previous research.⁴² Alzheimer's patients had shorter telomeres in T-cells than controls, and T-cell telomere length was inversely correlated with a serum marker of neuroinflammation. In posthoc analyses, we further investigated these possibilities by looking for similar *POT1* modification of the relationship between Ptau and both cognitive performance and levels of neuroinflammatory cytokines in serum.

4.2 Posthoc Analyses: Cognitive Performance and Neuroinflammation

Beyond the increased variance explained in ventricular dilation in our primary analyses, our posthoc analyses suggested the *POT1* $\times$ PTau interaction nominally predicts cognitive performance. The rs4728029 SNP modified the relationship between PTau and memory performance, though it did not have an effect on executive function performance. If such an effect is indeed driven by the ventricular dilation observed in our primary analysis, then it is not surprising that the effect is present in relation to memory rather than executive function, particularly given the crucial memory structures that surround the lateral inferior ventricles

including the hippocampus. Moreover, the observed interaction was consistent with our primary finding as A/A allele carriers showed both more dilation in the inferior lateral ventricle in relation to P τ load and poorer memory performance at baseline in relation to P τ . These two factors are, of course, highly correlated (Pearson's $r = -0.55$), and for that reason should not be interpreted as independent signals as both are likely related to a single underlying factor. In both cases, P τ was related to dilation and memory performance in all genotype subgroups, but showed the strongest relationship in the A/A group. Given the relationship between *POT1* and neuroinflammatory cytokines previously mentioned⁴² and the relationship between neuroinflammation and brain volume⁴³ we chose to investigate whether our observed effect may be driven by such a neuroinflammatory response.

Previous research has demonstrated a relationship between telomere length and tumor necrosis factor alpha (TNF- α), Interleukin-6 and Interleukin-1.^{42,44} For that reason we chose to focus on the TNF- α and Interleukin cytokines available in our dataset. We were underpowered to look for such gene-biomarker interactions in relation to cytokines (only available in a sub-sample of ADNI-1 participants), so our results should be interpreted with caution. We were able to observe a trend gene $\times$ P τ interaction in line with our cognitive and brain volume findings, as A/A allele carriers of rs4728029 showed lower levels of the Interleukin-6 receptor (IL-6R) in relation to increased levels of P τ . IL-6R has previously been suggested to be an anti-inflammatory marker,⁴⁵ and indeed, the association observed in this post-hoc analysis suggests in persons with lower levels of this anti-inflammatory marker (and possibly higher neuroinflammation), P τ leads to even higher levels of neurodegeneration. Soluble IL-6R appears to play a role in a large number of both pro- and anti-inflammatory activities mediated by IL-6,⁴⁶ and these posthoc results must be interpreted within the many actions of the IL-6/IL-6R complex. The present results suggest that future work clarifying the relationship between P τ load, neuroinflammation, and neurodegeneration is warranted.

4.3 Previous GWAS of CSF markers and Resilience in ADNI

Previous GWAS analyses have looked for single marker predictors of Tau and P τ levels in CSF using data from ADNI. The earliest studies highlighted strong single marker effects in and around the *APOE* gene.¹⁸ Although we did not use P τ as our outcome, we did verify that *APOE* was strongly associated with P τ ($p = 1.9 \times 10^{-11}$). As highlighted in our hierarchical model, the effect of *APOE* on LILV slopes was more modest, particularly when including P τ in the prediction model ($p = .013$). This is consistent with the modest predictive power of *APOE* in relation to longitudinal change in bilateral hippocampal volume and ventricular volume reported previously.^{36,47} An additional GWAS reported a strong association between a SNP within *APOE* and a variety of baseline volume endophenotypes including the right hippocampus.⁴⁸ This suggests future interaction analyses using a variety of smaller regions of interest may be warranted.

The most comprehensive GWAS of CSF biomarkers to date included data from ADNI and four other datasets,¹⁵ and reported a strong relationship between signals on chromosome 19 and biomarker levels. Interestingly, they also highlighted particular signals within the *APOE* region that are related to Tau pathology independent of A β 42 pathology or clinical

status. In the present manuscript, the strong relationship between PTau and *APOE*, along with the moderate association between *APOE* and ventricular volume might explain why the strongest gene interaction effects identified do not cluster around the *APOE* signal. Moreover, this key difference highlights the strength of gene-biomarker interactions in identifying novel candidates for future analyses.

In addition, previous work by Cruchaga et al. demonstrated that genetic variation associated with CSF biomarker levels can meaningfully predict disease progression.¹⁴ Taken together, the results of the current manuscript and both Cruchaga et al. manuscripts suggest that genetic risk factors must be considered at all stages of the Alzheimer's disease cascade in order to fully elucidate the genetic etiology of LOAD. Genetic variants may increase susceptibility to the primary pathologies of LOAD, to neurodegeneration in the presence of a given pathology as presented in this manuscript, or even to cognitive decline in the presence of neurodegeneration (as reported most recently by Mukherejee et al.).⁴⁹

4.4 Strengths and Weaknesses

The present manuscript presents a novel genetic interaction approach making use of MRI, CSF, and genotyping data. Combining the two independent data sources allowed us to maximize our power to detect genetic effects, and the stratified post-hoc analysis provides support for the consistency of the observed effect. The lack of consistency for some of the SNP interactions may be due to differences in the ADNI-1 and ADNI-2/GO protocols. For example, the longitudinal follow-up scans were slightly more frequent in ADNI-2/GO (approximately every 3 months v. approximately every 6 months in ADNI-1) but covered a shorter period of time. Certainly this longer follow-up interval in ADNI-1 could explain the cohort differences we observe, but other differences, such as the distribution of diagnostic categories in the two cohorts, cannot be ruled out.

In addition, it is possible that differences in CSF batches between ADNI-1 and ADNI-2/GO could be driving the differences in effect size between the two groups. In the current analysis, it is difficult to distinguish batch effects from group differences because we used the first CSF observation for each subject (thus the batches align roughly with ADNI-1 subjects v. ADNI-2/GO subjects). However, it should be noted that previous work has demonstrated the test-retest reliability of the biomarker measures from CSF in the ADNI dataset.³¹ An additional independent sample with a comparable longitudinal follow-up interval to that of ADNI-1 could help clarify whether the cohort differences observed are simply due to longitudinal power, or differences due to batch effects, MRI follow-up interval, or sample characteristics.

Our posthoc analyses highlight a possible mechanism of the observed genetic interaction based on alterations in the neuroinflammatory response. It should be noted that these posthoc effects did not survive correction for multiple comparisons. The current project was not powered to look for all possible SNP effects across the entire proteomic panel, and the trend seen with IL-6R should be interpreted conservatively as a hint at a potential mechanism. Future functional work confirming the mechanism of the observed SNP interaction will be necessary to the underlying mechanism.

Future work replicating our findings in an independent sample with MRI, CSF, and genotype data is needed to confirm the observed SNP effect. To avoid possible confounding factors related to population substructure we chose to restrict all analyses to Caucasian individuals, and thus our results may not generalize to other ancestral populations. The statistical approach taken in the present manuscript provides a blue-print for future exploratory analyses aimed at identifying genetic modifiers of disease risk using other AD biomarkers such as amyloid load measured using PET and provides some possible targets for future functional analyses.

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Appendix A

Genotyping

Quality control (QC) and statistical analyses were performed using PLINK software (version 1.07).²² During QC, we excluded single nucleotide polymorphisms (SNPs) with a genotyping efficiency < 90%, a minor allele frequency < 10%, or deviation from Hardy-Weinberg Equilibrium ($p < 1e^{-6}$). This left 515,383 SNPs in ADNI-1 and 605,317 SNPs in ADNI-2/GO. Finally, we merged the two genotyping files for our analyses and again applied filters for genotyping efficiency < 90% and minor allele frequency < 10%. Subjects were excluded if they had a call rate < 98%, if there was a reported versus genetic sex inconsistency, or if relatedness to another sample was established ($PI_HAT > 0.5$).

Quantification of Ventricular Dilation

The MRI processing pipeline has been described elsewhere.²³ Cortical reconstruction and volumetric segmentation were performed with the Freesurfer image analysis suite version 4.3 (<http://surfer.nmr.mgh.harvard.edu/>).²⁶⁻³⁰ Freesurfer processing in ADNI has been described in detail elsewhere.²⁴ An early version of the longitudinal image processing framework was used to process the sequential scans.³⁰ We excluded volumetric data from

subjects who did not pass ventricle QC as outlined in the ADNI Freesurfer processing pipeline from the ADNI MRI core (http://adni.loni.ucla.edu/wp-content/uploads/2010/12/ADNI_UCSF_Freesurfer-Overview-and-QC.pdf). Past work has demonstrated that, when measured longitudinally, the left inferior lateral ventricle shows greater dilation than the right, both in AD patients and in controls.¹⁹ For that reason we chose to focus on the left ventricle to reduce the total number of comparisons, while allowing for maximal power in detecting genetic effects related to progression. Slopes of change in left ventricular volume over time were calculated in SAS 9.3 (SAS Institute Inc., Cary, NC) using mixed model regression (PROC MIXED) to leverage the longitudinal data available. In the mixed model regression, time was modeled based on days from baseline for each subject. This was then rescaled so that slopes would represent annual change (days from baseline/365.25). On average we had 4 MRI scans for each subject (3.66 for subjects in ADNI1 and 4.62 for subjects in ADNI2/ADNIGO).

CSF P_{Tau} Preprocessing

We compiled a dataset across the UPENN1 – UPENN5 data sources available for download on the ADNI site. For the present analyses we used the first measure of P_{Tau} for each subject, given its previous association with neurofibrillary tangle pathology.²⁵ Two subjects had P_{Tau} values greater than three standard deviations beyond the mean levels in both the AD and MCI groups and were excluded from our analyses.

Hierarchical Linear Regression to Identify Variance Explained

In order to place our observed SNP interaction effects in the context of known predictors of brain volume, we used hierarchical linear regression to calculate the change in R^2 when adding in the SNP main effect, and after adding in the SNP $\times$ P_{Tau} interaction term. For this model we also chose to include the *APOE* genotype as a predictor to determine whether our observed SNP effects explained variance beyond this known major genetic predictor. Although we restricted analyses to Caucasian individuals, we also included population structure covariates in this model (calculated using Structure: <http://pritch.bsd.uchicago.edu/structure.html>)³³ to ensure there was no effect of ancestry contributing to the observed interaction.

Slopes of ventricular volume were again set as the quantitative outcome. Our first block included baseline age, gender, years of education, baseline ICV, baseline diagnostic category, and P_{Tau}. Our second block added in the *APOE* genotype (number of E4 alleles). Our third block added in the population structure eigenvectors. Our fourth block added in the SNP main effect term, and our final block added in the SNP $\times$ P_{Tau} interaction term. We report the variance explained by the final block in this model, which is the variance explained by the SNP $\times$ P_{Tau} interaction term.

Posthoc Cognitive Performance Analysis

Composite measures of memory and executive function were calculated previously in ADNI and are described elsewhere.^{34,35} We performed one cognitive analysis using memory as the

outcome (ADNI-MEM) and one analysis using executive function as the outcome (ADNI-EF) to test whether the observed interaction effects were associated with cognitive performance. The outcome measure was either baseline ADNI-EF or baseline ADNI-MEM, and predictors included baseline age, gender, years of education, diagnostic group, *APOE*, PTau, SNP, and the SNP $\times$ PTau interaction term.

Posthoc Neuroinflammatory Cytokine Analysis

Plasma based proteomic markers have been previously calculated in ADNI and are described elsewhere (http://adni.loni.ucla.edu/wp-content/uploads/2010/11/BC_Plasma_Proteomics_Data_Primer.pdf). For posthoc analyses we chose to focus on all interleukin (IL) and tumor necrosis factor alpha (TNF- α) markers that passed QC leaving 7 markers for analysis: TNF- α , IL-3, IL-8, IL-13, IL-17, IL-18, and IL-6 receptor (IL-6R). Each of these was a quantitative outcome measure in regression models using the same predictors as the cognitive post-hoc analyses. We again corrected for multiple comparisons using the Bonferroni procedure.

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Research in Context

Systematic Review

The authors performed a comprehensive review of existing work categorizing the relationship between phosphorylated tau (PTau) and brain volume, as well as genetic modifiers of this relationship. Previous research had been somewhat mixed with some studies finding only trend level associations between CSF tau/PTau and brain volume. No work to date has investigated genetic modifiers of this relationship beyond the *APOE* genotype.

Interpretation

Our results further clarify the relationship between CSF PTau and brain volume, identify a novel genetic interaction that explains substantial variance in brain atrophy, and identify a possible mechanism of the observed interaction related to a neuroinflammatory response.

Future Directions

Although subdividing our dataset into ADNI-1 and ADNI-2/GO suggested the interaction was consistent, a replication of this gene-biomarker interaction is warranted. Future functional analyses investigating the relationship between *POT1* and the neuroinflammatory response is also necessary to confirm our suggested mechanism of action.

Figure 1. POT1 (rs4728029) modifies the relationship between PTau and Lateral Inferior Ventricle Dilation

The Y axis represents annual change in ventricular volume in mm³. The x axis represents CSF phosphorylated tau in pg/mL. Points and lines are color coded by rs4728029 genotype. The R² linear for A/A carriers is 0.17, for A/G or G/A carriers it is 0.06 and for G/G carrier it is 0.02.

Figure 2. POT1 (rs4728029) modifies relationship between PTau and Baseline Memory Performance

The Y axis represents baseline memory performance. The X axis represents CSF phosphorylated tau in pg/mL. Points and lines are color coded by the rs4728029 genotype. The R^2 linear for A/A carriers is 0.15, for A/G or G/A it is 0.141, and for G/G it is 0.167.

Figure 3. POT1 (rs4728029) modifies relationship between PTau and Interleukin 6 Receptor
 The Y axis represents baseline plasma interleukin 6 receptor levels in ng/mL. The X axis represents CSF phosphorylated tau levels in pg/mL. Points and lines are color coded by the rs4728029 genotype. The R^2 linear for A/A carriers is 0.062, for G/A or A/G carriers it is 0.0004, and for G/G carriers it is 0.0005.

Table 1

Demographic Information

		Baseline Clinical Diagnosis ^a		
		Normal Control	Mild Cognitive Impairment	Alzheimer's Disease
ADNI-1 Dataset	Number of Patients	102	180	92
	Number of APOE- ε4 Carriers	24	99	64
	Number of Females	48	60	38
	Mean Baseline Age (<i>SD</i>)	75.75 (4.99)	74.58 (7.61)	75.01 (7.97)
	Mean Years of Education (<i>SD</i>)	15.92 (2.71)	15.76 (3.01)	15.25 (3.22)
	Mean Number of Visits	5.09 (1.42)	5.17 (1.74)	3.34 (0.89)
	Mean Interval in Days	1320 (549)	1032 (542)	585 (252)
	Phosphorylated Tau pg/mL (<i>SD</i>)	25.77 (15.23)	35.11 (17.09)	41.47 (18.48)
ADNI-2/GO Dataset	Number of Patients	95	208	23
	Number of APOE- ε4 Carriers	22	90	15
	Number of Females	46	92	9
	Mean Baseline Age (<i>SD</i>)	74.66 (5.63)	71.39 (7.32)	74.96 (10.67)
	Mean Years of Education (<i>SD</i>)	16.47 (2.56)	15.96 (2.69)	15.48 (2.76)
	Mean Number of Visits	3.55 (1.03)	3.94 (0.89)	3.35 (0.93)
	Mean Interval in Days	366 (150)	470 (202)	313 (143)
	Phosphorylated Tau pg/mL (<i>SD</i>)	21.42 (7.94)	24.76 (12.42)	35.46 (12.86)
Combined Dataset	Number of Patients	197	388	115
	Number of APOE- ε4 Carriers	45	189	79
	Number of Females	94	152	47
	Mean Baseline Age (<i>SD</i>)	75.22 (5.32)	72.86 (7.62)	75.00 (8.53)
	Mean Years of Education (<i>SD</i>)	16.19 (2.65)	15.87 (2.84)	15.30 (3.13)
	Mean Number of Visits	4.35 (1.46)	4.51 (1.48)	3.34 (0.90)
	Mean Interval in Days	860 (628.06)	731 (486)	530 (258)
	Phosphorylated Tau pg/mL (<i>SD</i>)	23.68 (12.43)	29.56 (15.63)	40.26 (17.61)

Mild Cognitive Impairment subjects had a MMSE score between 24 and 30, objective memory impairment, subjective memory impairment, and a CDR score of 0.5.

Alzheimer's Disease subjects met clinical criteria for dementia, had an MMSE of between 20 and 26, and had CDR score of .5 or 1.

^bSUVr - Standardized uptake value ratio for amyloid tracer

^aNormal Control subjects had a Mini-Mental Status Examination (MMSE) score between 24 and 30, a Clinical Dementia Rating (CDR) score of 0, and were not depressed (Geriatric Depression Scale score < 6).

Table 2

PTau-Gene Interaction Analysis

SNP × SNP Interactions							
Genes	ADNI-1 Dataset		ADNI2/GO Dataset		Combined Datasets		
	t	p-value	t	p-value	t	p-value	FWE*
<i>POT1</i> (rs4728029) × PTau	4.26	0.00003	2.33	0.020	5.36	1.11×10^{-7}	.045
<i>SHB</i> (rs1097368) × PTau	4.10	0.00005	1.40	0.164	5.44	7.26×10^{-8}	.019
<i>SHB</i> (rs7859035) × PTau	4.41	0.00001	1.33	0.184	5.44	7.60×10^{-8}	.019
<i>TBCA</i> (rs10514128) × PTau	4.25	0.00003	1.52	0.130	5.39	9.52×10^{-8}	.024
<i>EML1</i> (rs2273704) × PTau	4.39	0.00001	1.23	0.220	2.28	1.74×10^{-7}	.029

* FWE – p value when correcting for total number of comparisons using the bonferroni procedure.

Table 3

Post-hoc Hierarchical Linear Regression Result

Change Statistics								
Model	R	R²	Adj. R²	R² Change	F Change	df1	df2	Sig. F Change (P value)
1 ^a	0.589	0.347	0.341	0.347	31.35	6	693	4.33 × 10 ⁻⁶
2 ^b	0.594	0.353	0.346	0.006	6.27	1	692	0.013
3 ^c	0.597	0.356	0.347	0.004	1.28	3	690	0.282
4 ^d	0.598	0.357	0.347	0.001	1.10	1	689	0.296
5 ^e	0.618	0.382	0.371	0.025	27.67	1	688	1.92 × 10⁻⁷

^a Predictors: Constant, Intracranial Volume, Phosphorylated Tau, Age, Education, Diagnosis, Gender

^b Predictors: Constant, Intracranial Volume, Phosphorylated Tau, Age, Education, Diagnosis, Gender, *APOE*

^c Predictors: Constant, Intracranial Volume, Phosphorylated Tau, Age, Education, Diagnosis, Gender, *APOE*, **eigenvector1**, **eigenvector2**, **eigenvector3**

^d Predictors: Constant, Intracranial Volume, Phosphorylated Tau, Age, Education, Diagnosis, Gender, *APOE*, eigenvector1, eigenvector2, eigenvector3, **rs4728029**

^e Predictors: Constant, Intracranial Volume, Phosphorylated Tau, Age, Education, Diagnosis, Gender, *APOE*, eigenvector1, eigenvector2, eigenvector3, rs4728029, **rs4728029 × PTau**