Apolipoprotein E (APOE) ε4 allele is the strongest genetic risk factor for late-onset Alzheimer’s disease mainly by modulating amyloid-β pathology. APOE ε4 is also shown to exacerbate neurodegeneration and neuroinflammation in a tau transgenic mouse model. To further evaluate the association of APOE genotype with the presence and severity of tau pathology, we express human tau via an adeno-associated virus gene delivery approach in human APOE targeted replacement mice. We find increased hyperphosphorylated tau species, tau aggregates, and behavioral abnormalities in mice expressing APOE ε2/ε2. We also show that in humans, the APOE ε2 allele is associated with increased tau pathology in the brains of progressive supranuclear palsy (PSP) cases. Finally, we identify an association between the APOE ε2/ε2 genotype and risk of tauopathies using two series of pathologically-confirmed cases of PSP and corticobasal degeneration. Our data together suggest APOE ε2 status may influence the risk and progression of tauopathy.
Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by the presence of extracellular amyloid plaques and intracellular aggregates of hyperphosphorylated tau protein (encoded by microtubule-associated protein tau gene (MAPT)). Apolipoprotein E (apoE) is a major cholesterol carrier in the brain. The APOE ε4 allele is the strongest genetic risk factor for late-onset AD, increasing brain amyloid burden in an allele dose-dependent manner. AD also has extensive tau pathology and is considered a “secondary tauopathy,” with primary tauopathies referring to disorders in which tau pathology is not accompanied by amyloid. In AD, tau protein aggregates form neurofibrillary tangles (NFTs) and neuritophil threads (NTs), as well as dystrophic neurites in senile plaques. Most tau pathology in AD is in neurons, but in primary tauopathies such as progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and frontotemporal lobar degenerations with tau pathology, tau aggregates are also found in glia. As a consequence of the extensive amyloid deposition in AD, it is difficult to evaluate the effect of APOE alleles on tau pathology in the setting of AD. To exclude the confounding effects of amyloid-β (Aβ), it is critical to investigate the impact of APOE alleles in primary tauopathies such as PSP and CBD, which share clinical and pathological mechanisms of tau dysfunction, or use pure tau transgenic mouse model system. It is noteworthy that the major genetic risk for many of the primary tauopathies is the common variation in MAPT defined by two major haplotypes, with H1 haplotype being more common in primary tauopathies. The H1 haplotype has also been implicated in AD and synucleinopathies.

A recent study showed that APOE ε4 exacerbates neurodegeneration and neuroinflammation in a tau transgenic mouse model. To further examine the association of APOE genotype with the presence and severity of tau pathology, we established a gene delivery approach by which adenovirus expressing human tau protein bearing P301L mutation (AAV-TauP301L) was bilaterally injected into the cerebral lateral ventricles of neonatal human apoE-targeted replacement (TR) mice with different isoforms. We found that the apoE2-TR mice expressing human tau (TauP301L-apoE2) exhibited significant increases in hyperphosphorylated tau species, thioflavin S-positive tau aggregates, astrocytosis, and behavioral abnormalities. These effects were smaller in apoE3-TR mice and absent in the apoE4-TR mice. We confirmed that the APOE ε2 allele is associated with increased tau pathology in the brains of human PSP patients. Finally, we identified a genetic association between APOE ε2/ε2 genotype and the risk of PSP and CBD. Together, our results support a pathogenic risk of APOE ε2 gene allele with primary tauopathies.

Results
Augmented tau pathology and astrogliosis in TauP301L-apoE2 mice. To generate a tauopathy mouse model in the background of different human apoE isoforms, we bilaterally injected AAV-TauP301L into the cerebral lateral ventricles of apoE2-TR, apoE3-TR, and apoE4-TR pups at postnatal day 0. The efficiency of AAV vector transduction and human MAPT gene expression were evaluated in mice at 6 months of age. No differences were found in the copy numbers of AAV vectors (Supplementary Fig. 1a) or the levels of MAPT mRNA (Supplementary Fig. 1b) in the cortex of TauP301L-apoE2-, apoE3-, and apoE4-TR mice. High levels of human tau expression, reflected by immunoreactivity for V5-tag, were broadly distributed in TauP301L-apoE mice (Supplementary Fig. 2a, b). No differences in total human tau expression were found in cerebral cortex, hippocampus or amygdala among apoE2-, apoE3-, and apoE4-TR mice (Supplementary Fig. 2c–f). Given that NFT contain abnormally hyperphosphorylated tau protein, we first determined the degree of tau phosphorylation in the apoE-TR mice expressing human TauP301L at 6 months of age. Importantly, TauP301L-apoE2 mice showed an approximately twofold increase in phosphorylation of tau at Ser202 and Thr205 sites (AT8 immunoreactivity) compared with TauP301L-apoE3 or TauP301L-apoE4 mice (Fig. 1a, b; Supplementary Fig. 3a). Consistently, the phosphorylation of tau at Ser199 site was also increased in TauP301L-apoE2 mice compared with TauP301L-apoE3 or TauP301L-apoE4 mice examined by enzyme-linked immunosorbent assay (ELISA) (Supplementary Fig. 4). To further define tau pathology in these mice, we evaluated the formation of tau aggregates monitored by thioflavin S fluorescence, which is a sensitive method to demonstrate inclusions with amyloid-like properties. Consistently, we found that the thioflavin S-positive NFT immunoreactivity in the neocortex of TauP301L-apoE2 mice was twofold higher than TauP301L-apoE3 and TauP301L-apoE4 mice (Fig. 1a–c; Supplementary Fig. 3b). We did not detect significant differences in the numbers of Nissl-positive cells (Supplementary Fig. 5a–c) and NeuN-positive nuclei (Supplementary Fig. 6a, b) between control and TauP301L mice suggesting there was no difference in neuronal loss in the two models at 6 months of age.

Several lines of evidence indicate that tau pathology is associated with gliosis, suggesting a toxic interplay between gliosis and tau pathology in disease progression. We histologically examined astrogliosis with glial fibrillary acidic protein (GFAP) immunohistochemistry in the AAV-TauP301L mouse model (Fig. 1a; Supplementary Fig. 3c). Significantly elevated expression of GFAP-positive astrocytes was noted in the cortex of TauP301L-apoE2 and TauP301L-apoE3 mice compared with their AAV-GFP controls, whereas no increase was found in the TauP301L-apoE4 mice (Fig. 1d). Iba1 immunoreactivity was significantly reduced in TauP301L-apoE4 mice, which might be due to the morphological changes of the microglia, and not different in TauP301L-apoE2 mice or TauP301L-apoE3 mice compared with their own controls, respectively (Supplementary Fig. 7a, c). The mRNA level of Aif1 gene showed no difference between control and TauP301L mice in all apoE genotype groups (Supplementary Fig. 7e). There were no significant differences in the staining of CD68, a marker of phagocytic microglia, nor its mRNA levels, between the control and TauP301L mice in different apoE genotype groups (Supplementary Fig. 7b, d, f). Thus, these results suggest that the microglia activation is not associated with tau pathology in this model.

Impaired behavior and synaptic integrity in TauP301L-apoE2 mice. To determine the effects of apoE isoforms on the behavioral performance of AAV-TauP301L model, we evaluated exploration and anxiety-related behaviors, as well as learning and memory that are often abnormal in various human tauopathies. In the open-field assay (OFA), apoE-TR mice expressing human TauP301L did not exhibit any hyperactivity as assessed by total distance traveled and time mobile compared with their controls (Supplementary Fig. 8a, b). TauP301L-apoE2 and TauP301L-apoE3 mice displayed a decreased tendency in exploring the center of the field (Fig. 2a), a typical characteristic of increased anxiety. In the elevated plus maze (EPM) task, TauP301L-apoE2 mice spent an increased amount of time in the open arms, whereas TauP301L-apoE3 and TauP301L-apoE4 mice were not compared with their
own controls, respectively (Fig. 2b), indicating that TauP301L-apoE2 exhibited aberrant exploratory behavior and disinhibition. Additionally, in a contextual fear conditioning paradigm, TauP301L-apoE2 mice showed significant impairment in the auditory cue-associated memory (Fig. 2d), although there was no significant change in context-associated memory (Fig. 2c). Such impairment was less severe in TauP301L-apoE3 mice and not detectable in TauP301L-apoE4 mice. Taken together, our finding demonstrated that expression of human TauP301L in the brain of apoE2 mice is detrimental to behavior, to a lesser extent of apoE3 mice, consistent with the observed effects tau pathology in apoE3 and apoE2 mice.

To determine whether synaptic abnormalities were present in TauP301L-apoE mice, we evaluated the expression of the postsynaptic proteins, including GluR2, the subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), and the postsynaptic density protein 95 (PSD95). The levels of GluR2 (Fig. 2e, f) and PSD95 (Fig. 2e–g) were both significantly decreased in TauP301L-apoE2 mice compared with their AAV-GFP controls, but such effects were not observed in TauP301L-apoE3 and TauP301L-apoE4 mice (Fig. 2e–g).

**Increased insolubility of tau and apoE in TauP301L-apoE2 mice.** We biochemically determined the distribution of soluble and insoluble tau and apoE proteins in the TauP301L-apoE mice by sequentially extracting cortical brain tissues with RAB (non-detergent-soluble fraction), RIPA (detergent-soluble fraction), and formic acid (FA; detergent-insoluble fraction) buffers. We found that the levels of insoluble tau and apoE were increased in the FA fraction of TauP301L-apoE2 mice (Fig. 3d), while the soluble tau and apoE in both RAB (Fig. 3a, b) and RIPA fractions (Fig. 3a–c) were decreased in TauP301L-apoE2 mice compared with TauP301L-apoE4 mice. The insolubility of tau and apoE protein in TauP301L-apoE3 mice also trended higher compared to that in TauP301L-apoE4 mice although not as pronounced as TauP301L-apoE2 mice. Additionally, the apoE mRNA level was significantly increased in apoE2 mice after TauP301L expression, whereas no such significant difference was found in apoE3 and apoE4 mice (Supplementary Fig. 9). It has been reported that tau can bind to apoE3, but not to apoE4, forming a biomolecular complex; however, it remains unclear whether apoE2 can directly form a complex with human tau. To address this, we examined whether recombinant apoE isoforms differentially form a complex with recombinant tau in a cell-free system. Recombinant human apoE protein was incubated with recombinant human tau protein for 1 h at 37 °C in phosphate-buffered saline (PBS). The incubation was ended by addition of sample buffer with or without 2-mercaptoethanol (2-ME), followed by 5 min of boiling before western blotting. In the absence of the reducing agent 2-ME, we observed tau/apoE complexes (bands A and B) that are immune-reactive to both tau
and apoE antibodies (Fig. 4a). Based on stoichiometric calculations, the size of band “A” was consistent with two tau molecules forming a complex with one apoE molecule, while that of band “B” was comprised of one tau and two apoE molecules. ApoE2 formed complexes with tau to a much greater extent than apoE3, whereas such complex was virtually absent when apoE4 was incubated with tau (Fig. 4b, c). The tau/apoE complexes were disrupted in the presence of reducing agent 2-ME (lane 8 and 9), confirming that apoE binds to tau through the formation of intermolecular disulphide bonds. We also incubated the recombinant tau protein with the astrocyte-secreted apoE lipoprotein particles. Similarly, the tau/apoE complexes were observed in the presence of apoE2 or apoE3 lipoprotein particles, but not in apoE4 (Supplementary Fig. 10). However, we did not observe an increase of tau/apoE complex when tau was incubated with apoE2 particles compared with apoE3 particles. We attempted to detect an in vivo interaction between tau and apoE by co-immunoprecipitation studies using brain lysates of our experimental mice. Unfortunately, we could not detect an association between tau and apoE, likely reflecting that such an interaction either does not exist in vivo or is not robust enough for detection by this co-immunoprecipitation method (data not shown). Taken together, our findings suggest that apoE2, non- lipided form in particular, could form a complex with tau in vitro.

**Increased severity of tau pathology in human PSP patients with APOE ε2 allele.** To assess whether APOE genotype influences the severity of tau pathology in human PSP patients, we analyzed the association between APOE genotype and tau lesion subtypes (CB, NFT, TA, and NT) in PSP brains. The severity of tau pathology for CB (median score = 1.6), NFT (median score = 2.2), TA (median score = 1.0), and NT (median score = 2.2) is shown in Supplementary Table 1. Consistent with our findings using animal models, after adjustment for multiple testing (P ≤ 0.0125 considered significant), the presence of APOE ε2 was significantly associated with more severe tau pathology (evaluated by overall tau pathology scores) for TA (P = 0.004), with similar nominally significant (P ≤ 0.05) associations observed for CB (P = 0.045) and and NT (P = 0.029) (Table 1). There was a similar trend for NFT, though it was not quite nominally significant (P = 0.059). These findings appear to be driven primarily by the ε2/ε3 genotype, which was more strongly associated with a greater severity of tau pathology for CB (P = 0.004), TA (P = 0.002), and NT (P = 0.010). No significant associations with the severity of tau pathology were noted for the APOE ε2/ε2 genotype likely due to an inadequate statistical power for this rare genotype. Given the significant or nominally significant associations between APOE ε2 and greater overall tau pathology scores for CB, TA, and NT, we further evaluated the associations of presence of ε2 with the severity of tau pathology for CB, TA, and NT in each separate brain region (Supplementary Tables 2a-c, 3). APOE ε2 was most strongly associated with severe CB lesion scores in the inferior olive (P = 0.003) and the pontine base (P = 0.050), while TA lesions were associated in the basal nucleus of Meynert (P = 0.001), globus pallidus (P = 0.008), subthalamic nucleus (P = 0.005), ventral thalamus (P = 0.023), midbrain tectum (P = 0.001), and pontine tegmentum (P = 0.027), and NT lesions associated with the basal nucleus of Meynert (P = 0.012), subthalamic nucleus (P = 0.018), medullary tegmentum (P = 0.023), and substantia nigra (P = 0.022). These results provide further evidence that
**APOE ε2 affects the severity of tau pathology in human primary tauopathies.** As expected, in comparison to the common ε3/ε3 genotype, APOE ε4 (in particular the ε4/ε4 genotype) was strongly associated with a higher Thal phase ($P < 0.0001$), while conversely ε2/ε3 was associated with a lower Thal phase (Supplementary Table 4)\(^\text{18}\). No association was found between APOE genotype and presence of TDP-43 pathology (Supplementary Table 4).

**Increased risk of PSP and CBD in carriers of the APOE ε2/ε2 genotype.** To further investigate the effects of APOE genotype on the frequency of tauopathy in humans, we studied 994 PSP patients, 134 CBD patients, and 1406 controls (Supplementary Table 5). As displayed in Table 2, after correction for multiple testing ($P \leq 0.00625$ considered as significant), the only significant association between APOE and risk of PSP that we observed occurred for the ε2/ε2 genotype when compared to all other
genotypes; APOE e2/e2 was significantly more frequent in PSP compared to controls (1.3% vs. 0.4%, odds ratio (OR) = 4.41, P = 0.0057). Results were similar when comparing e2/e2 to a reference e3/e3 genotype (OR = 4.38, P = 0.009), although this finding was not quite significant, likely due to the lower power to detect an association with the lower sample size given the similar OR estimate. Interestingly, although not significant, this same trend was also observed in the smaller series of CBD patients, where e2/e2 was more common in patients compared to controls (1.5% vs. 0.4%, OR = 4.86, P = 0.083). Noteworthy, although not significant, the e2/e2 genotype was associated with a lower risk of PSP (OR vs. e3/e3 = 0.69) suggesting a recessive disease model under the risk paradigm.

The MAPT gene has two conserved haplotypes H1 and H2, and the common H1 haplotype is significantly over-represented in PSP [10,19,20] and CBD [11,21,22]. To assess the contribution of APOE genotype to PSP and CBD susceptibility in the absence of the strong MAPT H1 risk factor, we examined the aforementioned associations when excluding individuals who were homozygous for that MAPT H1/H1 genotype (Table 2). Notably, the presence of APOE e2/e2 dramatically increased the risks of both PSP (3.7% vs. 0.3%, OR: 9.56, P = 0.010) and CBD (5.9% vs. 0.3%, OR: 21.40, P = 0.018) in this subgroup of 109 PSP patients, 17 CBD patients, and 599 controls. Taken together, these data indicate that APOE e2/e2 genotype increases the risk of both PSP and CBD and may act as a stronger determinant in the absence of MAPT H1/H1 status.

**Discussion**

In the present study, we demonstrated a significantly increased accumulation of hyperphosphorylated tau species, thioflavin S-positive tau aggregates, and tauopathy-related astroglisis in a TauP301L apoE2 disease mouse model, leading to behavioral abnormalities in anxiety, exploration, as well as learning and memory. Furthermore, we showed that APOE e2 was associated with increased severity of tau pathology in the postmortem human brain tissues from PSP patients. Finally, we detected an increased frequency and risk of the recessive APOE e2/e2 genotype in a large series of PSP and CBD cases.

We introduced mutant TauP301L via viral delivery in humanized mouse models for each APOE allele (e2, e3, and e4). In these viral-induced tau mouse models, we found severe tau pathology and behavioral deficits in the presence of APOE...
A recent study showed that APOE ε4 exacerbates neurodegeneration and neuroinflammation in a tau transgenic mouse model. In our model system, APOE ε4 did not affect tau pathology, behavior, or neuroinflammation. Further, we did not observe neurodegeneration in any of the APOE genotypes at 6 months of age, which is consistent with the previously reported phenotypes using AAV-tau as tauopathy mouse model. Thus, it is possible that the discrepancy of APOE genotype effects between our model system and that of Shi et al. is due to the different experimental model systems used in each study. For example, the total amounts of tau expressed (AAV-mediated vs. transgenic system), the toxicity of different tau species (P301L vs. P301S mutation), the amount of tau aggregation and pathology within certain time periods (6 vs. 9 months of age), and the absence or presence of tau-mediated neurodegeneration are among the variations between these mouse models. These factors together may contribute to the differences observed between these two model systems. However, our human genetic and pathological data supporting an APOE ε2 effect on tauopathy are consistent with the findings from our model studies. Future studies are needed to assess dynamic effects of APOE genotype in different model systems, but more importantly to interrogate these findings in human study cohorts.

The apoE protein is primarily expressed by astrocytes in the brain and delivers cholesterol and other lipids to neurons through apoE receptor LRPI and perhaps LDLR. Several features of apoE2 distinguish it from apoE3 and apoE4 isoforms, which might cause apoE2 to behave differently. ApoE2 has a vastly reduced binding ability to LDLR, contributing to an increased risk of type III hyperlipoproteinemia in APOE ε2/ε2 individuals. The level of apoE2 protein is the highest compared with other apoE isoforms in cerebrospinal fluid (CSF), interstitial fluid (ISF), brain parenchyma, and plasma. Regarding amyloid pathology, apoE2 and to a lesser extent apoE3 are hyperepididymal, leading to reduced Apβ aggregation and faster clearance of Apβ from the brain. How apoE isoforms affect tau pathology is unclear. Although tau is a cytoplasmic protein, recent evidence has shown that tau protein is also found in extracellular space, including CSF and brain ISF. Intracellular tau aggregates are in equilibrium with extracellular tau, whose aggregation might mediate spreading of pathologic species of tau between cells. Since apoE is naturally a secreted protein and readily present in the extracellular fluids, it is possible that apoE may form a complex with tau and modulate its metabolism in the brain. Alternatively, apoE has been reported to be present in the cytoplasm, where it can also modulate tau aggregation and related pathologies. The higher amount of apoE2 protein in the brain might also contribute to the increased tau aggregation compared with other apoE isoforms. Additionally, the potentially different complex forming properties between tau and apoE isoforms might impact tau metabolism. The exact mechanisms by which apoE isoforms differentially regulate tau aggregation under normal and pathological conditions require further investigation.

We observed the APOE ε2 allele is associated with greater tau burden in PSP brains, indicating that APOE ε2 may enhance tau pathology not only in mouse models but also in humans. While it has been reported that APOE ε4 is associated with NFT pathology in postmortem human brains whenever Apβ is present, no such association is observed in brains without Apβ. Most patients with PSP have minimal or no Alzheimer-type pathology (median Braak NFT stage of II–III and median Thal amyloid phase of 1), but some PSP patients have concomitant Alzheimer-type pathology. Interestingly, the APOE ε4 allele frequency is similar between pure PSP patients with minimal or no Alzheimer-type pathology (11%) and controls, whereas it is significantly higher in PSP patients with concomitant Alzheimer-type pathology (64%) or with pathologic aging (38%). Also, a recent publication showed that although APOE ε4 carriers had more severe amyloid pathology, the total tau burden and the global cognitive impairment index did not differ from APOE ε4 non-carriers in 121 autopsy-confirmed PSP patients. These results suggest that APOE ε4 is a risk factor for Alzheimer-type amyloid pathology but not necessarily tau burden in PSP.

APOE ε2 is protective in the setting of AD; however, in the absence of amyloid pathology, it has been previously implicated as a risk factor for tau-related neurodegeneration (e.g., NFT-predominant senile dementia). Although AD patients who are APOE ε4 carriers have greater tau pathology compared to non-carriers, the APOE ε4 genotype is not associated with primary age-related tauopathy (PART), which has medial temporal neurofibrillary pathology but no (“definite PART”) or only minimal (“possible PART”) Apβ deposition, implying that apoE4 might only impact tau pathology in the presence of amyloid. Susceptibility to agyrophilic grain disease, a common aging-related medial temporal primary tauopathy, is variably associated with APOE ε2 allele in mouse model systems used in each study. For example, the total amounts of tau expressed (AAV-mediated vs. transgenic system), the toxicity of different tau species (P301L vs. P301S mutation), the amount of tau aggregation and pathology within certain time periods (6 vs. 9 months of age), and the absence or presence of tau-mediated neurodegeneration are among the variations between these mouse models. These factors together may contribute to the differences observed between these two model systems. However, our human genetic and pathological data supporting an APOE ε2 effect on tauopathy are consistent with the findings from our model studies. Future studies are needed to assess dynamic effects of APOE genotype in different model systems, but more importantly to interrogate these findings in human study cohorts.

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In summary, our study suggests that APOE ε2 plays a role in the severity of tau pathology in PSP and CBD, in mouse models and humans. Our results indicate that APOE ε2 is not benign with respect to risk for neurodegenerative diseases, which should be taken into consideration in future clinical trial design and early prevention strategies for tau-related disorders.

**Methods**

**Animals.** The three APOE genotypes ε2, ε3, and ε4 encode three isoforms of human apoE protein, which differ at amino-acid positions 112 and 1584. ApoE-TR mice in which murine ApoE gene locus is replaced with human APOE ε2/ε2, APOE ε3/ε3, or APOE ε4/ε4 gene were obtained from Taconic. Animals were housed under controlled conditions of temperature and lighting and given free access to food and water. In all, 12–22 mice/genotype were injected with AAV vectors and used for behavioral tests. Six to eight mice/genotype were randomly selected for further biological analysis. Sample sizes were adequately powered to observe the effects on the basis of past experience. All animal procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committee and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Intracerebroventricular viral injections in animals.** V5-tagged TauP301L or green fluorescent protein (GFP) expression plasmids in AAV1 vectors (abbreviated as AAV-TauP301L and AAV-GFP, respectively) were prepared as described. Briefly, AAV vectors expressing GFP or TauP301L under the control of the cytomegalovirus enhancer/chicken β-actin promoter were generated by plasmid transfection with AAV helper plasmids in HEK293T cells. The genomic titer of each virus was measured using ABI3730 with Big Dye chemistry following manufacturer’s protocols (Applied Biosystems, Foster City, CA, USA). ApoE2-, apoE3-, and apoE4-TR pups at postnatal day 0 were intracerebroventricularly injected with 2 µl of AAV-GFP or AAV-TauP301L viruses into both hemispheres. Briefly, newborn pups were cryoanesthetized and subsequently placed on a cold metal plate. A 30-gauge needle...
which served as the unconditioned stimulus (US). After 1 min, another CS was presented. The mouse was removed 30 s after the second CS auditory CS test, environmental and contextual cues were changed as described15.

Enzyme-linked immunosorbent assay. Levels of apoE and tau in FA fractions and phospho tau (pS199) in RIPA fraction were determined by ELISAs for apoE ELISA, WUE4 capture antibody (Cat# NB110-60531, Novus, 1,100) and biotin-conjugated mouse monoclonal detector antibody (Cat# K74180B, Meridian Life Science, 1,100) and anti-β-actin (Cat# A2282, Sigma, 1,000) antibodies. The scans of the membrane are shown in Supplemental Fig. 11.

Genomic DNA extraction and AAV copy number analysis. DNA isolation from the cortex of the AAV-Tau301L-apoE-TR mice (n = 8 mice per group) was performed using the Puragene kit (Gentra Systems, Minneapolis, MN) in accordance with the manufacturer’s protocol. For measurement of apoE content, DNA was isolated from 50 µl of tissue by spectrophotometry using Nanodrop, and 10 ng of DNA from each sample was used as the template material for real-time PCR. Real-time PCR was performed on each sample for both the human MAPT gene in order to determine copies of the viral genome, and the mouse Actb gene to standardize for number of mouse genomes present in each sample. The primers used for human MAPT gene were: 5′-TGAAGCAGGATGCTGACC-3′ (forward) and 5′-TCTGATCCTGCTACATGC-3′ (reverse). The primers used for Actb gene were: 5′-AGTTTGAGCTTGAATCCT-3′ and 5′-GCCAGAGGATTAATCACTCCT-3′ (reverse). The accumulation of PCR products for each gene was measured using SYBR green (Thermo Fisher Scientific, Waltham, MA). All the samples were run on a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). The absolute amount of each gene was obtained by reformatting to a standard curve consisting of mouse actin plasmid DNA (Origene, Rockville, MD) or human Tau301L plasmid DNA at 10 pg, 5 pg, 1 pg, 100 fg, and 10 fg. All samples and standard plasmid DNA were run in duplicate.

RNA isolation and real-time PCR analysis. Total RNA was isolated by using Trizol (QIAGEN, Hilden, Germany), RNeasy Mini Kit (QIAGEN, Hilden, Germany) and subjected to DNAse I digestion to remove contaminating genomic DNA. Reverse transcription was performed using Script™ Reverse Transcription Supermix (Bio-Rad, Hercules, CA). CDNA was added to a reaction mix (20 µl final volume containing gene-specific primers and SYBR green) on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Relative gene expression was normalized to actin controls and assessed using the 2−ΔΔCT method. Primer sequences are as follows (5′–3′): Actb: AGTTTGAGCTTGAATCCT (forward) and GCCAGAGGATTAATCACTCCT (reverse); human MAPT: TGAACGAGGTGCTGACC (forward) and TTCTGATCCTGCTACATGC (reverse); human CD68: MG411122 (forward) and GCCAGAGGATTAATCACTCCT (reverse); human CTSC: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse); human APOE: TGTCAGAGGTGCTGACC (forward) and GCCAGAGGATTAATCACTCCT (reverse);

Purification of labeled apoE particles from culture medium. Immortalized mouse astrocytes derived from apoE2-TR, apoE3-TR, and apoE4-TR mice were cultured and conditioned with serum-free medium for 48 h. Conditioned medium was concentrated using Amicon centrifugal filter unit (Millipore), and run through a HiTrap Heparan column on an AKTA PPLc system (GE Healthcare). Heparin-bound apoE was eluted with NaCl gradient from 0 to 1 M in Tris buffer. The peak fractions containing apoE that is associated with cholesterol were described previously46. The concentration of apoE was quantified by apoE ELISA.

Solution binding of tau and apoE. Recombinant human Tau-40 protein (1 µg, Sigma) and recombinant apoE protein (1 µg, Fitzgerald) or liposomal apoE particles (1 µg) were incubated for 1 h at 37 °C in 20 µl of PBS (pH 7.4). The incubation was ended by addition of 20 µl of PBS to a 2 µl aliquot of the 1:2 suspension, and concentrations of tau and apoE were determined by the Bradford assay following incubation in 10 µg of boiling. Proteins were electrophoretically separated by SDS-PAGE gel and transferred to PVDF membranes. After blocking, the membrane was incubated in
primary antibody overnight. The primary antibodies were monoclonal anti-tau antibody (Cat# MN1000B, H17, Thermo Fisher Scientific, 1:1000) and monoclonal anti-apolipoprotein E (ApoE) antibody (Cat# K74180B, Meridian Life Science, 1:1000). Membrane was then probed with HRP-conjugated secondary antibody, and detected using the Odyssey infrared imaging system (LI-COR).

Statistical analyses for animal study. In order to ensure that results are valid in the presence of non-normal distributions or differing variances between groups, nonparametric Mann-Whitney tests followed by Bonferroni correction for multiple comparisons were used to compare outcomes between apoE2, apoE3, and apoE4 groups, and also between control and TauP301L separately for apoE2, apoE3, and apoE4 groups. All statistical tests were two-sided.

Subjects and statistical methods for tau pathology in PSP. A total of 858 cases of PSP were obtained from the Mayo Clinic brain bank for neurodegenerative disorders approved by the Mayo Clinic Institutional Review Board. The PSP brains were evaluated with standardized histopathologic methods and phospho-tau immunohistochemistry, and all met neuropathologic criteria for PSP.66 The 858 PSP cases represent all cases with available semiquantitative tau pathology measures. Quantitative lesion scores as part of the neuropathologic evaluation (performed by a single observer (D.W.D.)) and are included in this analysis (Supplementary Table 1). Semiquantitative tau pathology measures were assessed on a four-point severity scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe). All sections from all cases were processed in an identical manner with phospho-tau monoclonal antibody (CP13, from Dr. Peter Davies, Feinstein Institute, Long Island, NY) and immunohistochemistry using a DAKO Autostainer. Four lesion types were scored (NFT, CT, TA, and NT) in 17–20 different neuroanatomical regions that are vulnerable to PSP (Supplementary Tables 2a–2c). An overall score was created for each separate tau pathology measure (NFT, CT, TA, and NT) by calculating the mean of the semiquantitative measures (0, 1, 2, or 3) for each PSP patient across all anatomical regions, where a higher overall score indicates more severe tau pathology. The overall tau pathology scores are displayed in Supplementary Table 1. There was a relatively small amount of missing data for the four tau pathology measures (median: 1.9% missing, range: 0.1–22.0% missing, Supplementary Tables 2a–2c). All cases had assessment of Alzheimer-type pathology with thioflavin S fluorescent microscopy, and based upon the density and distribution of plaques and tangles, Braak NFT stage35 and Thal amyloid phase.28/pathology (Table 5). The numbers of cases with each category in each group are given in Supplementary Table 4. In three of the PSP cases (N = 795), TDP-43 pathology was assessed as previously described65 using a polyclonal antibody to a neopterin in pathologic TDP-43. Information was collected regarding age (age at death), sex, and APOE genotype (ε2/ε2, ε2/ε3, ε2/ε4, or ε3/ε3). Genotyping for APOE (rs429358 C/T and rs7412 C/T) and MAPT (rs8070723 A/G) alleles was performed using custom TaqMan Allelic Discrimination Assay on an ABI 7900HT Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). All subjects were non-Hispanic Caucasian and unrelated within and between sample groups. Characteristics of the PSP and CBD patients, and neurologically normal controls are displayed in Supplementary Table 5.

For primary analysis, after controlling control genotype/allele frequencies were in Hardy-Weinberg equilibrium (P > 0.05), the associations of APOE genotype with risk of PSP and CBD (i.e. each disease separately vs. controls) were evaluated using ORs and 95% CIs from logistic regression models that were adjusted for age, sex, and number of H1 alleles. Initially, we examined associations with risk of PSP and CBD for both presence of the ε2 allele and presence of the ε4 allele. Subsequently, associations with PSP and CBD were examined when considering each APOE genotype separately in order to examine whether there may be specific risk or protective genotypes. The APOE ε3/ε3 genotype was used as the reference genotype for all comparisons, although in some cases we also examined the association between presence of ε2/ε2 vs. all other genotypes given the strong risk effects for both PSP and CBD that were observed for ε2/ε2. In secondary analysis, all of the aforementioned logistic regression association analysis was also performed in the subset of subjects who did not carry the MAPT H1/H1 genotype (MAPT genotype was not adjusted for in this sub-analysis). In order to avoid multiple sample testing in the primary analysis, we applied a Bonferroni correction for the eight statistical tests that were performed in the separate PSP vs. controls and CBD vs. controls analyses, after which P-values of 0.00625 or lower were considered as statistically significant. All statistical tests were two-sided. All statistical analyses were performed using SAS (version 9.2; SAS Institute, Inc., Cary, North Carolina).

Data availability
All relevant data are available from the authors upon reasonable request.

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PSP and CBD cases; N.Z., C.-C.L., O.A.R. and G.B. wrote the manuscript with critical inputs and edits by co-authors.

**Additional information**

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