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Authors
Farrell, Kurt
Kim, SoongHo
Han, Natalia
et al.

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Genome-wide association study and functional validation implicates JADE1 in tauopathy

Kurt Farrell1,2,3 · SoongHo Kim1,2,3 · Natalia Han1,2,3 · Megan A. Iida1,2,3 · Elias M. Gonzalez4 · Marcos Otero-Garcia5 · Jamie M. Walker6 · Timothy E. Richardson6 · Alan E. Renton2,7 · Shea J. Andrews2,7 · Brian Fulton-Howard2,7 · Jack Humphrey2,7 · Ricardo A. Viale2,7 · Kathryn R. Bowles7 · Katia de Paiva Lopes2,7 · Kristen Whitney1,2,3 · Diana K. Dangoor1,2,3 · Hadley Walsh1,2,3 · Edoardo Marcara2,7 · Marco M. Hefti8 · Alicia Casella1,2,3 · Cheick T. Sissoko1,2,3 · Manav Kapoor2,7 · Gloriia Novikova2,7 · Evan Udine2,7 · Garrett Wong2,7 · Weijjing Tang31 · Tushar Bhangale9 · Julie Hunkapiller9 · Gai Ayalon10 · Robert R. Graham11 · Jonathan D. Cherry12 · Etty P. Cortes1 · Valery Y. Borukov2,7 · Ann C. McKee12 · Thor D. Stein12 · Jean-Paul Vonsattel13 · Andy F. Teich13 · Marla Gearing14 · Jonathan Glass14 · Juan C. Troncoso15 · Matthew P. Frosch16 · Bradley T. Hyman16 · Dennis W. Dickson17 · Melissa E. Murray17 · Johannes Attems18 · Margaret E. Flanagan19 · Qinwen Mao19 · M.-Marcel Mesulam19 · Sandra Weintraub19 · Randy L. Wolter20 · Thao Pham20 · Julia Kofler21 · Julie A. Schneider22 · Lei Yu22 · Dushyant P. Purohit1,23 · Vahram Haroutunian2,23 · Patrick R. Hof2 · Sam Gandy23,39 · Mary Sano23 · Thomas G. Beach24 · Wayne Poon25 · Claudia H. Kawa26 · Maria M. Corrada26 · Robert A. Rissman26 · Jeff Metcalfe26 · Sara Shulder26 · Bahar Salehi26 · Peter T. Nelson27 · John Q. Trojanowksi28 · Edward B. Lee28 · David A. Wolk29 · Corey T. McMillan29 · C. Dirk Keene30 · Caitlin S. Latimer30 · Thomas J. Montine30 · Gabor G. Kovacs32,33,34 · Mirjam I. Lutz34 · Peter Fischer41 · Richard J. Perrin35 · Nigel J. Cairns38 · Erin E. Franklin35 · Herbert T. Cohen36 · Towfique Raj2,7 · Inma Cobos31 · Bess Frost4 · Alison Goate2,7 · Charles L. White III37 · John F. Crary1,2,3

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Abstract

Primary age-related tauopathy (PART) is a neurodegenerative pathology with features distinct from but also overlapping with Alzheimer disease (AD). While both exhibit Alzheimer-type temporal lobe neurofibrillary degeneration alongside amnestic cognitive impairment, PART develops independently of amyloid-β (Aβ) plaques. The pathogenesis of PART is not known, but evidence suggests an association with genes that promote tau pathology and others that protect from Aβ toxicity. Here, we performed a genetic association study in an autopsy cohort of individuals with PART (n = 647) using Braak neurofibrillary tangle stage as a quantitative trait. We found some significant associations with candidate loci associated with AD (SLC24A4, MS4A6A, HS3ST1) and progressive supranuclear palsy (MAPT and EIF2AK3). Genome-wide association analysis revealed a novel significant association with a single nucleotide polymorphism on chromosome 4 (rs56405341) in a locus containing three genes, including JADE1 which was significantly upregulated in tangle-bearing neurons by single-soma RNA-seq. Immunohistochemical studies using antisera targeting JADE1 protein revealed localization within tau aggregates in autopsy brains with four microtubule-binding domain repeats (4R) isoforms and mixed 3R/4R, but not with 3R exclusively. Co-immunoprecipitation in post-mortem human PART brain tissue revealed a specific binding of JADE1 protein to four repeat tau lacking N-terminal inserts (0N4R). Finally, knockdown of the Drosophila JADE1 homolog rhinoceros (rno) enhanced tau-induced toxicity and apoptosis in vivo in a humanized 0N4R mutant tau knock-in model, as quantified by rough eye phenotype and terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) in the fly brain. Together, these findings indicate that PART has a genetic architecture that partially overlaps with AD and other tauopathies and suggests a novel role for JADE1 as a modifier of neurofibrillary degeneration.
Introduction

Primary age-related tauopathy (PART) is nearly ubiquitously observed to varying degrees of severity in the brains of aged individuals. PART develops in the absence Aβ plaques yet manifests as neurofibrillary tangles (NFT) composed of abnormal aggregates of the microtubule-associated protein tau that are morphologically, biochemically and ultrastructurally identical to those in early to moderate-stage Alzheimer Disease (AD) [23]. A neuropathological diagnosis of PART does not imply cognitive impairment; individuals with PART can be normal, mildly cognitively impaired (MCI) or demented [40, 101]. Most individuals with PART remain cognitively normal; however, those clinically affected usually develop amnestic cognitive changes [7, 10, 40, 69]. Given the similarities between PART and AD, PART as a diagnostic construct would have greater value if it were shown to arise independently [16, 28]. The observation that some individuals of advanced age at autopsy show substantial NFT pathology without evidence of amyloid deposition provides strong evidence that it does. However, the argument can be made that PART might be a component of the AD spectrum, and Aβ pathology might have eventually developed in such individuals had they lived longer [28, 53]. Thus, the question remains to what extent patients with a neuropathological diagnosis of PART have divergent or overlapping risk factors with AD and other dementias [8, 48].

Much of the mechanistic knowledge surrounding tauopathy stems from genetic studies [95]. Autosomal dominant mutations in the microtubule-associated protein tau gene (MAPT) in coding regions can interfere with microtubule binding or promote transition to toxic forms [38]. Mutations in splice sites disrupt alternative mRNA splicing of the tau mRNA transcript, modifying the ratio of three repeat (3R) and four repeat (4R) tau isoforms leading to downstream pathological sequelae [38], while alternative splicing of the N-terminal exons may also play a role in modulating toxic tau specifically through regulation of microtubule stabilization [21, 26, 72]. However, MAPT mutations are rare. In contrast, PART occurs sporadically and nearly ubiquitously with advanced age in individuals without MAPT mutations. Thus, studying PART may reveal common genetic drivers of tauopathy and their association with abnormalities in tau proteostasis and isoform expression. The MAPT 17q21.31 locus is defined by a large ~900 kb inversion consisting of two distinct common haplotypes, H1 and H2. The more frequent H1 has been associated with increased risk for PART and several other sporadic tauopathies including APOE ε4-negative AD, progressive supranuclear palsy, corticobasal degeneration, as well as Parkinson disease which is not classically considered a tauopathy [18, 35, 46, 49, 68, 83]. Research focusing on the genetics of PART has consistently failed to show an association with the APOE ε4 allele, the strongest risk locus in AD [43, 65, 69, 83]. This is not surprising, given the strong association of APOE ε4 with early deposition of amyloid beta (Aβ) peptide. Outside of PART, one of the largest AD GWAS has identified 21 risk loci [52]. However, APOE, the strongest AD risk locus may be confounding associations independently related to tauopathy [46]. Another study confirmed APOE ε4 was not associated with PART but did find associations in PART with AD alleles in the BIN1, PTK2B, and CR1 loci [61]. Taken together, these data suggest an unexplored genetic risk driving tauopathy that might be revealed by conducting association studies in PART.

The neuropathological overlap between PART and AD allows for a unique opportunity to study mechanisms of tau-mediated AD-type neurodegeneration independently of Aβ. However, PART cannot be reliably diagnosed pre-mortem because of non-specific symptomology and accompanying age-related comorbid dementing pathologies. Therefore, discovering unique drivers is challenging antemortem [43, 45, 70, 71, 80, 85]. Assembling an autopsy cohort large enough to assess its genetics on a genome-wide scale has not yet been attempted. In collaboration with 21 domestic and international brain biorepositories, we performed the first GWAS in PART, using tau pathology as a quantitative trait, and compared our findings to known tauopathy risk loci. We then examined expression of candidate genes in our strongest risk locus (chromosome 4q28.2) using bulk RNA sequencing, single cell RNA-sequencing, and immunohistochemistry. We identified gene for apoptosis and differentiation in epithelia (JADE1) as a potential candidate gene. Finally, we validated our findings biochemically in human postmortem brain and identified a JADE1-tau protein interaction. Studies in Drosophila suggest that JADE1 plays a neuroprotective role in tauopathy. The work presented here not only further informs the genetics of PART, but also suggests a novel role for JADE1 in tauopathy.

Materials and methods

Cohort

Fresh-frozen brain tissue was obtained from the contributing centers (Supplementary Table 1, online resource). All tissue was used in accordance with the relevant guidelines and regulations of the respective institutions. Inclusion criteria were individuals with normal cognition, mild cognitive impairment (any type) and dementia. Cognitive status was determined either premortem or postmortem by a clinical chart review, mini-mental score, or clinical dementia rating...
Neuropathological assessments were performed at the respective centers using standardized criteria including Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) neuritic plaque assessment and Braak neurofibrillary tangle staging [15, 62]. Neuropathological inclusion criteria were Braak tangle stage of 0–IV and neuritic plaque severity CERAD score of 0 [15, 62]. In addition, formalin fixed paraffin-embedded tissue sections were obtained and reevaluated by the study investigators to confirm the lack of Aβ and degree of PART tau pathology as described by Walker et al. [97]. Clinical exclusion criteria were motor neuron disease, parkinsonism, and frontotemporal dementia. Neuropathological exclusion criteria were other degenerative diseases associated with NFTs (i.e., AD, progressive supranuclear palsy [PSP], corticobasal degeneration [CBD], chronic traumatic encephalopathy [CTE], frontotemporal lobar degeneration-tau [FTLD-tau], Pick disease (PiD), Guam amytrophic lateral sclerosis/parkinsonism–dementia, subacute sclerosing panencephalitis, globular glial tauopathy). Individuals with aging-related tau astrogliapathy (ARTAG) were not excluded [51]. In addition to PART cases, neuropathologically confirmed cases of AD, PSP, CTE, CBD, argyrophilic grain disease (AGD) and PiD (n = 3 for each, Supplemental Table 6) were examined for convergent or divergent staining patterns.

Genotyping

High-throughput isolation of DNA was performed using the MagMAX DNA Multi-Sample Ultra 2.0 Kit on a KingFisher Flex robotic system (ThermoFisher, Waltham, MA). Fresh frozen cortical brain tissue (20–40 mg) was placed into a deep-well plate and treated with 480 µl of Proteinase K mix (Proteinase K, phosphate buffered saline [pH 7.4], Binding Enhancer) and incubated overnight at 65 °C at 800 rpm on a shaking plate. Genomic DNA was isolated and purified using magnetic particles. DNA quality control was performed using a nanodrop spectrophotometer (concentration > 50 ng/µl, 260/280 ratio 1.7–2.2). Genotyping was performed using single nucleotide polymorphism (SNP) microarrays (Infinium Global Screening Array v2.4. or Infinium OmniExpress-24; Illumina, San Diego, CA). Raw genotype files were converted to PLINK-compatible files using GenomeStudio software (Illumina, San Diego, CA).

Genetic analysis

PLINK v1.9 was used to perform quality control [78]. SNP exclusion criteria included minor allele frequency < 1%, genotyping call-rate filter less than 95%, and Hardy–Weinberg threshold of $1 \times 10^{-6}$ [3]. Individuals with discordant sex, non-European ancestry, genotyping failure of > 5%, or relatedness of > 0.1 were excluded. A principal component analysis (PCA) was performed to identify population substructure using EIGENSTRAT v6.1.4 and the 1000 genomes reference panel [20, 76]. Samples were excluded if they were five standard deviations away from the European population cluster (Supplementary Fig. 1, online resource). PCA was performed again on the genomic data with non-Europeans excluded to generate new PCs, of which the first four were used as covariates in the regression model. All data was imputed on the University of Michigan server using minima3 and HRC reference panel [24, 60]. Imputed variants with MAF < 0.01 and a dosage $R^2$ < 0.7 were excluded. A quantitative trait association test was run on 647 PART cases vs. Gaussian-normalized Braak stage using conditional linear regression and age, sex, principal component 1–4 and SNP chip array (batch) as covariates. The analysis was run separately on each genotyping array and a meta-analysis was performed using METAL (2011 release) [99]. Regional genome-wide association plots were created with LocusZoom [77] (GRCh37) and other plots were created using R v4.0 and ggplot2 v3.3.5.

Expression quantitative trait loci (eQTL) analysis

For eQTL analysis, publicly available Religious Orders Study and Rush Memory and Aging Project (ROS–MAP) whole genome sequencing (WGS) and bulk RNA sequencing data were obtained from the Synapse portal (syn3219045 and syn8456629, respectively) and analyzed using techniques described elsewhere [25, 88]. Briefly, RNA was analyzed from the dorsolateral prefrontal cortex of 452 postmortem samples and a linear regression was performed stratified by the lead SNP’s genotype and JADE1 mRNA expression. Statistics were performed using R v4.0 and plotted in ggplot2 v3.3.5.

Single-cell mRNA profiling in tangle-containing neurons

Identification of differentially expressed genes in single neuronal somata with and without NFTs was performed by analyzing a transcriptomic data set of isolated neurons from Brodmann area 9 post-mortem human brain from individuals with AD (Braak stage VI) previously reported by Otero-Garcia et al. [73]. This data set consists of single neuronal soma transcriptomes obtained by mechanical dissociation of frozen tissue, followed by fluorescence-activated cell sorting (FACS) using p-tau (AT8) and MAP2 antisera to isolate single NFT-positive and NFT-negative neurons, mRNA capture using 10x Chromium v2 and sequenced with Illumina Novaseq 6000. Briefly, the data was normalized using the global-scaling function “LogNormalize” which normalizes the gene expression measurements for each cell by the total expression, multiplies this by a scale factor (10,000), and
log-transforms the result (natural log). For dimensionality reduction and clustering, Seurat v.2.4 MultiCCA was used with the top 4200 highly variable genes, selecting the first 26 canonical correlation vectors aligned by sample as grouping variable. For visualization, Seurat v.2.4 FindClusters function was used with the following parameters: 1:22 PCs; 1.0 resolution; 100 random start positions and 10 iterations per random start; and 30 k for the k-nearest neighbor algorithm. Gene cluster-specific markers were identified by comparing the gene expression levels for each specific cluster to all other cells using the Wilcoxon rank-sum test. Genes detected in ≥25% of cells with positive log fold changes > 0.25 and adjusted p values < 0.05 were included. Clusters containing cells with nonspecific identities were removed from further analysis. Differential gene expression (DGE) between neurons with and without NFTs for each cell-type cluster was assessed using the MAST generalized linear model, selecting an adjusted p value of <0.05 as cutoff [31].

**Immunohistochemistry**

Formalin-fixed paraffin-embedded (FFPE) tissue sections (5 μm) on charged slides were baked at 70 °C and immunohistochemistry (IHC) was performed on a Ventana Benchmark XT automatic stainer (Rouche, Tucson, AZ). Antigen retrieval was done using citric acid buffer (CC1) for 1 h followed by primary antibody incubation for approximately 40 min. A secondary antibody, 3,3′-diaminobenzidine (DAB) was then applied. For slides that were double-labeled, DAB and alkaline phosphatase were used for visualization. Slides were stained with antibodies to JADE1 (1:100, Proteintech, Rosemont, IL) and hyperphosphorylated tau (p-tau, AT8, 1:1000, Invitrogen, Waltham, MA). To ensure specificity of the JADE1 antisera, a peptide competition was performed using a blocking peptide. The JADE1 antibody was combined with a fivefold (by weight) excess of blocking peptide (Proteintech, Rosemont, IL) in 500 μl of PBS and incubated overnight at 4 °C. Whole slide images (WSI) were visualized and scanned using an Aperio CS2 digital slide scanner (Leica Biosystems, Wetzlar Germany).

**Biochemical analysis**

Western blots were performed using homogenized fresh-frozen brain tissue. Samples were placed in a micro-tube homogenizer (SP Bel-Art, Wayne, NJ) in 10 volumes (wt/vol) of ice-cold Pierce RIPA buffer (Thermo Fisher Scientific, Waltham, MA) containing Halt protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific), and incubated on ice for 30 min. For each sample, 20 μg of proteins were boiled in 1×Laemmli sample buffer (Bio-Rad, Hercules, CA) for 5 min, run on 10% Criterion TGX Pre-cast Gels (Bio-Rad, Hercules, CA), blotted to nitrocellulose membranes, and stained with JADE1 antisera (1:2000). Horseradish peroxidase-labeled secondary anti-rabbit antiser (1:20,000; Vector Labs, Burlingame, CA) was detected by SuperSignal West Pico PLUS Chemiluminescent Substrate or Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific). To quantify and standardize protein levels, GAPDH was detected with GAPDH antisera (6C5, 1:20,000; Abcam, Cambridge, MA) and total protein was detected with Amido Black (Sigma-Aldrich, St. Louis, MO) as previously described [2]. Chemiluminescence was measured in a ChemiDoc Imaging System (Bio-Rad), and relative optical densities were determined using AlphaEaseFC software, version 4.0.1 (Alpha Innotech, San Jose, CA), normalized to GAPDH and total protein loaded.

Co-immunoprecipitation (IP) assays were performed using fresh-frozen brain tissue homogenized in a glass-Teflon homogenizer at 500 rpm in 10 volumes (wt/vol) of ice-cold lysis buffer containing 50 mM Tris, pH 7.8, 0.5% NP40, 150 mM NaCl, 1 mM EDTA, and Halt protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Samples were incubated on ice for 30 min, and centrifuged at 1000×g for 10 min. The supernatant was collected and used for immunoprecipitation. In a microcentrifuge tube, 70 ul of supernatant. Lysis buffer (930 μl) and 2 μg of either JADE1 antisera or anti-ON tau antisera (EPR21726, Abcam, Cambridge, MA) were combined and incubated overnight at 4 °C. Antisera-negative and IgG isotype controls, either normal rabbit IgG (PeproTech, Rocky Hill, NJ) or Mouse IgG1 kappa, (clone: P3.6.2.8.1, Thermo Fisher Scientific, Waltham, MA), were carried out. 20 ul of Pierce Protein A/G Agarose beads (Thermo Fisher Scientific, Waltham, MA) was added to each reaction, and the mixture was incubated for 1 h at 4 °C. Agarose beads were pelleted at 1000×g for 5 min at 4 °C, supernatant was removed, 1 ml of ice-cold lysis buffer was added, and pellet was washed by inverting tube several times. Beads were washed 4 times, each time repeating the centrifugation step above. After the final wash, pelleted beads were resuspended in 40 μl of 1×Laemml sample buffer (Bio-Rad, Hercules, CA) and boiled for 5 min. The samples were then centrifuged to pellet the agarose beads followed by SDS-PAGE analysis of the supernatant. Fifteen μl of samples for JADE1 detection and 5 μl for tau isoform ladders (rPeptide, Watkinsville, GA) were run on 10% PROTEAN TGX Pre-cast Gels (Bio-Rad, Hercules, CA), blotted to nitrocellulose membranes, and stained with JADE1 antisera (1:2000), total tau antisera (HT7, 1:3000; Thermo Fisher Scientific), three microtubule repeat domain tau antisera (3R, 8E6/C11, 1:2000; MilliporeSigma, St. Louis, MO), four microtubule binding domain repeat tau antisera (4R, 1:2000; Cosmo Bio, Carlsvad, CA), pThr231 tau antisera (RZ3, 1:200; a gift from Dr. Peter Davies), pSer396/pSer404 tau antisera (PHF1, 1:500; a gift from Dr. Peter Davies), pSer202 tau antisera (CP13,
1:500; a gift from Dr. Peter Davies), pSer202/pThr205 tau antisera (AT8, 1:1000; Thermo Fisher Scientific), pSer214 tau antisera (S214; 44-742G, 1:1000; Thermo Fisher Scientific, Waltham, MA), or pSer409 tau antisera (PG5, 1:200; a gift from Dr. Peter Davies). Horseradish peroxidase-labeled conformation-sensitive secondary anti-mouse IgG for IP or anti-rabbit VeriBlot for IP Detection antibody (both at 1:20,000; Abcam, Cambridge, MA) was detected by SuperSignal West Femto Maximum Sensitivity substrate (Thermo Fisher Scientific).

A dephosphorylation assay was performed using fresh-frozen brain tissue homogenized with a glass-Teflon homogenizer at 500 rpm in 10 volumes (wt/vol) of ice-cold lysis buffer containing 50 mM Tris, pH 7.8, 0.5% NP40, 150 mM NaCl, and Halt protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA). Samples were incubated on ice for 30 min, centrifuged at 1000 ×g for 10 min, and supernatant was collected. Reaction mixtures (51 µl) consisted of 39 µl of supernatant, 1 µl of protease inhibitor cocktail, 5 µl of 10X NEBuffer for protein metallophosphatases, 5 µl of 10 mM MnCl2, 1 µl of lambda protein phosphatase (New England BioLabs, Ipswich, MA). Each mixture was incubated at 30 °C for either 1, 2, 3, or 4 h. Additional 1 µl of lambda protein phosphatase and 1 µl of protease inhibitor cocktail were added in each mixture every hour.

Proximity ligation assay

5 µm-thick FFPE hippocampal sections were mounted on charged slides and stained using a Duolink kit (Millipore-Sigma, St. Louis, MO). Sections were deparaffinized and incubated in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) at 95 °C for 20 min, washed in running water, incubated in 0.2% Tween 20 in PBS at room temperature for 20 min, and washed in PBS 3 times for 5 min. Blocking assays were performed using Duolink In Situ Red Starter Kit Mouse/Rabbit (MilliporeSigma, St. Louis, MO) according to the manufacturer’s protocol with JADE1 antisera (1:20) and 0N tau antisera (1:500; BioLegend, San Diego, CA). Two control assays were also performed, one with JADE1 antisera only, and the other with anti-0N tau antisera. All samples were counterstained with 4’,6-diamidino-2-phenylindole (DAPI). Sections were imaged using an Axioview fluorescent microscope (Carl Zeiss, Oberkochen, Germany) and processed using Zen Blue software v3.4 (Carl Zeiss).

In vivo Drosophila model

Drosophila stocks, crosses, and aging were performed at 25 °C for the duration of the experiment and an equal number of male and female flies were used. The GAL4-UAS expression system and the pan-neuronal elav-GAL4 driver were used to control transgenic gene expression. Analyses were run on four fly groups (Bloomington stock rnoRNAi line number 57774): elav-GAL4 driver in the heterozygous state (control, elav-GAL4/+), elav-GAL4 positive plus rnoRNAi positive group (control + rnoRNAi, elav-GAL4/+;UAS-rnoRNAi/+), elav-GAL4 positive transgenic human UAS-tauR406W tau group (0N4R Tau, elav-GAL4/+;UAS-TauR406W), elav-GAL4 positive human transgenic UAS-tauR406W tau plus rnoRNAi positive group (0N4R Tau + rnoRNAi, elav-GAL4/+;UAS-TauR406W/UAS-rnoRNAi). An additional rnoRNAi line was tested but did not produce progeny when crossed to tau transgenic Drosophila, as often occurs with strong enhancers (Bloomington stock rnoRNAi line number 62880). Flies were aged to 10 d, at which point terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay was performed in the brain (n = 6 per genotype) and a blinded assessment of the fly eye phenotype was performed (n = 16 per genotype). TUNEL was performed on 4 µm FFPE fly heads. Quantification of TUNEL-positive nuclei was performed throughout the entire brain using DAB-based detection and bright field microscopy and representative images were taken in the fly cortex. Fly eye phenotype scoring was performed blindly using light microscopy. The blinded rater semi-quantitatively evaluated the eye for four distinct qualities including roughness, size, shape and conical shape on a 1–5 scale (five being the most severe phenotype) and an average summary score was calculated. Representative fly eye images were taken using a scanning electron microscope (JEOL, Peabody, MA) using an accelerating voltage of 20 kV.

Statistical analysis

For GWAS, statistical analysis was performed in PLINK and our genome-wide significance value was < 5 × 10–8, which is Bonferroni-corrected for all the independent common SNPs across the human genome. Genome-wide suggestive significant value was set at < 5 × 10–6. All other statistical analyses were performed in R v4.0. For non-normally distributed data a Wilcoxon test was used to test for significance, and an ANOVA was used for normally distributed data.

Results

We assembled a cohort of aged individuals to enable our genetic study of primary age-related tauopathy (PART) in which all were neuropathologically confirmed to be devoid of neuritic plaques. Brain tissue samples were obtained from 21 biorepositories (n = 857). While each center had performed a comprehensive neurodegeneration workup, we restated and reassessed these cases as a component of our ongoing histological studies [97]. Because some subjects...
Upon restaining were found to have a mild neuritic plaque burden (CERAD 0), the final number of subjects in the study was reduced to 647. Neurofibrillary tangles (NFTs) were assessed using the Braak staging system, ranging from 0 to IV with all stages well represented, as is consistent with PART (Table 1). It should be noted that many Braak stage 0 subjects were observed to have rare tau pathology when assessed using immunohistochemistry which is compatible with that stage. The average age of death was 83 years, and the number of male and females was approximately equal. Braak stage II was the most frequent (n = 189), with relatively equal number of donors with Braak stages III (n = 152) and I (n = 142), and the fewest with Braak stages 0 (n = 71) and IV (n = 93). Braak staging performed at each center was not disproportionally skewed (Supplementary Fig. 2a, online resource). Sixty-six percent of the donors were cognitively normal. However, amongst Braak stages I–IV, there was an equal number of cognitively impaired subjects across each stage. We evaluated the association of age at death with Braak stage with respect to cognitive status and found a positive correlation that does not significantly change when cognitive status is accounted for in the model (Supplementary Fig. 3a, online resource). Comorbid cerebrovascular disease, which is common in this cohort, is likely responsible for the variation in cognitive impairment [39]. In summary, our cohort consists of older individuals, with a range of dementia severity, restricted regional tau distribution and varying severity of burden, demonstrating the diversity of both the clinical and neuropathological features of the condition.

Using this cohort, we ran a quantitative trait association analysis across the entire genome to characterize genetic risk for PART. Our model adjusted for age, sex, genotyping platform, and principal components 1–4 and produced an inflation λ of 1.04 (Supplementary Fig. 3a, online resource). Using Braak stage as a quantitative trait revealed suggestive signals in 14 loci and a nominally genome-wide significant signal on chromosome 4q28.2 (Fig. 1a, rs56405341; linear regression β = 0.35, standard error = 0.06, p = 4.82 × 10−8, Table 2, Supplementary Table 3, online resource). The genome-wide significant variant (rs56405341) has a minor allele frequency of 0.27. Assessment of this region indicated this SNP is not within the footprint of any specific gene, but is near C4orf33, SCLT1 and JADE1 (Fig. 1b), and in high linkage disequilibrium with 22 other SNPs (r² > 0.8, Supplementary table 4, online resource). Further examination of the homozygous and heterozygous alleles using strip chart showed a significant relationship between higher Braak stage and homozygous minor allele carriers (Fig. 1c, AA–AG p = 0.024, AA–GG p = 3.3 × 10−5, AG–GG p = 7.2 × 10−5). Analysis of data from each independent genotyping array separately confirmed that the signal was apparent in both subsets of the data (Infinium OmniExpress-24, n = 440, β = 0.27, SE = 0.05, p = 1.11 × 10−6, λ = 1.03, Global screening array n = 207, β = 0.20, SE = 0.08, p = 1.42 × 10−2, λ = 1.01, Supplementary Table 2 and Fig. 5a–d, online resource). The individual summary statistics derived from the separate chip analysis were then combined to run a meta-analysis, and the resulting p value was similar to the combined analyses, as well as in agreement in the direction of effect tested allele (p = 5.61 × 10−8).

Replication in an independent cohort proved challenging given the lack of data sets containing similarly neuropathologically ascertained individuals with PART. To our knowledge, the only available autopsy based study performed in a cohort of AD subjects using Braak as a quantitative trait identified two other SNPs (rs4975209 R² = 0.0072, D’ = 0.0967 and rs10009321 R² = 0.0091, D’ = 0.1061) in a region distant (≈ 150,000 bp) from 4q28.2, and consequently these SNPs are not in linkage disequilibrium with our lead SNP rs56405341 [6]. Another case/control AD GWAS used a cerebral spinal fluid (CSF) Aβ42/40 ratio threshold to dichotomize their study into 2 groups (normal and abnormal Aβ42/40 ratios) and found another unique SNP in the region (rs13129839) which was genome-wide suggestive (p = 9.0 × 10−6), had a positive (protective) odds ratio (OR = 0.043), and was in high R² and D’ with our lead as well as supporting SNPs (> 0.8 and 0.1, respectively) [37]. It should be noted the Aβ42/40 ratio in the CSF is highly relevant in the context of PART given Aβ42/40 Ratios can be used as a proxy for lower levels of brain amyloid levels which is

| Braak stage | n   | Average age of death in years (range) | Male (%) | Female (%) | Normal (%) | MCI (%) | Dementia (%)* |
|-------------|-----|--------------------------------------|----------|------------|------------|---------|---------------|
| 0           | 71  | 71.2 (51–104)                        | 48 (7.5) | 23 (3.5)   | 58 (8.9)   | 7 (1.0) | 6 (0.9)       |
| I           | 142 | 78.1 (53–108)                        | 88 (13.6) | 54 (8.3)   | 99 (15.3)  | 17 (2.6) | 22 (3.4)      |
| II          | 189 | 84.1 (51–107)                        | 98 (15.1) | 91 (14.0)  | 126 (19.4) | 40 (6.1) | 21 (3.2)      |
| III         | 152 | 88.8 (68–105)                        | 59 (9.1)  | 93 (14.8)  | 96 (14.8)  | 29 (4.4) | 26 (4.0)      |
| IV          | 93  | 91.2 (67–106)                        | 29 (4.4)  | 64 (9.8)   | 50 (7.7)   | 22 (3.4) | 21 (3.2)      |
| Total (647) | 647 | 83.5 (51–108)                        | 322 (49.7) | 325 (50.2) | 429 (66.3) | 115 (17.7) | 96 (14.8)     |

*7 samples did not have cognitive status available, MCI mild cognitive impairment
observed in PART. Taken together, these two independent genetic studies show signals near the 4q28.2 loci, with the latter using the same quantitative trait described here (Braak stage), and the former accounting for amyloidosis but lacking a measurement of tau burden.

We then examined candidate SNPs previously found to be associated with AD and progressive supranuclear palsy (PSP) in prior GWAS studies to explore convergent and divergent genetic risk (Table 3, Supplementary table 5, online resource). Of the 52 candidates investigated, we found five also suggestively associated with Braak stage in PART. rs12590654, which is in the SLC24A4 locus, had the highest significance level ($p = 0.001$). rs1582763, rs2081545, rs7935829, within the MS4A6A locus ($p = 0.01, 0.01, 0.02$, respectively). The remaining AD SNP, rs7657553, was within HS3ST1 ($p = 0.02$) locus. We found two variants that overlapped with PSP risk: rs242557 ($p = 0.02$) in the MAPT locus, and rs7571971 ($p = 0.03$) in the EIF2AK3 locus. In summary, seven of the 52 probed risk AD and PSP SNPs showed significant associations ($p < 0.05$) in PART; however, they do not survive multiple test corrections.
Next, we refocused on the nominal association at the 4q28.2 locus. While the strength of this association is relatively weak, it passed the most conservative Bonferroni threshold for genome-wide significance; therefore, we investigated the 4q28.2 locus as previous studies have shown that many nominal associations are replicated in subsequent studies [74]. Examination of RNA expression quantitative trait loci (eQTL) using the Religious Orders Study (ROS) and Memory and Aging Project (MAP) data yielded a significant lead SNP (rs56405341) eQTL ($p = 0.038$) for \textit{JADE1} (Fig. 2a) [25, 88]. As our GWAS quantitative trait was specific to tau pathology and our SNP eQTL was only modestly significant, we then examined mRNA expression levels of genes in the locus using a novel single-cell soma RNA sequencing data set which measured transcriptomic changes specifically in tangle-bearing neurons and non-tangle-bearing neurons (Fig. 2b–d). Using this data set, we first looked at the overall change in \textit{JADE1} expression and found that tangle-bearing excitatory neurons significantly differentially upregulated \textit{JADE1} compared to non-tangle-bearing excitatory neurons ($p = 1.04 \times 10^{-61}$). Conversely \textit{C4orf33} and \textit{SCLT1}, the other genes in the locus, had low levels of expression regardless of cell type and tangle status. Given that the cell-type composition is slightly different in each type of neuronal group, we then looked at individual cell subclusters and observed two subpopulations of excitatory neurons in which \textit{JADE1} was significantly differentially expressed (adjusted $p = 4.55 \times 10^{-15}$, $7.82 \times 10^{-8}$). We did not observe differences in \textit{JADE1} expression in the remaining neuronal populations due to low cell numbers which decreased our statistical power (Supplemental Fig. 2a). We also examined the relative average expression and percentage of cells expressing \textit{JADE1} and observed increases in both metrics in tangle-containing neuronal populations compared to non-tangle-containing neuronal populations (Fig. 2e–g). \textit{SCLT1} and \textit{C4orf33} had negligible expression levels in a substantially smaller percentage of neurons (Supplemental Fig. 5b, c). Taken together, these data suggest

### Table 2

| Chr | Base pair | SNP   | Genes                          | A1  | Beta  | SE  | L95  | U95  | $t$ statistic | $p$  |
|-----|-----------|-------|--------------------------------|-----|-------|-----|------|-----|---------------|------|
| 2   | 11,935,322| rs78580932 | GREB1, NTSR2, LPIN1, MIR4262 | C   | -0.54 | 0.10 | -0.76 | -0.34 | -5.15        | 3.56E-07 |
| 2   | 235,768,810| rs74600760 | ARL4C, SH3BP4                | T   | -0.53 | 0.11 | -0.74 | -0.32 | -4.86        | 1.46E-06 |
| 3   | 61,518,166| rs111610565 | FHT1, PTPRG                  | C   | -0.49 | 0.10 | -0.70 | -0.30 | -4.82        | 1.81E-06 |
| 3   | 140,216,029| rs349509 | CLSTN2, CLSTN2-AS1, TRIM42    | A   | -0.77 | 0.16 | -1.08 | -0.47 | -4.97        | 8.64E-07 |
| 3   | 179,019,705| rs13323081 | PIK3CA, KCMNBS, ZNF639, MFN1  | G   | 0.24  | 0.05 | 0.15  | 0.33  | 5.17         | 3.16E-07 |
| 4   | 130,085,480| rs56405341 | C4orf33, SCLT1, JADE1        | A   | 0.25  | 0.05 | 0.16  | 0.35  | 5.52         | 4.82E-08 |
| 4   | 137,329,297| rs77506227 | –                              | A   | -0.79 | 0.16 | -1.12 | -0.48 | -4.88        | 1.34E-06 |
| 5   | 150,473,104| rs6579838  | GPX3, TNIP1, ANXA6            | C   | 0.23  | 0.05 | 0.14  | 0.32  | 5.10         | 4.57E-07 |
| 10  | 124,487,608| rs150945906 | DMBT1, C1orf120, DMBT1P1      | A   | -0.64 | 0.14 | -0.91 | -0.38 | -4.77        | 2.31E-06 |
| 15  | 24,515,682| rs147462127 | PWRN2, PWRN1                  | T   | -0.84 | 0.17 | -1.18 | -0.51 | -4.98        | 8.37E-07 |
| 15  | 36,865,219| rs185845364 | C15orf41, CSNK1A1P1           | G   | -0.79 | 0.16 | -1.11 | -0.48 | -4.94        | 9.79E-07 |
| 17  | 1,879,293 | rs12946465 | RPA1, RTN4RL1, DPH1, OVCA2    | C   | -0.21 | 0.05 | -0.31 | -0.13 | -4.75        | 2.51E-06 |
| 19  | 45,719,790| rs10415392 | TRAPP6A, BLOC135, EXO3L2, MARK4 | T  | 0.34  | 0.07 | 0.21  | 0.48  | 4.85         | 1.59E-06 |
| 20  | 56,754,110| rs78923929 | C20orf85, PPP4R1L, RAB22A     | A   | -0.65 | 0.13 | -0.92 | -0.40 | -4.95        | 9.76E-07 |

### Table 3

| Associated disease | Chr | Base pair | SNP   | Gene | A1  | Beta  | SE  | L95  | U95  | $t$ statistic | $p$  |
|--------------------|-----|-----------|-------|------|-----|-------|-----|------|-----|---------------|------|
| AD                 | 14  | 92,938,855| rs12590654 | SLC24A4 | A   | -0.14 | 0.044 | -0.23 | -0.05 | -3.19        | 0.0015 |
| AD                 | 11  | 60,021,948| rs1582763  | MS4A6A  | A   | -0.11 | 0.044 | -0.19 | -0.02 | -2.47        | 0.0138 |
| AD                 | 11  | 59,958,380| rs2081545  | MS4A6A  | A   | -0.10 | 0.044 | -0.19 | -0.02 | -2.36        | 0.0185 |
| AD                 | 4   | 11,723,235| rs7657553  | H3ST1   | A   | 0.11  | 0.048 | 0.01  | 0.20  | 2.24         | 0.0255 |
| PSP                | 17  | 44,019,712| rs242557   | MAPT    | A   | 0.10  | 0.047 | 0.01  | 0.20  | 2.24         | 0.0256 |
| AD                 | 11  | 59,942,815| rs7935829  | MS4A6A  | G   | -0.10 | 0.043 | -0.18 | -0.01 | -2.21        | 0.0274 |
| PSP                | 2   | 88,895,351| rs7571971  | EIF2AK3 | T   | -0.10 | 0.047 | -0.19 | -0.01 | -2.09        | 0.0373 |

AD = Alzheimer disease, PSP = progressive supranuclear palsy, Chr = chromosome, SNP = single nucleotide polymorphism

Genome-wide significant difference in bold, $chr$ = chromosome, SNP = single nucleotide polymorphism, SE = standard error
Fig. 2 Bulk and single-cell sequencing reveals JADE1 mRNA is modulated by rs56405341 and upregulated in tangle-bearing neurons. a Bulk RNA sequencing data yielded a significant lead SNP (rs56405341) eQTL ($p = 0.038$) in the dorsolateral prefrontal cortex of 452 postmortem human brain samples. Expression of JADE1 decreases with minor allele. Single cell sequencing data of populations of neurons with and without neurofibrillary tangles isolated from human post-mortem brain samples separated using fluorescence-activated cell sorting and transcriptomic profiles from single-cell RNA-sequencing were subsequently analyzed. 

b In 2 unique excitatory neuronal populations (Ex1 and Ex2) JADE1 mRNA was significantly differentially expressed in the tangle-bearing neurons (adjusted $p = 7.82 \times 10^{-8}$, $4.55 \times 10^{-15}$); in comparing the overall population of tangle-bearing excitatory neurons (Ex-total) to non-tangle bearing neurons the difference is highly significant (adjusted $p = 1.04 \times 10^{-61}$). 

c, d Other two genes in the locus, C4orf33 and SCLT1, were overall nominally expressed in both excitatory neuronal groups, as well as subclusters (Supplementary Fig. 5 b, c, online resource). e Dot plot showing average relative expression and percent expression of the candidate genes in the locus. Both JADE1 relative average expression and percentage of cells expressed was higher than C4orf33 and SCLT1. f, g t-Distributed stochastic neighbor embedding (tSNE) plots showing the different populations neurons, tangle bearing status, and relative expression of JADE1 in neuronal subpopulations.
increased $JADE1$ mRNA expression is a characteristic of tangle-bearing neurons.

Given our evidence of a genetic signal in the 4q28.2 locus, and single cell transcriptomic data indicating modulation of $JADE1$ expression in neurons with NFT pathology, we conducted an immunohistochemical study using specific antisera to $JADE1$ in our collection of post-mortem tauopathy brain tissue (Fig. 3). We assessed tauopathies that are known to involve preferentially tau isoforms with 3 microtubule-binding domain repeats (3R), 4 microtubule-binding domain repeats (4R) or a mixture of the two [50]. We found strong and specific $JADE1$ immunopositivity in structures

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**Fig. 3** Selective immunolabeling of tau aggregates containing tau with four microtubule-binding domain repeats (4R), but not three (3R), in post-mortem human tauopathy brains with antisera targeting $JADE1$ protein. Immunohistochemical staining with phospho-tau (p-tau) specific antisera (AT8) and $JADE1$ specific antisera demonstrates neurofibrillary tangle (NFT) formation marked by the presence of $JADE1$ in specific populations of neurons and glia. 

- **a, b** Primary age-related tauopathy (PART, $n=3$) NFTs contain $JADE1$ positive staining in the soma and neurites in the entorhinal cortex.
- **c, d** Alzheimer disease (AD, $n=3$) individual with Aβ and AT8-positive neuritic plaques and NFTs (subiculum) also display $JADE1$ immunopositivity in dystrophic neurites and NFTs.
- **e, f** Chronic traumatic encephalopathy (CTE, $n=3$) contains positive p-tau staining around a blood vessel in the depth of a neocortical sulcus that is immunopositive for $JADE1$.
- **g, h** AT8 positive tufted astrocytes, oligodendroglial coiled bodies, and NFTs are positive in the subthalamic nucleus in an individual with progressive supranuclear palsy (PSP, $n=3$) which are also in immunopositive for $JADE1$.
- **i, j** Astrocytic plaques in corticobasal degeneration (CBD, $n=3$) and extensive thread-like pathology positive for p-tau and $JADE1$ in the neocortex.
- **k, l** In the cornu ammonis 1 (CA1) sector in an individual with argyrophilic grain disease (AGD, $n=3$), abundant grains that are immunopositive for p-tau and $JADE1$ are evident.
- **m, n** Pick disease (PiD), a 3R tauopathy, with Pick bodies in the dentate gyrus that are immunopositive for p-tau but negative for $JADE1$.
- **o, p** Double staining of a PART entorhinal cortex showing the absence of $JADE1$ (brown) staining in early pre-tangles, but the presence of p-tau (pink, see inset).
- **Peptide competition** Negative control using a peptide competition despite the presence of a tangle (inset). Scale bar, 100 µm.
morphologically indicative of mature aggregate containing intracellular NFT in not only PART, but also the other mixed 3R/4R tauopathies (i.e., AD and chronic traumatic encephalopathy, Fig. 3a–f). In the 4R tauopathies (PSP, corticobasal degeneration, and amyrophic lateral sclerosis), NFT were also immunopositive for JADE1 (Fig. 3g–l). Notably, gliofibrillary pathology in these diseases (i.e., aging-related tau astrogliopathy, tufted astrocytes, and astrocytic plaques) were also immunopositive. Surprisingly, no signal was detected in Pick bodies in Pick disease (PiD), a predominately 3R tauopathy (Fig. 3m, n). Double-labeling experiments showed that early pre-NFT that consist predominantly of prefibrillary soluble oligomers were negative for JADE1 suggesting that this factor begins to coalesce into NFT at the transition to the aggregate stage (Fig. 3o). To ensure the antibody was specifically targeting the JADE1 protein rather than binding non-specifically to NFT, we added blocking peptides with the JADE1 antibody and did not observe any staining (Fig. 3p). These findings indicate that JADE1 protein expression is localized specifically in mature NFTs. Furthermore, translocation of JADE1 to NFTs does not occur in PiD, the only 3R tauopathy examined, suggesting selective tau isoform interactions.

We then biochemically examined JADE1 protein expression by immunoblot using lysates derived from the entorhinal cortex and hippocampus proper (cornu ammonis 1–4 and dentate gyrus) of PART and AD individuals. JADE1 exists as 2 isoforms, JADE1 long (JADE1L) and JADE1 short (JADE1S), both of which contain proline, glutamic acid, serine, threonine (PEST) domains and 2 PHD fingers. However, the long form is 333 amino acids longer and contains an additional PEST domain as well as a nuclear localization signal (Fig. 4a). Immunoblot analysis revealed expression of JADE1S in both brain regions and diseases, but no bands were observed at the expected molecular weight for JADE1L, indicating the signal observed in IHC may be specific to the short isoform of JADE1 (Fig. 4b).

Cytoplasmic colocalization of JADE1 with NFTs immunohistochemically raises the possibility that they form a functional complex in tauopathy brains. To examine this, co-immunoprecipitation experiments were conducted using postmortem brain lysate from individuals with PART. We first immunoprecipitated JADE1 and then performed immunoblot using antisera to tau and observed a ~45 kDa isoform that corresponds to 0N4R (Fig. 4c). We then reverse co-immunoprecipitated using 0N tau antisera and observed a ~50 kDa single band when probing with our JADE1 antisera (Fig. 4d). We treated our pellets with protein phosphatase and observed a slight band shift (Fig. 4e), indicating the JADE1 bound to tau is phosphorylated. Immunoblotting isoform specific anti-tau antisera (3R and 4R tau) confirmed that the coimmunoprecipitated tau was predominately 4R, thus 0N4R (Fig. 4f, g). To further validate our findings, we repeated the same coimmunoprecipitation experiments using a non-commercially available JADE1S-specific antibody raised against a different epitope and observed the same results [106]. We then ran immunoblots on the immunoprecipitated JADE1 that we probed using a panel of phospho-tau site-specific antibodies. The strongest signals relative to the input were with pThr231 (RZ3) and pSer409 (PG5), of which both have been reported to mark pre- and intercellular tangles, but not mature tangles [5] (Fig. 4h). Finally, to confirm the interaction of JADE1 and tau, proximity–ligation assays (PLA) were performed in fixed hippocampus from PART individuals. JADE1 and tau antibodies were labeled with oligonucleotides. The red fluorescent signal indicates the close proximity (maximum 30–40 nm) of these two proteins (Fig. 4i). Controls did not show signal (Supplementary Fig. 7e, f, online resource). Therefore, JADE1S likely forms a complex with 0N4R tau in tangle bearing neurons.

Finally, given the association between JADE1 and 0N4R tau, we asked whether JADE1 plays a functional role in tau pathology. We used a Drosophila model that overexpresses human mutant 0N4R tau [100] as well as RNAi-mediated reduction of rhinoceros (rno), the highest matched JADE1 Drosophila ortholog. We blindly evaluated the fly eye phenotype using a semi-quantitative assessment of size, roughness, overall shape, and conical shape and observed a significant increase in severity between tau transgenic Drosophila and tau transgenic Drosophila with rno knockdown (Fig. 5a–e, p = 8.7 × 10^{-5}). We did not observe significant differences between rnoRNAi and controls in the absence of transgenic tau. To assess toxicity, we quantified TUNEL-positive cells throughout the Drosophila brain. We find that rno knockdown significantly enhances neurotoxicity in tau transgenic Drosophila but not controls based on TUNEL staining at day 10 of adulthood (p = 0.008, Fig. 5f–i). These data provide in vivo evidence that JADE1/rno loss plays a mechanistic role in promoting neurotoxicity in tauopathy and suggest that proper functioning of JADE1/rno is protective.

**Discussion**

Genome-wide association studies (GWAS) have enabled advances in our understanding of sporadic tauopathies [17, 35, 42, 49, 81, 95, 102]. Yet, in the context of the growing numbers of genes associated with Alzheimer disease (AD) [44], direct links with tau proteinopathy have been challenging to pinpoint as association signals show minimal overlap with factors classically implicated in tauopathy (e.g., proteostasis, tau protein kinases, prions, etc.) [34, 50, 59]. This is not surprising given the ubiquity and heterogeneity of tau and other pathological changes in the aging human brain that are variably associated with cognitive impairment [63].
While dementia is the phenotype focused on in most genetic studies, it is non-specific. Here, we performed an autopsy-based neuropathology-based GWAS, which minimizes classification errors and other issues, assembling the largest cohort of post-mortem brain tissues from aged individuals devoid of Aβ neuritic plaque pathology with a goal of identifying factors independently associated with primary age-related tauopathy (PART). In doing so, we sought to obtain genetic evidence that might clarify the controversial relationship between PART and AD, which are neuropathologically
Despite its strong association with AD [4, 44, 52, 54, 58], locus JADE1, homolog rhinoceros (rno) expressed its transcript with sporadic late-onset AD and replicated the consistency of PART but occurs in AD and all tauopathies with specific anti-tau antisera are the 0N4R isoform and not the 3R isoform. Co-immunoprecipitated tau was positively stained with a panel of phospho-tau specific antibodies with the most signal coming from pThr231 (RZ3), pSer396/pSer404 (PHF-1), pSer214 (S214), and pSer409 (PG5). Proximity–ligation assay showing positive fluorescence signal (red) around the nucleus of neurons (blue) indicating the close association between JADE1 and 0N tau detected using the corresponding two primary antibodies in the soma (inset). Scale bar, 20 μm.

Similar. We failed to find an association with APOE ε4, the strongest common risk allele for sporadic late-onset AD, but did observe modest signals in other candidate risk loci. We also found a novel, albeit nominal, genome-wide significant association at the chromosome 4q28.2 locus. Our data indicate that among genes in this locus, only the gene for apoptosis and differentiation in epithelia 1 (JADE1), a member of a small family of multifunctional adaptor proteins implicated in renal and other cancers [19, 104–106], is upregulated in tangle-bearing neurons at both the mRNA and protein levels. This accumulation of JADE1 protein in NFT is not specific to PART but occurs in AD and all tauopathies with accumulation of 4R isoforms, but not in Pick disease, a 3R tauopathy. We also show that JADE1 binds 0N4R tau, an isoform proposed to be a critical driver of tau pathology [86, 98]. Finally, experiments in Drosophila show that reducing expression of the JADE1 homolog rhinoceros (rno) exacerbates tau-induced neurotoxicity in vivo. Together, these findings strongly argue that JADE1 is a factor broadly capable of protecting neurons from neurofibrillary cell death that links PART to the tauopathic component of AD.

We confirm that the genetics of PART has a partial overlap with sporadic late-onset AD and replicated the consistent finding showing the lack of a signal in the APOE locus despite its strong association with AD [4, 44, 52, 54, 58]. It has been shown previously that individuals with PART have a higher APOE ε2 allele frequency which distinguishes PART from AD both neuropathologically and genetically [41, 45, 79]. We have reported that the frequency of the APOE ε4 allele is lower in PART [83] and other studies have found similar results in independent cohorts [7, 61]. It should be noted that these values can fail to reach significance when comparing groups with varying degrees of neuritic plaque pathology [9, 61]. Recent studies in mice and humans have indicated that the APOE ε4 allele may exacerbate tau pathology independently of Aβ plaques [87], while other human studies failed to show an interaction [1, 30]. These results reinforce prior evidence that PART occurs independently of APOE ε4 irrespective of Aβ plaque pathology. Alternatively, given the higher APOE ε2 and lower APOE ε4 allele frequency observed in PART, this enrichment could suggest a functional epistatic interaction with JADE1 and APOE which also provides context to the lack of signal in the observed in the 4q28.2 locus in other large AD GWAS studies.

17q21.31 MAPT locus is the strongest genetic risk factor for PSP [35], which we and others had previously reported is associated with PART [43, 83]. The MAPT H1 haplotype has also been associated with AD [66, 82, 93]. However, this region has a complex haplotype structure and may be more important in specific AD subgroups given the modest signal and variable findings in these association studies [11, 92]. Intriguingly, in one AD GWAS using clinically ascertained individuals, removal of APOE ε4 carriers enhanced signals in the 17q21.31 locus [46]. In the present study, there was only a modest association of MAPT with PART. This result may stem from differences in cohort selection in case–control studies focusing on pathological extremes, while our study design included a range of pathological severity, specifically including mild to moderately affected individuals. Together, these data highlight that further investigation of the role of 17q21.31 MAPT locus in PART is warranted.

Beyond APOE and MAPT, we found four additional association signals in PART that overlap with either AD or PSP. Eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3) encodes an endoplasmic reticulum (ER) membrane protein critical for the unfolded protein response (UPR) [91, 94]. Activation of the UPR has been observed and positively correlated with tau pathology, but not with Aβ plaque burden, in the hippocampus of aged cognitively normal individuals [94]. Solute carrier family 24 member 4 (SLC24A4), a gene in the locus most strongly associated with PART and AD, is a member of the potassium-dependent sodium/calcium exchanger protein family and is involved in neural development; however, little is known about its possible function in AD [55, 103]. We identified an association of PART with the membrane spanning 4-domains A6A (MS4A6A) locus, which contains the binding regions for the transcription factor PU.1 which is selectively expressed in brain microglia and macrophages [29]. The last overlapping genetic locus contains heparan sulfate-glucosamine 3-sulfotransferase 1 (HS3ST1), which has been suggested to modulate heparan sulfate proteoglycans as receptors for the spreading of tau [36, 54]. Taken together, these data are compatible with the hypothesis that the candidate genes
we identified in our GWAS are possible modulators of tau pathology.

We identified a novel locus on chromosome 4q28.2 associated with PART pathology. This locus was previously associated with Braak NFT stage in an AD autopsy GWAS, as well as a second GWAS focusing on dichotomized CSF Aβ positivity [6, 37]. This prompted us to focus on this locus for further validation and functional studies. Because we only identified a nominally significant expression quantitative trait locus signal to \( JADE1 \), and given that our trait was tangle-specific, we reasoned that modulation of mRNA expression of genes in the locus might also be cell-type specific. This hypothesis was also motivated by the increase in genetic to transcriptomic associations found in cell specific populations in other contexts [47, 56, 107]. Our results indicate that of the genes in the 4q28.2 locus, only \( JADE1 \) mRNA was significantly and differentially expressed in tangle-bearing neurons. Our immunohistochemical studies revealed \( JADE1 \) protein accumulation in both neuronal and glial tau-immunopositive cells, validating these findings.

Thus, \( JADE1 \) is most likely responsible for the GWAS signal at this locus; however, further genetic and expressions studies on perturbated cell populations are required in the future to validate these findings.

Our immunohistochemical studies indicate that \( JADE1 \) is potentially involved broadly in 4R and mixed 3R/4R tauopathies. We observed immunopositivity not only in PART tangles, but also in tangles of tauopathies with aggregates containing 4R tau and in mixed tauopathies with aggregates containing both 3R and 4R tau. The absence of staining in Pick disease, the only tauopathy with 3R tau aggregates examined, was surprising. Our biochemical studies suggest that \( JADE1 \) protein specifically interacts with 0N4R tau that is phosphorylated on epitopes known to be hyperphosphorylated in NFT. Our proximity ligation assay confirms the direct interaction between \( JADE1 \) protein and tau. Studies using cryo-EM and mass spectrometry have shown the ultrastructure of tau aggregates at unprecedented resolution, and it has been reported that 0N4R has a unique single β-sheet conformation for the fibril core [27, 32, 84]. Intriguingly,
recent mass spectrometry profiling studies of human post-mortem brain tissues have suggested that changes in 0N4R tau isoform specifically is an early event in tauopathy [98]. Double-labeling experiments indicate that JADE1 increases shortly after the pre-tangle stage, accumulating alongside insoluble tau aggregates during the transition to the intracellular tangle stage possibly reflecting a reactive/protective compensatory role [14].

Our findings provide clues as to how JADE1 may be functioning in tauopathy. JADE1S protein has been previously implicated as a renal tumor suppressor involved in apoptosis, an inhibitor of Wnt signaling by ubiquitylating β-catenin, and an inhibitor of pro-survival kinase AKT and interacting with cell cycle regulators [12, 13, 19, 75, 90, 105]. Of the two JADE1 isoforms, the short form lacks a nuclear localization signal, consistent with the cytoplasmic localization of the protein we observed by immunohistochemistry. This prompted us to hypothesize that JADE1S functions in the cytoplasm to modulate tauopathy. Our in vivo studies in which we reduced rno (the closest JADE1 ortholog) levels in Drosophila overexpressing mutant human 0N4R tau significantly enhanced the tau-induced rough eye phenotype as well as TUNEL-positive cells, a marker of apoptotic DNA fragmentation. While JADE1 has been shown to promote apoptosis in some contexts, RNAi knockdown experiments suggest that proper functioning of JADE1 may be neuroprotective. Other studies have demonstrated that loss of rno function attenuates apoptosis in Drosophila [96]. Because previous studies have found JADE1 to be stabilized by the von Hippel–Lindau tumor suppressor which is a component of an E3 ubiquitin–protein ligase activity and JADE1 is itself a single-subunit ubiquitin ligase for beta-catenin, JADE1 may be working with 0N4R tau through a similar mechanism to promote ubiquitin-mediated clearance of tau [57, 89, 106].

Limitations of our study include small sample size for GWAS standards; however, it should be noted that it is still the largest study of its kind and limited by the availability of tissue that meet our diagnostic criteria for PART, recognizing that PART is currently only reliably diagnosed postmortem. To amass sufficient donors, our study relies on neuropathological assessments performed at multiple centers that could introduce batch effects (although none were detected). In addition, we were only able to identify a modestly significant eQTL signal between our lead SNP and JADE1 mRNA expression. However, our analysis of perturbed single soma RNA sequencing stratified by the presence or absence of NFTs allude to the fact that our lead SNP could be modulating JADE1 expression specifically in tangle containing neurons. Our attempt to replicate our findings only identified candidate SNPs ($p < 5 \times 10^{-8}, > 5 \times 10^{-6}$) which were not in strong linkage disequilibrium with our lead SNP. We hypothesize this is likely a product of these studies containing subjects with amyloid pathology, and for these reasons future replication is required to validate our findings. Finally, while Braak staging is highly reproducible across neuropathologists and institutions, with one report showing that across brains and raters the kappa score was greater than 0.90 [67], it is a semiquantitative (ordinal) variable. Braak NFT staging is also modeled after a subset of AD cases with patterns of NFT formation that may differ somewhat from the pattern seen in PART. Furture studies using a more quantitative approach to measuring tau burden that we have shown more closely aligns with functional clinical measures in PART may reveal additional candidates [39].

In conclusion, drug development efforts are increasingly targeting tau due to the lack of clinical efficacy of Aβ modulating therapeutic approaches [22]. Here, by focusing on individuals with PART who lack Aβ neuritic plaques, we enriched our cohort for signals related to tau proteinopathy. Our analysis provides additional evidence that PART overlaps with but has considerable differences from AD. This interdisciplinary approach led to the identification of JADE1, which interacts with 0N4R tau, and is protective in vivo. Additional studies in experimental models are necessary to further validate these findings. Further understanding the genetics of PART will provide opportunities for rationally designed therapeutics for tauopathies.
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31 Department of Pathology, Stanford University, Palo Alto, USA
32 Laboratory Medicine Program, Krembil Brain Institute, University Health Network, Toronto, ON, Canada
33 Tanz Centre for Research in Neurodegenerative Disease and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
34 Institute of Neurology, Medical University of Vienna, Vienna, Austria
35 Department of Pathology and Immunology, Department of Neurology, Knight Alzheimer Disease Research Center, Washington University School of Medicine, St. Louis, MO, USA
36 Departments of Medicine, Pathology, and Pharmacology, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA
37 Department of Pathology (Neuropathology), University of Texas Southwestern Medical School, Dallas, TX, USA
38 College of Medicine and Health, University of Exeter, Exeter, UK
39 Department of Neurology, Center for Cognitive Health, Icahn School of Medicine at Mount Sinai, New York, NY, USA
40 Department of Neurology, Department of Neurobiology and Behavior, Institute for Memory Impairments and Neurological Disorders, UC Irvine, Irvine, CA, USA
41 Department of Psychiatry, Danube Hospital, Vienna, Austria