

ORIGINAL ARTICLE

# Susceptibility to neurofibrillary tangles: role of the *PTPRD* locus and limited pleiotropy with other neuropathologies

LB Chibnik<sup>1,2,3,4</sup>, CC White<sup>1,3</sup>, S Mukherjee<sup>5</sup>, T Raj<sup>1,3</sup>, L Yu<sup>6</sup>, EB Larson<sup>7</sup>, TJ Montine<sup>8</sup>, CD Keene<sup>8</sup>, J Sonnen<sup>9</sup>, JA Schneider<sup>6</sup>, PK Crane<sup>5</sup>, JM Shulman<sup>10,11</sup>, DA Bennett<sup>6,12</sup> and PL De Jager<sup>1,2,3,12</sup>

Tauopathies, including Alzheimer's disease (AD) and other neurodegenerative conditions, are defined by a pathological hallmark: neurofibrillary tangles (NFTs). NFT accumulation is thought to be closely linked to cognitive decline in AD. Here, we perform a genome-wide association study for NFT pathologic burden and report the association of the *PTPRD* locus (rs560380,  $P=3.8 \times 10^{-8}$ ) in 909 prospective autopsies. The association is replicated in an independent data set of 369 autopsies. The association of *PTPRD* with NFT is not dependent on the accumulation of amyloid pathology. In contrast, we found that the *ZCWPW1* AD susceptibility variant influences NFT accumulation and that this effect is mediated by an accumulation of amyloid  $\beta$  plaques. We also performed complementary analyses to identify common pathways that influence multiple neuropathologies that coexist with NFT and found suggestive evidence that certain loci may influence multiple different neuropathological traits, including tau, amyloid  $\beta$  plaques, vascular injury and Lewy bodies. Overall, these analyses offer an evaluation of genetic susceptibility to NFT, a common end point for multiple different pathologic processes.

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## INTRODUCTION

Multiple neurologic diseases can lead to loss of cognitive function with older age. Several of them can be grouped by neuropathologic changes found at autopsy, such as neurofibrillary degeneration, which is best characterized by the accumulation of neurofibrillary tangles (NFTs). 'Tauopathies' share the accumulation of aggregates consisting of the microtubule-associated protein tau (Tau) but have different clinical manifestations and distinct etiologies that ultimately converge in the abnormal accumulation of Tau species. Tauopathies include neurodegenerative disorders such as Alzheimer's disease (AD), some forms of frontotemporal degeneration, progressive supranuclear palsy and corticobasal degeneration, as well as forms of neurodegeneration occurring secondary to environmental insults, such as in chronic traumatic encephalopathy.<sup>1</sup> In an older population, it is possible that multiple different disease mechanisms interact to lead to the accumulation of NFT. Identifying risk factors that contribute to the accumulation of pathologic Tau is therefore meaningful, not just for the vast majority of the population that is at risk for AD but even more broadly for informing our understanding of other tauopathies.

Here, we leverage neuropathologic data collected from two longitudinal cohorts of aging whose participants are non-demented at the time of enrollment; in both the Religious Order Study (ROS) and the Rush Memory and Aging Project (MAP), brain donation is a condition of enrollment. Importantly, these subjects represent a sample of the older population in which multiple different neuropathologic changes are present, including NFT but

also amyloid  $\beta$  plaques, vascular injury, Lewy bodies and other pathologic changes. We focused on factors that influence NFT accumulation from all central nervous system (CNS) insults commonly experienced by older subjects. Thus, in our discovery study, we performed a quantitative trait analysis to identify susceptibility loci for NFT and replicated our main result in an independent longitudinal cohort study of aging that collects neuropathologic data.

In individuals selected as AD cases or AD controls, a recent genome-wide association study (GWAS)<sup>2</sup> sought genetic factors associated with multiple individual neuropathologies, using correlations in  $\beta$ -estimates to discuss shared effects across the various pathologies. In this paper we propose a different approach. We leverage the data collected on multiple pathologies within each individual in the ROS and MAP studies to identify loci that display pleiotropic effects on multiple pathologies within the same individual and thus influence multiple insults and pathways leading to neurodegeneration. Such loci could be excellent targets of further investigation both in understanding Tau pathology and in exploring the shared pathways leading to the CNS's response to different disease processes.

## MATERIALS AND METHODS

### Participants

The subjects consisted of participants from two longitudinal cohort studies operated out of the Rush Alzheimer's Disease Center in Chicago, the ROS and the MAP. All participants are required to sign an informed consent and

<sup>1</sup>Program in Translational NeuroPsychiatric Genomics, Departments of Neurology and Psychiatry, Institute for the Neurosciences, Brigham and Women's Hospital, Boston, MA, USA; <sup>2</sup>Department of Neurology, Harvard Medical School, Boston, MA, USA; <sup>3</sup>Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA; <sup>4</sup>Department of Epidemiology, Harvard TH Chan School of Public Health, Boston, MA, USA; <sup>5</sup>Department of Medicine, University of Washington, Seattle, WA, USA; <sup>6</sup>Rush Alzheimer's Disease Center, Department of Neurology, Rush University Medical Center, Chicago, IL, USA; <sup>7</sup>Group Health Research Institute, Seattle, WA, USA; <sup>8</sup>Department of Pathology, University of Washington, Seattle, WA, USA; <sup>9</sup>Department of Pathology, University of Utah, Salt Lake City, UT, USA; <sup>10</sup>Departments of Neurology, Molecular and Human Genetics, Neuroscience and Program in Developmental Biology, Baylor College of Medicine, Houston, TX, USA and <sup>11</sup>Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, TX, USA. Correspondence: Dr PL De Jager, Program in Translational NeuroPsychiatric Genomics, Departments of Neurology and Psychiatry, Institute for the Neurosciences, Brigham and Women's Hospital, 77 Avenue Louis Pasteur, NRB 168C, Boston, MA 02115, USA.

E-mail: pdejager@rics.bwh.harvard.edu

<sup>12</sup>These authors contributed equally to this work.

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an Anatomical Gift Act at enrollment, agreeing to donate their brains (ROS) or brains, spinal cords, and select nerves and muscles (MAP) to the study upon death. Both studies were approved by the Institutional Review Board of Rush University Medical Center.

The ROS and MAP studies are maintained and the data are collected by a single group of researchers at the Rush Alzheimer's Disease Center. Data collectors in both studies are cross-trained to allow efficient merging of data and all analyses are performed on the two cohorts combined. A more comprehensive description of the ROS and MAP studies can be found in previous publications.<sup>3,4</sup>

At the time of analysis there were over 1200 deceased participants combined in the ROS and MAP cohorts. Of these, 909 were successfully genotyped, all of whom had non-missing values for all seven pleiotropic phenotypes and covariates: neuritic plaque (NP), NFTs, diffuse plaque (DP), amyloid angiopathy, micro- and macroinfarct, and Lewy bodies, age at death and sex.

The Adult Changes in Thought (ACT) cohort, used for replication in this analysis, is a community-based study of brain aging and dementia, which collects data on neuropathology on a subsample of their participants' post mortem. Participants in ACT are aged 65 years or older at enrollment, randomly sampled from a multicenter health maintenance organization from King County, Washington. More detailed information on the ACT cohort is published elsewhere<sup>5-7</sup>

### Pathological phenotypes

The neuropathological phenotypes analyzed included NP, NFTs, DP, presence of Lewy bodies, microscopic and gross (macroscopic) infarcts and cerebral amyloid angiopathy (CAA).

For ROS and MAP, brain removal was performed at either Rush University or at one of 12 designated sites across the country and sent to the Rush Alzheimer's Disease Center where all post-mortem data collection occurred. Detailed descriptions of the autopsy procedure are published elsewhere.<sup>8-11</sup> In brief, NPs, DPs and NFTs were observed with a modified Bielschowsky silver stain and counted in five regions of the brain: midfrontal, temporal, inferior parietal, entorhinal and hippocampus. For each measure in each region, counts were normalized by dividing by the standard deviation, and a z-score of the counts within each region was calculated. For the global measure of each AD pathology, we averaged these scores over the five regions, followed by a square root transformation that allowed us to treat it as a normally distributed continuous variable.<sup>12</sup> CAA was evaluated in four neocortical regions of the brain: midfrontal, midtemporal, angular and calcarine cortices. Three monoclonal anti-human  $\beta$ -amyloid antibodies with the following dilutions were used for the assessment: (1) 6F/3D (1:50; Dako North America, Carpinteria, CA, USA), (2) 10D5 (1:600; Elan Pharmaceuticals, San Francisco, CA, USA) and (3) 4G8 (1:9000; Covance Labs, Madison, WI, USA). A score for  $\beta$ -amyloid deposition ranging from 0 (no deposition) to 4 (circumferential deposition in over 75% of vessels in the total region) was determined from meningeal and parenchymal vessels in each region separately, with the final score for the region defined as the maximum score between meningeal and parenchymal CAA scores. Finally, a final CAA score was created using the average score across the four regions in each individual and classified as none, mild, moderate and severe using consistent thresholds determined by the neuropathologist.<sup>13</sup> Lewy bodies present in either the nigra or cortex was defined using consensus guidelines established by the international consortium on dementia with Lewy bodies.<sup>14</sup> Details of microscopic and macroscopic infarct measurements are detailed in the previous literature.<sup>15</sup> Briefly, all suspected or visualized gross infarcts were further dissected for histological confirmation. Microscopic infarct measures were taken from examination of a minimum of nine regions in one hemisphere. Both macroscopic and microscopic infarcts were analyzed as binary, presence in at least one region or absence in all regions. Braak staging was performed according to consensus criteria for ROS, MAP and ACT cohorts,<sup>16,17</sup> with detailed methods reported in previously published literature.<sup>9,18</sup>

**Genotyping.** DNA used for genotyping ROS and MAP participants was collected from post-mortem brain tissue, whole blood or lymphocytes. The final QCed data set that consisted of 1709 participants were genotyped on the Affymetrix GeneChip 6.0 Platform (Santa Clara, CA, USA) at the Broad Institute's Center for Genotyping or the Translational Genomics Research Institute and 384 were genotyped on the Illumina OmniQuad Express platform at Children's Hospital of Philadelphia, of these 799 and 110, respectively, had pathology information and were included in these

analyses. The EIGENSTRAT software (<https://www.hsph.harvard.edu/alkes-price/software/>) was used to calculate principal components used to control for population substructure and analysis was limited to only those of European descent.<sup>19</sup> All DNA samples go through the same rigorous quality control process before and after genotype generation, and we see no difference in data quality based on source of DNA. Further information regarding genotyping and imputation can be found in previous publications.<sup>20,21</sup>

RNA-Seq expression data were generated from frozen dorsolateral prefrontal cortex tissues following the construction of complementary DNA libraries. The paired-end reads were mapped using the Ensemble human genome transcriptomic database (<http://www.ensembl.org>). RNA expression of the associated AD genes was queried and examined for an association with AD pathologies. Details on the RNA-Seq expression profiling are provided in the Supplementary Methods.

### Statistical analysis

We used *t*-tests and  $\chi^2$  tests to compare ROS and MAP participants on demographic characteristics and pathological traits

For each of the continuous pathological phenotypes (NP, DP and NFT), we performed univariate GWAS using linear regression in PLINK. We analyzed dichotomous outcomes (Lewy body dementia and micro- and macroinfarctions) in PLINK with logistic regression. We used R to analyze an ordinal CAA outcome (<http://www.r-project.org>) using ordered logistic regression with the function *polr* from the package *MASS* (<https://cran.r-project.org/web/packages/MASS/MASS.pdf>).<sup>22</sup> To minimize artifacts of imputation, we analyzed samples from the Affymetrix and Illumina platforms independently and then meta-analyzed the findings using a fixed-effects method weighting by the inverse of the standard error. We used PLINK to meta-analyze the continuous and dichotomous phenotypes, and METAL to meta-analyze CAA.<sup>23</sup> For the *cis*-expression quantitative trait analysis, we used linear regression to assess the association between significant loci from the NFT analyses and mRNA expression within 1 Mb of the analyzed single-nucleotide polymorphism (SNP).

We used the *mPhen* function in the R package *MultiPhen* 2.0 to perform the pleiotropic GWAS.<sup>24</sup> *MultiPhen* performs an inverted ordered logistic regression with SNP as the outcome and the phenotypes and covariates as the independent variables. The value for SNP used in *mPhen* is the dosage rounded to its nearest integer 0, 1 or 2. A  $\chi^2$  test statistic is calculated from the comparison of two models with a likelihood ratio test: one model containing all pleiotropic phenotypes plus covariates against a second model containing only covariates. The *P*-value is based on the resulting  $\chi^2$  statistic, where the number of pleiotropic phenotypes equals the number of degrees of freedom.

In the pleiotropy GWAS, we pooled the data and adjusted for platform within the regression. As the pathological phenotypes are associated with age at death and sex, all eight GWASes (seven univariate and one pleiotropic), were adjusted for age at death, sex, cohort (ROS or MAP) and the first three principal components to account for population substructure.<sup>25</sup>

## RESULTS

### Discovery study for susceptibility to NFT accumulation

Characteristics of the participants ( $n = 909$  with full data), including means (s.d.) and frequency (%) of the pathology measures are reported in Table 1. The results of the primary analysis, a GWAS for NFT burden, are presented in Table 2. As expected, we found that the *APOE* locus on chromosome 19 is the top result, consistent with prior studies in this cohort<sup>8</sup> and studies in other populations.<sup>2,26</sup> The Q-Q and Manhattan plots for the GWAS of NFT burden are presented in Supplementary Figures S1 and S2.

Outside *APOE*, we found a SNP, rs560380, that exceeds a threshold of genome-wide significance in an intron of *PTPRD* ( $P = 3.1 \times 10^{-8}$ ) (Figure 1). The variance explained by the *PTPRD* locus is 3%, which is second only to the *APOE* locus (rs429358) at 9% in terms of contribution to overall variation in NFT burden. We were able to replicate this result (with the same direction of effect) in an independent data set generated from participants in the ACT study, a longitudinal study of older participants who are non-demented at enrollment. ACT participants do not have the same quantitative NFT measure, but we found  $P = 0.02$  for association with the  $n = 380$  participants (46% male and average age at death

of 87.2 years (s.d.=6.4)) with a complementary phenotype of the same pathology: Braak stage, a semiquantitative rating scale based on the topographic progression of NFT. For completeness, we also evaluated the Braak stage in ROS and MAP participants in a secondary analysis, and we found that rs560380 is also associated with the Braak phenotype. Using an ordinal logistic regression analysis, we see consistency across the three studies (ROS:  $\beta=0.31$ ,  $P=0.0099$ ; MAP:  $\beta=0.41$ ,  $P=0.0002$ ; ACT:  $\beta=0.30$ ,  $P=0.021$ ,  $\beta$  for each additional 'A' (major) allele): together, the three sets of subjects have a meta-analyzed  $P$ -value of  $5.3 \times 10^{-7}$ . As expected, the quantitative NFT burden and Braak stage are strongly correlated (Spearman's  $r=0.867$ ) in the ROS and MAP cohorts. Having replicated evidence of association between a variant and NFT, we initiated additional analyses to investigate possible mechanisms of the association.

Besides NFT accumulation, many other neuropathologic traits are assessed in ROS and MAP, which we used to further

characterize the impact of the *PTPRD* locus. rs560380 had a nominal association with NP ( $\beta=0.07$ ,  $P=0.005$ ). NP are the form of amyloid  $\beta$  plaques most closely associated with AD dementia, and the co-occurrence of NP and NFT neuropathologically characterizes AD. We found that rs560380 was nominally associated with a pathologic diagnosis of AD (OR=1.27, 95% confidence interval (CI) (1.04, 1.55),  $P=0.018$ ). As amyloid  $\beta$  accumulation is thought to precede the accumulation of NFT in the context of AD,<sup>27</sup> we evaluated whether the *PTPRD* association with NFT was driven by this specific pathophysiologic process. We first performed a mediation analysis that compares a model without NP to one with NP included as a covariate. In the basic model, rs560380 has a  $\beta$  of 0.10 ( $P=3.8 \times 10^{-8}$ ) for the association with NFT, and, in the model that also includes NP, we see a  $\beta$  of 0.07 ( $P=1 \times 10^{-6}$ ), a reduction of 33% of the effect size of the SNP. This indicates partial mediation of the SNP's effect by NP accumulation; however, the majority of the SNP's effect is not mediated through NP. Results are similar when using measures of amyloid  $\beta$  and Tau pathology derived from immunohistochemistry as previously reported for *APOE*<sup>28</sup> (data not shown). Consistent with the effect of the *PTPRD* locus being due to more than one pathophysiologic process, we found that rs560380 remains associated with NFT burden in the absence of NP: in the subset of 132 participants with no NP on neuropathologic examination, we found a  $\beta$  of 0.06 ( $P=0.02$ ), which is similar to the SNP's effect size ( $\beta=0.07$ ) in the analysis adjusting for NP (Figure 2).

We also found modest evidence that rs560380 is associated with other neuropathologic traits: rs560380 is weakly associated with CAA ( $P=0.002$ ), a form of amyloid  $\beta$  vasculopathy. We repeated these analyses adjusting for *APOE*  $\epsilon 4$  burden and with the exception of the *APOE* haplotype, we found no meaningful change in effect sizes or  $P$ -values. (Supplementary Table 1)

We assessed whether the rs560380 variant influences mRNA expression in RNA sequence data available from the dorsolateral prefrontal cortex of 494 ROS and MAP participants, a subset of our data set. We did not find evidence that rs560380 or the single SNP in LD (rs324543,  $R^2 > 0.5$ ) had an effect on either the level of *PTPRD* expression or the abundance of its different isoforms in our sample (data not shown). As these data are generated from cerebral cortical samples, they do not exclude the possibility that the SNP has effects elsewhere in the CNS: as with many susceptibility variants,<sup>29</sup> its effect may be cell- or context-specific

**Table 1.** Characteristics of the ROS and MAP participants included in analyses

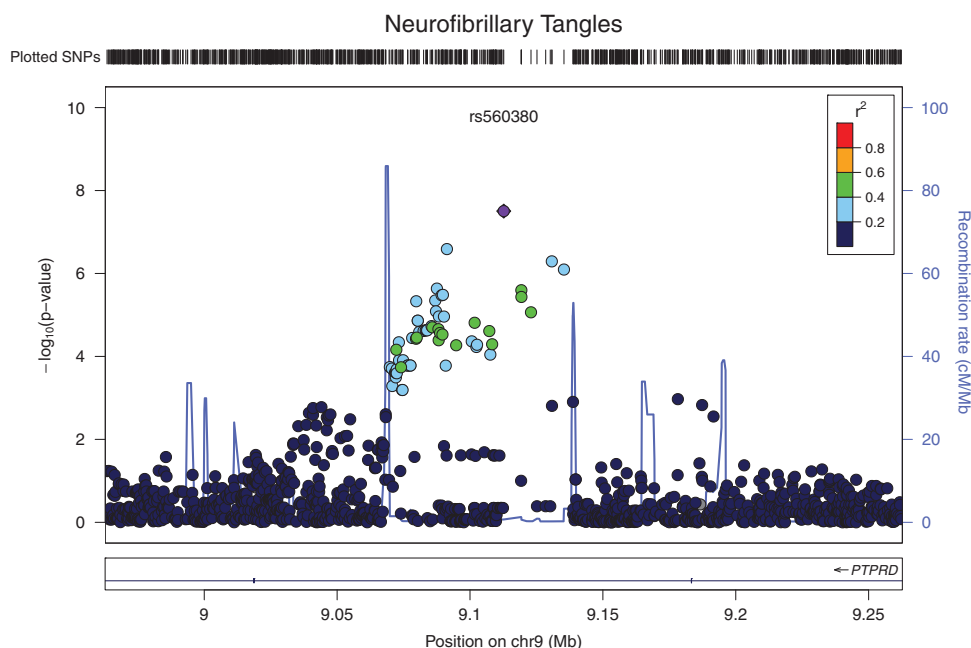
|                                 | MAP, n=438    | ROS, n=471    | P-value  |
|---------------------------------|---------------|---------------|----------|
| <i>Variable</i>                 |               |               |          |
| Age at death                    | 89.4 ± (5.8)  | 87.5 ± (6.9)  | < 0.0001 |
| Male                            | 151 (34%)     | 174 (37%)     | 0.480    |
| Pathology dx of AD <sup>a</sup> | 275 (63%)     | 295 (63%)     | > 0.99   |
| <i>Pathology</i>                |               |               |          |
| Lewy body                       | 87 (20%)      | 108 (23%)     | 0.296    |
| Neurofibrillary tangles         | 0.70 ± (0.41) | 0.66 ± (0.41) | 0.171    |
| Neuritic plaque                 | 0.74 ± (0.53) | 0.74 ± (0.54) | 0.993    |
| Diffuse plaque                  | 0.64 ± (0.48) | 0.71 ± (0.50) | 0.029    |
| Microinfarct                    | 113 (26%)     | 140 (30%)     | 0.213    |
| Macroinfarct                    | 162 (37%)     | 165 (35%)     | 0.586    |
| CAA                             |               |               | 0.272    |
| None                            | 78 (18%)      | 105 (22%)     |          |
| Mild                            | 204 (47%)     | 200 (42%)     |          |
| Moderate                        | 105 (24%)     | 104 (22%)     |          |
| Severe                          | 51 (12%)      | 62 (13%)      |          |

Abbreviations: AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; MAP, Rush Memory and Aging Project; NIA, National Institute on Aging; ROS, Religious Order Study. <sup>a</sup>NIA-Reagan Criteria.

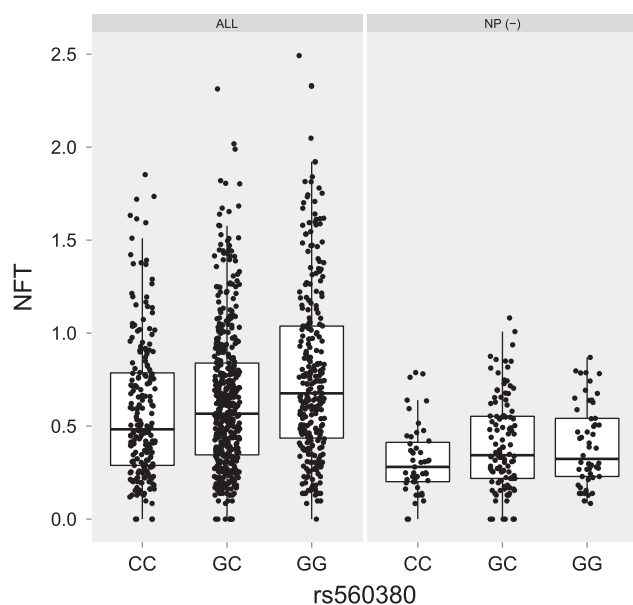
**Table 2.** Significant ( $P < 5.0 \times 10^{-8}$ ) and suggestive ( $P < 1 \times 10^{-5}$ ) results from the GWAS of neurofibrillary tangles

| SNP            | Chr | BP          | Nearest gene <sup>a</sup> | Region     | A1 | A2 | Af <sup>b</sup> | OR (95% CI)          | P-value  |
|----------------|-----|-------------|---------------------------|------------|----|----|-----------------|----------------------|----------|
| rs429358       | 19  | 45 411 941  | <i>APOE/TOMM40</i>        | Intronic   | T  | C  | 0.85            | -0.27 (-0.21, -0.33) | 4.06E-20 |
| rs560380       | 9   | 9 112 698   | <i>PTPRD</i>              | Intronic   | A  | C  | 0.55            | 0.10 (0.07, 0.14)    | 3.14E-08 |
| rs2446485      | 6   | 21 140 299  | <i>CDKAL1</i>             | Intronic   | G  | A  | 0.82            | -0.13 (-0.08, -0.17) | 1.82E-07 |
| rs9739162      | 12  | 1 838 008   | <i>ADIPOR2</i>            | Intronic   | G  | T  | 0.46            | -0.09 (-0.05, -0.12) | 2.56E-06 |
| Rs2104198      | 20  | 16 653 076  | <i>SNRBP2</i>             | Intergenic | C  | T  | 0.50            | -0.08 (-0.05, -0.12) | 4.88E-06 |
| rs2964044      | 5   | 173 720 716 |                           | Intergenic | A  | T  | 0.89            | -0.13 (-0.08, -0.19) | 5.50E-06 |
| rs11022147     | 11  | 1 341 815   | <i>TOLLIP</i>             | Intergenic | G  | A  | 0.93            | -0.25 (-0.14, -0.36) | 5.50E-06 |
| chr5:167502615 | 5   | 167 502 615 | <i>ODZ2</i>               | Intronic   | T  | C  | 0.97            | -0.26 (-0.15, -0.37) | 5.85E-06 |
| rs11743956     | 5   | 6 292 456   | <i>FLJ33360</i>           | Intergenic | C  | T  | 0.91            | -0.20 (-0.11, -0.28) | 6.40E-06 |
| rs12609752     | 19  | 56 448 708  | <i>NLRP13</i>             | Intergenic | C  | T  | 0.98            | -0.30 (-0.17, -0.44) | 6.52E-06 |
| rs13425639     | 2   | 184 588 957 |                           | Intergenic | G  | A  | 0.82            | -0.11 (-0.06, -0.16) | 6.57E-06 |
| rs1494971      | 8   | 108 650 188 |                           | Intergenic | T  | C  | 0.83            | -0.15 (-0.09, -0.22) | 6.72E-06 |
| rs10025919     | 4   | 146 242 907 |                           | Intergenic | T  | C  | 0.88            | 0.16 (0.09, 0.23)    | 7.25E-06 |
| Chr6:53236216  | 6   | 53 236 216  | <i>ELOVL5</i>             | Intergenic | A  | G  | 0.94            | -0.26 (-0.14, -0.37) | 7.80E-06 |
| rs73035809     | 11  | 131 606 984 | <i>NTM</i>                | Intergenic | G  | A  | 0.97            | -0.39 (-0.22, -0.56) | 8.18E-06 |
| chr3:98769677  | 3   | 98 769 677  |                           | Intergenic | T  | C  | 0.91            | -0.21 (-0.12, -0.3)  | 9.63E-06 |
| rs73183521     | 8   | 2 955 118   | <i>CSMD1</i>              | Intronic   | T  | C  | 0.98            | -0.42 (-0.23, -0.6)  | 1.01E-05 |

Abbreviations: BP, base pairs; Chr, chromosome; CI, confidence interval; GWAS, genome-wide association study; OR, odds ratio; SNP, single-nucleotide polymorphism. <sup>a</sup>Within 100 kb of SNP. <sup>b</sup>Allele frequency of A1.



**Figure 1.** Regional association plot for *PTPRD* and neurofibrillary tangles (NFTs). The x axis is the base pair position and the y axis is the  $-\log(P\text{-value})$  for the association with NFT. The blue line represents the recombination rate. SNP, single-nucleotide polymorphism.



**Figure 2.** Association between *PTPRD* single-nucleotide polymorphism (SNP), rs560380 and neurofibrillary tangles (NFT). The left plot includes all  $n = 909$  Religious Order Study (ROS) and Rush Memory and Aging Project (MAP) brains and the right plot includes a subset of  $n = 132$  from participants with no neuritic plaques at death.

and is simply not appreciated in this particular location and mixture of cells. Further, given that many AD susceptibility alleles influence gene expression in myeloid cells, we also checked the expression quantitative trait results generated from healthy participants of the ImmVar project,<sup>29</sup> but we found that the rs560380 variant has no effect on mRNA expression of *PTPRD* in *ex vivo* monocytes or naïve CD4+ T cells. As is standard in *cis*-expression quantitative trait analyses, we examined the expression of all other genes within a 1 Mb radius of the SNP,

but *PTPRD* is the only gene found in this genomic segment. Thus, rs560380 does not appear to influence mRNA expression of *PTPRD* in the samples that we have queried to date, and, as is the case for a majority of disease-associated SNPs,<sup>29</sup> its mechanism of action remains unclear

#### Leveraging correlated neuropathologic traits to enhance gene discovery for NFT

As the participants in the ROS and MAP data sets have information on a wealth of different but related neuropathologic phenotypes, we chose to pursue a complementary strategy for further gene discovery: we implemented a secondary analysis for pleiotropy to identify variants that affect not only NFT but also other neuropathologies. Such pleiotropic effects are likely in the context of neurodegeneration in which different insults may ultimately trigger similar responses in neurons and other CNS cells.

We first calculated a correlation matrix between seven neuropathologic outcomes that are available in almost all of our participants. Results are presented in Supplementary Table 2, showing, as expected, modest to strong correlations between findings, ranging from  $\rho = 0.41$  ( $P < 0.001$ ) between NFT and diffuse amyloid plaques (DP) to  $0.69$  ( $P < 0.001$ ) between NP and DP. Lewy body pathology was not correlated ( $\rho < 0.1$ ) ( $ps > 0.05$ ) with the other traits, and the two neurovascular pathology traits (microscopic and macroscopic strokes) showed only weak ( $\rho = 0.25$ ,  $P < 0.001$ ) correlation with each other. Thus, the observed level of correlation, while substantial in certain cases, remains within the range in which a pleiotropic analysis can be considered: a prior study showed that, even in the presence of correlations as high as 0.9, the MULTIPHEN method shows no inflation in the type I error.<sup>24</sup>

We therefore used MULTIPHEN to implement a pleiotropy analysis integrating results from association studies of seven neuropathologic traits (see details in the Supplementary Methods section). The *APOE* locus serves as a positive control since it is known to influence several of these traits.<sup>30</sup> Results are presented in Table 3; Q-Q and Manhattan plots for the MULTIPHEN GWAS are presented in Supplementary Figures S3 and S4. Overall, the most

**Table 3.** Significant ( $P < 5.0 \times 10^{-8}$ ) and suggestive ( $P < 1 \times 10^{-5}$ ) results from pleiotropic analyses.

| SNP            | Chr | BP          | Nearest gene <sup>a</sup> | Region     | A1 | A2 | Af <sup>b</sup> | P-value  |
|----------------|-----|-------------|---------------------------|------------|----|----|-----------------|----------|
| rs429358       | 19  | 45 411 941  | <i>APOE/TOMM40</i>        | Intronic   | T  | C  | 0.85            | 2.88E-33 |
| chr3:197113961 | 3   | 197 113 961 | <i>LOC100507086</i>       | Intergenic | C  | G  | 0.96            | 6.60E-08 |
| chr15:41258121 | 15  | 41 258 121  | <i>CHAC1</i>              | Intergenic | C  | T  | 0.96            | 1.02E-06 |
| rs4145953      | 11  | 74 331 879  | <i>POLD3</i>              | Intronic   | A  | G  | 0.80            | 1.07E-06 |
| chr6:44535225  | 6   | 44 535 225  |                           |            | G  | T  | 0.95            | 1.18E-06 |
| rs2291354      | 15  | 25 952 889  | <i>ATP10A</i>             | Intronic   | A  | G  | 0.48            | 1.23E-06 |
| rs11584308     | 1   | 14 805 993  |                           |            | C  | G  | 0.80            | 1.46E-06 |
| rs72723271     | 4   | 188 308 255 | <i>LOC339975</i>          | Intronic   | T  | G  | 0.94            | 1.63E-06 |
| rs2446485      | 6   | 21 140 299  | <i>CDKAL1</i>             | Intronic   | G  | A  | 0.82            | 2.18E-06 |
| rs59008350     | 20  | 18 219 685  | <i>ZNF133/CSRP2BP</i>     | Intergenic | C  | T  | 0.96            | 2.21E-06 |
| rs11765038     | 7   | 5 336 105   | <i>SLC29A4</i>            | Intronic   | A  | G  | 0.32            | 3.55E-06 |
| chr3:70929100  | 3   | 70 929 100  | <i>FOXP1</i>              | Intergenic | G  | T  | 0.91            | 4.97E-06 |
| rs7107464      | 11  | 36 584 944  | <i>RAG1</i>               | Intergenic | T  | C  | 0.91            | 4.98E-06 |
| rs6540796      | 1   | 213 821 667 |                           |            | A  | G  | 0.70            | 5.45E-06 |
| rs9321429      | 6   | 134 475 749 | <i>SGK1</i>               | Intergenic | C  | G  | 0.64            | 5.50E-06 |
| chr1:245947879 | 1   | 245 947 879 | <i>SMYD3</i>              | Intronic   | A  | C  | 0.99            | 6.52E-06 |
| rs10836184     | 11  | 34 325 250  | <i>ABTB2</i>              | Intronic   | T  | C  | 0.21            | 6.68E-06 |
| Rs348147       | 8   | 73 353 197  |                           |            | G  | A  | 0.89            | 7.27E-06 |
| rs66657970     | 11  | 134 377 829 | <i>LOC283177</i>          | Intergenic | C  | T  | 0.97            | 8.60E-06 |
| rs7206188      | 16  | 26 152 155  | <i>HS3ST4</i>             | Intergenic | T  | C  | 0.56            | 9.45E-06 |
| rs12142743     | 1   | 146 585 620 | <i>PRKAB2</i>             | Intergenic | G  | T  | 0.89            | 9.66E-06 |
| rs560380       | 9   | 9 112 698   | <i>PTPRD</i>              | Intronic   | A  | C  | 0.55            | 9.92E-06 |

Abbreviations: BP, base pair; Chr, chromosome; SNP, single-nucleotide polymorphism. <sup>a</sup>Within 100 kb of SNP. <sup>b</sup>Allele frequency of A1.

significant SNP in the *APOE* locus is rs429358 ( $P = 2.9 \times 10^{-33}$ ), which is in partial LD with the *APOE*  $\epsilon 4$  haplotype. This variant is strongly associated with four of the seven neuropathologic outcomes (NP,  $P = 3.0 \times 10^{-24}$ ; DP,  $P = 1.3 \times 10^{-20}$ ; NFT,  $P = 4.1 \times 10^{-20}$ , and CAA,  $P = 5.2 \times 10^{-25}$ ), but it had no significant association with microscopic infarcts, macroscopic infarcts or Lewy bodies ( $P_s > 0.05$ , results not shown). Although our pleiotropy results for rs429358 are similar to what is reported in a recent GWAS of neuropathologic traits,<sup>2</sup> we do not replicate their association with our Lewy body measure ( $P = 0.46$ , ever vs none). However, we were able to replicate their findings for rs6857 using similar phenotypes; NP as a continuous trait in our analyses ( $P = 6.2 \times 10^{-20}$ ) vs an ordinal trait in Beecham ( $3.1 \times 10^{-47}$ ), NFT as a continuous trait in our analyses ( $P = 5.0 \times 10^{-18}$ ) vs an ordinal Braak score in Beecham ( $4.7 \times 10^{-47}$ ), CAA as an ordinal trait in our analyses ( $P = 1.9 \times 10^{-21}$ ) vs binary in Beecham ( $P = 2.9 \times 10^{-21}$ ) (Supplementary Table 3).

Looking at the top non-*APOE* results that are not significant but have a  $P < 1 \times 10^{-5}$  in the pleiotropy analysis, we note a few interesting results. First, there are a few examples where a SNP such as rs12597858, near the *HS3ST4* gene, has a modest association with NFT burden ( $P = 0.0039$ ) in the primary analysis and a much stronger pleiotropy score ( $P = 9.5 \times 10^{-6}$ ) (Table 3) that is driven by a broad effect on five of the remaining six neuropathologic traits: NP burden ( $P = 0.003$ ), DP burden ( $P = 0.03$ ), Lewy bodies ( $P = 0.03$ ), CAA ( $P = 0.03$ ) and macroscopic infarcts ( $P = 0.0003$ ) (Figure 3). *HS3ST4* encodes a protein involved in heparan sulfate biosynthesis. Notably, heparan sulfate proteoglycans have been implicated in many different biological and pathologic processes, including AD and the cellular propagation of both Tau and  $\alpha$ -synuclein pathologies.<sup>31-33</sup> Another example of a SNP whose effect becomes appreciable only when considering multiple neuropathologic traits is in the *POLD3* gene: the top SNP, rs4145953, has a modest effect on NFT burden ( $P = 2.4 \times 10^{-4}$ ) and a stronger pleiotropic score ( $P = 1.1 \times 10^{-6}$ ) (Table 3), which is driven by additional, modest associations with NP burden ( $P = 1.1 \times 10^{-4}$ ), DP burden ( $P = 2.7 \times 10^{-5}$ ) and macroscopic infarcts ( $P = 0.01$ ).

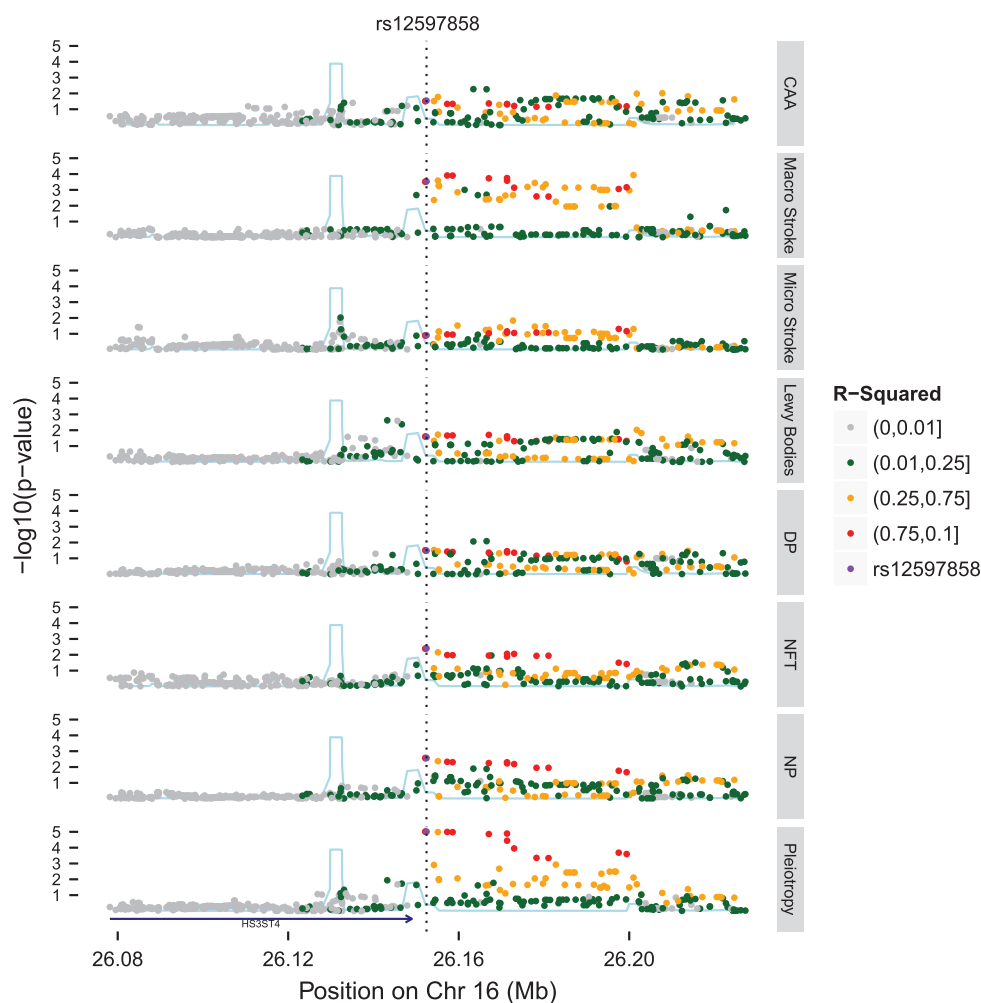
As compared with *HS3ST4* whose pleiotropic association is driven by a modest effect on many neuropathologies, a second type of locus, like *PTPRD*, has a strong effect on one trait and modest effects on two other traits: NP ( $P = 0.005$ ) and CAA ( $P = 0.002$ ). Thus, not surprisingly, this SNP is suggestive but not significant in the pleiotropy analysis ( $P = 9.7 \times 10^{-6}$ ), with this association being driven primarily by the NFT trait. In another example, SNP chr3:197 113 961 in the *SLC29A4* gene is nearly significant genome wide in the pleiotropic analysis ( $P = 6.6 \times 10^{-8}$ ) and is driven by effects on microscopic ( $P = 3.4 \times 10^{-6}$ ) and macroscopic ( $P = 9.3 \times 10^{-4}$ ) infarcts. However, this variant has no effects on non-vascular traits.

Tables with the top results for the GWAS for all the neuropathologies examined are presented in Supplementary Tables S4-S9, and Q-Q plots and Manhattan plots for each trait are presented in Supplementary Figures S5-S16.

#### Role of known disease-associated variants in NFT accumulation and pleiotropy

We also evaluated more closely the role of SNPs known to be associated with tauopathies (AD and progressive supranuclear palsy).<sup>26,34</sup> Results are displayed in Table 4. In the 24 susceptibility loci that we evaluate,<sup>34-37</sup> we found a significant NFT association (using a significance cutoff of  $0.002 = 0.05/24$ ) with the rs1476679 SNP in the *ZCWPW1* locus ( $\beta = 0.07$ ,  $P = 4.9 \times 10^{-4}$ ) (Table 4). As with the *PTPRD* variant, rs1476679 also influences NP ( $P = 0.0043$ ), and, when we adjust for NP, the  $\beta$  of the NFT association ( $\beta = 0.037$ ,  $P = 0.024$ ) was reduced by 47%. Thus, in this case, much of the SNP's effect appears to be mediated through pathologic processes that underlie NP. The only other locus to show some level of suggestive association with NFT in our data is rs10948363, a SNP in the *CD2AP* gene ( $P = 0.0085$ ), consistent with a recent report and our earlier results.<sup>2,38</sup>

Since both *ZCWPW1* and *CD2AP* are known AD susceptibility loci, we tested if this association is mediated through NFT pathology. In our data only the *ZCWPW1* SNP (rs1476679) is associated with AD risk ( $\beta = 0.32$ ,  $P = 0.003$ ), and the effect is attenuated 50% with the addition of NFT ( $\beta = 0.15$ ,  $P = 0.23$ ). No



**Figure 3.** Regional association plot for *HS3ST4*. The x axis is the base pair position centered around the top pleiotropy single-nucleotide polymorphism (SNP), rs12597858, and the y axis is the  $-\log(P\text{-value})$ . The blue line represents the recombination rate. The bottom level shows the pleiotropic results and the top level show results for each of the seven neuropathological phenotypes.

SNPs in *CD2AP* were associated with AD risk in our limited data set.

## DISCUSSION

Our primary analysis reports a significant association between the *PTPRD* locus and the accumulation of NFT in older participants. While the detailed molecular mechanism of this association remains unclear at present, we found that the effect of the rs560380 SNP on NFT accumulation is not dependent on a single pathophysiologic process. NP mediates part of the SNP's effect on NFT, but most of the effect is independent of NP, and the association is observed in individuals without substantial amyloid  $\beta$  pathology. This pattern of findings suggests that rs560380 may be a locus that influences the CNS's response to different insults that can lead to neuronal dysfunction and neurodegeneration.

*PTPRD* is a large gene that has previously been associated with restless leg syndrome<sup>39–41</sup> and, less convincingly, obsessive compulsive disorder.<sup>42</sup> The SNPs driving these associations are not found on the same susceptibility haplotype as the NFT-associated *PTPRD* variant and suggest the presence of allelic heterogeneity and phenotypic heterogeneity: *PTPRD* is implicated in NFT accumulation as well as neurologic disorders without Tau

pathology. The SNPs associated with obsessive compulsive disorder and restless leg syndrome—two conditions that are not tauopathies—do not display an association with NFT burden in our data (data not shown). In terms of tauopathies, an existing PSP GWAS does not provide evidence of significant association with *PTPRD*. In terms of AD, a suggestive association of *PTPRD* with AD dementia susceptibility has been reported previously ( $P=4.5 \times 10^{-5}$ )<sup>37</sup> in one AD GWAS, and in our own data, we see a modest association with AD dementia susceptibility ( $P=0.04$ , with clinical diagnosis of AD dementia). Overall, further studies are needed to evaluate the extent of the role of this locus to clinical manifestations associated with the accumulation of NFT. Our evidence to date highlights a role for *PTPRD* in the accumulation of this pathologic feature, irrespective of its clinical manifestations.

*PTPRD* encodes the protein tyrosine phosphatase receptor-type delta protein, which is reported to have protein tyrosine phosphatase activity *in vitro*.<sup>43</sup> It is expressed in the brain where it has been implicated in synaptic differentiation.<sup>44</sup> A null allele in mice leads to memory impairment and altered electrophysiological responses in the hippocampus.<sup>45</sup> Further, in our prior work, *PTPRD* was considered among other AD candidate genes for studies using a *Drosophila* model, and knockdown of *lar*, the fly orthologue of *PTPRD*, was discovered to enhance Tau-induced

**Table 4.** Associations of known AD and PSP SNPs with the neurofibrillary tangles and pleiotropic outcomes

| Gene   | Chr | SNP        | Pos         | Allele frequency | P-value (NFT)                 | P-value (pleiotropy)          |
|--|-----|------------|-------------|------------------|-------------------------------|-------------------------------|
| <i>Significant SNPs from AD GWAS<sup>a</sup></i> |     |            |             |                  |                               |                               |
| CR1  | 1   | rs6656401  | 207 692 049 | 0.815            | 0.2771                        | 0.3152                        |
| BIN1   | 2   | rs7561528  | 127 889 637 | 0.655            | 0.6676                        | 0.1816                        |
| BIN1   | 2   | rs6733839  | 127 892 810 | 0.397            | 0.2380                        | 0.2190                        |
| INPP5D   | 2   | rs35349669 | 234 068 476 | 0.463            | 0.1829                        | 0.7458                        |
| MEF2C  | 5   | rs190982   | 88 223 420  | 0.407            | 0.2733                        | 0.8914                        |
| CD2AP  | 6   | rs10948363 | 47 487 762  | 0.256            | <b>0.0086</b>                 | <b>0.0284</b>                 |
| TREML2   | 6   | rs9381040  | 41 154 650  | 0.279            | 0.2548                        | 0.0695                        |
| HLA-DRB5   | 6   | rs9271192  | 32 578 530  | 0.708            | 0.2342                        | 0.8329                        |
| ZCWPW1   | 7   | rs1476679  | 100 004 446 | 0.707            | <b>0.0005</b>                 | <b>0.0308</b>                 |
| NME8   | 7   | rs2718058  | 37 841 534  | 0.636            | 0.6656                        | 0.1890                        |
| EPHA1  | 7   | rs11771145 | 143 110 762 | 0.641            | 0.1856                        | 0.8026                        |
| CLU  | 8   | rs9331896  | 27 467 686  | 0.403            | 0.3164                        | 0.4114                        |
| PTK2B  | 8   | rs28834970 | 27 195 121  | 0.349            | 0.6440                        | 0.4197                        |
| CELF1  | 11  | rs10838725 | 47 557 871  | 0.694            | 0.8936                        | 0.0501                        |
| PICALM   | 11  | rs10792832 | 85 867 875  | 0.364            | 0.1130                        | 0.5884                        |
| SORL1  | 11  | rs11218343 | 121 435 587 | 0.969            | 0.5155                        | 0.6918                        |
| MS4A6A   | 11  | rs983392   | 59 923 508  | 0.572            | 0.5211                        | 0.6942                        |
| FERMT2   | 14  | rs17125944 | 53 400 629  | 0.912            | 0.0852                        | 0.0145                        |
| SLC24A4  | 14  | rs10498633 | 92 926 952  | 0.775            | 0.1696                        | 0.5026                        |
| DSG2   | 18  | rs8093731  |             | 0.006            | MAF < 1%, no results          |                               |
| APOE   | 19  | rs4420638  | 45 422 946  | 0.827            | <b>1.9 × 10<sup>-14</sup></b> | <b>2.3 × 10<sup>-26</sup></b> |
| APOE   | 19  | rs2075650  | 45 395 619  | 0.863            | <b>5.9 × 10<sup>-14</sup></b> | <b>9.5 × 10<sup>-23</sup></b> |
| ABCA7  | 19  | rs4148929  | 73 740 837  | 0.609            | 0.9686                        | 0.1547                        |
| CD33   | 19  | rs3865444  | 51 727 962  | 0.689            | 0.8673                        | 0.4297                        |
| CASS4  | 20  | rs7274581  | 55 018 260  | 0.919            | 0.9493                        | 0.5211                        |
| <i>Significant SNPs from PSP GWAS**</i>          |     |            |             |                  |                               |                               |
| STX6   | 1   | rs1411478  | 179 229 155 | 0.391            | 0.9068                        | 0.7213                        |
| EIF2AK3  | 2   | rs7571971  | 88 676 716  | 0.266            | 0.0851                        | 0.4059                        |
| MOBP   | 3   | rs1768208  | 39 498 257  | 0.706            | 0.3310                        | 0.3190                        |
| MAPT   | 17  | rs8070723  | 41 436 651  | 0.202            | 0.7335                        | 0.4593                        |
| MAPT   | 17  | rs242557   | 41 375 823  | 0.620            | 0.9564                        | 0.4059                        |

Abbreviations: AD, Alzheimer's disease; Chr, chromosome; GWAS, genome-wide association study; MAF, minor allele frequency; NFT, neurofibrillary tangle; Pos, position; PSP, progressive supranuclear palsy; SNP, single-nucleotide polymorphism. <sup>a</sup>Lambert *et al.*<sup>34</sup> and Hoglinger *et al.*<sup>50</sup> Bold and italic values signify  $P < 0.05$  in both NFT and pleiotropy analysis.

retinal degeneration, consistent with its association with NFT accumulation in humans.<sup>46</sup> Further work in flies and other model systems is now necessary to delineate the molecular consequences of perturbations in *PTPRD* expression that lead to Tau pathology.

Aside from *PTPRD*, we note that the validated AD susceptibility *ZCWPW1* allele has a significant effect on NFT accumulation (Figure 2). However, unlike *PTPRD*, the effect of this variant appears to be mediated more strongly through an effect on amyloid  $\beta$  accumulation.

The other locus that deserves some discussion is *HS3ST4*, which had a modest effect of NFT burden but a strongly suggestive association in the pleiotropy analysis because of its effect on six of the seven available neuropathologic measures. This association was also supported by our earlier work in *Drosophila*: the homolog of another gene encoding a heparan sulfate biosynthetic enzyme, *HS6ST3*, influenced Tau toxicity in a *Drosophila* transgenic model.<sup>47</sup> This result is consistent with the emerging potential role of heparan sulfate proteoglycans as receptors for the spread of Tau and synuclein pathologies.<sup>48</sup> Notably, variants at loci encoding other heparan sulfate sulfotransferases have been strongly suggested in AD (*HS3ST1*, rs448799,  $P = 6.6 \times 10^{-8}$ )<sup>34</sup> as well as a study of memory performance in non-demented individuals (*HS3ST4*, rs11074779  $P = 3.1 \times 10^{-8}$ ).<sup>49</sup>

Comparing our study to the recently published GWAS of multiple neuropathologies,<sup>2</sup> we showed similar findings with the *APOE* region being associated with the NFT, NP and CAA outcomes plus a strong pleiotropic association; however, we were unable to replicate their novel findings. This discordance can be explained by the multiple differences between the two studies, especially differences in phenotype definitions and sample

selection as our subjects come from prospective studies of aging and are not recruited in specialty-specific dementia clinics.

Our study of older participants has certain limitations, including the fact that participants are non-demented at study entry, biasing us toward a population of older individuals who have survived to an advanced age without dementia. On the other hand, we have the advantage of performing all autopsies using a single structured protocol at a single site, minimizing the phenotypic error. In contrast to the recent study that also performed a GWAS for NFT by repurposing genotypes generated for an AD study,<sup>2</sup> our participants were not originally selected to fit certain clinicopathologic criteria. Since such a selection of AD 'cases' and 'controls' will influence the distribution of pathology and may limit the generalizability of its results, it was not pursued here. These features and the moderate sample sizes used in this study and the earlier NFT GWAS of AD cases and controls<sup>26</sup> could explain differences in the results of the two studies. However, we do have similar findings with the *APOE* region: we found it to be associated with the NFT, NP and CAA outcomes, but we were unable to replicate the novel finding of association with Lewy bodies. We note that the present study has high internal validity as follow-up rates exceeded 95% and autopsy rates exceeded 90% and the limited power due to small sample size should not detract from the positive results that meet reasonable thresholds of statistical significance.

In sum, this NFT GWAS begins to uncover genetic variation that influences the accumulation of Tau pathology in older individuals. In addition, we present evidence supporting the utility of a pleiotropic analysis approach in identifying genetic variation with shared effects in common brain pathologies. These two approaches—focused and pleiotropic—are complementary and

will both be necessary to dissect the complex web of inter-related factors that lead impaired cognition and, ultimately, dementing syndromes.

### CONFLICT OF INTEREST

Dr Schneider is a consultant for Navidea Biopharmaceuticals and an advisor to Eli Lilly and Genetech. The remaining authors declare no conflict of interest.

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### AUTHOR CONTRIBUTIONS

CDK, JS and JAS collected, prepared and generated data from the samples. LBC, CCW, TR and LY performed analyses on the resulting data. SM, EBL, TJM, CDK, JS and PKC generated and analyzed the replication data. EBL, TJM and PKC designed the replication study. PLD and DAB designed the primary study. PLD, CCW, JMS and LBC wrote the manuscript. All of the authors critically reviewed the manuscript.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)