



Upregulation of Prickle2 Ameliorates Alzheimer's Disease-Like Pathology in a Transgenic Mouse Model of Alzheimer's Disease

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Alzheimer's disease (AD) is a devastating neurodegenerative disorder that has no effective therapies. Prickle planar cell polarity protein 2 (Prickle2), is an important cytoplasmic regulator of Wnt/PCP signaling. It has been reported that Prickle2 deficiency reduced neurite outgrowth levels in mouse N2a cells and led to autism-like behaviors and hippocampal synaptic dysfunction in mice. However, much less is known about the relationship of Prickle2 to AD pathogenesis. RT-qPCR, Western blot and IHC results showed that the mRNA and protein levels of Prickle2 were reduced in APP/PS1/Tau transgenic (3xTg) mice. Intravenous injection of Prickle2-overexpressing AAV-PHP.eB vectors improved the cognitive deficits in 3xTg mice. We also demonstrated that Prickle2 could repress oxidative stress and neuroinflammation, ameliorate the amyloid β (A β) plaque pathology and reduce Tau hyperphosphorylation in APP/PS1 mice. Further investigation of the mechanism of Prickle2 in AD revealed that Prickle2 inhibited Wnt/PCP/JNK pathway in vivo and in vitro. Our results suggest that Prickle2 might be a potential candidate for the diagnosis and treatment of AD.

Keywords: Prickle2, Alzheimer's disease, oxidative stress, neuroinflammation, Wnt/PCP pathway

INTRODUCTION

Alzheimer's disease (AD) is a devastating neurodegenerative disorder with a high incidence rate among the elderly worldwide. It is characterized by progressive memory loss, cognitive dysfunctions and dementia (Whitaker et al., 2014). AD is mainly caused by the accumulation of extracellular amyloid β (A β) deposits, intracellular neurofibrillary tangles (NFTs) and loss of neurons and synapses (Oboudiyat et al., 2013; Thal et al., 2015; Nisbet and Gotz, 2018). Although many studies have investigated the etiology of AD in the last few decades, the molecular mechanism

of this disease remains unclear, and effective therapy is still unsatisfactory (Barrera-Ocampo and Lopera, 2016; Agrawal et al., 2017; Suzuki et al., 2017; Cao et al., 2018; Panza et al., 2019). Therefore, it is urgent to unravel and understand the complex mechanisms of AD etiology in depth and to identify novel targets for AD diagnosis and treatment.

The Wnt/planar cell polarity (Wnt/PCP) signaling pathway, known as the non-canonical WNT signals independent β -catenin, was initially found to be involved in tissue polarity and cell movement (Veeman et al., 2003). Recently, Wnt/PCP signaling, which participates in development and a variety of human diseases (Katoh, 2005; Chen et al., 2018; Humphries and Mlodzik, 2018), is also associated with a higher risk of AD (Elliott et al., 2018; Sellers et al., 2018). Wnt/PCP signaling contains upstream canonical Wnt signaling pathway components, such as Wnt5A, Wnt5B, and Wnt11, for the transduction of Wnt/PCP signals, followed by several transmembrane protein receptors of the PCP core components, Frizzled (FZD), Dishevelled (DSH), Vangl (van Gogh-like) and the cytoplasmic regulator Prickle (Katoh, 2005; Barrow, 2006; VanderVorst et al., 2019). Most recently, intriguing studies have implicated the involvement of Prickle2 in the pathogenesis of nervous system diseases. For example, deletion of Prickle2 causes a lower seizure threshold and autism-like behaviors with hippocampal synaptic abnormalities (Tao et al., 2011; Ehaideb et al., 2014). Overexpression of Prickle2 promotes neurite outgrowth *in vitro* (Fujimura and Hatano, 2012). Accordingly, Prickle2 interacts with two autism spectrum disorder-related proteins, PSD-95 and the NMDA receptor in the postsynaptic density (Hida et al., 2011; Tao et al., 2011). Furthermore, the Prickle2 mRNA, predicted by an online tool AlzData (Xu et al., 2018), is markedly downregulated in the entorhinal cortex, temporal cortex and hippocampus of AD patients compared with that in normal people. However, the exact function of Prickle2 in the etiology of AD is poorly understood, and whether Prickle2 regulates A β generation and cognitive functions in AD is of interest.

In this study, we found that Prickle2 was downregulated in the brain tissue of 3 \times Tg-AD mice, suggesting that the reduction of Prickle2 may play a certain role in the etiology of AD. AAV-mediated upregulation of Prickle2 ameliorates cognitive deficits and AD-like pathology in 3 \times Tg transgenic mice by inhibiting the Wnt/PCP signaling pathway. Our results not only uncover the mechanism of Prickle2 in the etiology of AD but also provide a potential biomarker for the future diagnosis and treatment of AD.

MATERIALS AND METHODS

Animals

The C57BL/6J mice (25–28 g; 6 months) were obtained and approved by Beijing HFK Bioscience Co., LET. APP/PS1/TAU (3 \times Tg-AD; homozygous for the Psen1 mutation and homozygous for the co-injected APPSwe and tauP301L transgenes (Tg(APPSwe,tauP301L)1Lfa) transgenic mice with the same background were obtained from the Jackson Laboratory, being created via co-microinjecting two independent transgenes encoding human APPSwe and human TAUP301L

into single-cell embryos harvested from homozygous mutant PS1m146v knockin (PS1-KI) mice. All animal procedures were approved by the Animal Care and Use Committee of Tianjin Medical University.

Generation of AAV-PHP.eB Virus

The AAV-PHP.eB-Syn-mScarlet-P2A-MCS Rep-Cap trans plasmid was generated by gene synthesis (GenScript). Prickle2 (NM_001134461) was synthesized and cloned to AAV-PHP.eB-Syn-mScarlet-P2A-MCS to generate Prickle2 overexpressing vectors AAV-PHP.eB-Syn-mScarlet-P2A-MCS-Prickle2. Recombinant AAVs were generated by triple transfection of HEK293T cells using polyethylenimine (PEI) and purified by ultracentrifugation over iodixanol gradients as previously described (Deverman et al., 2016).

AAV-PHP.eB Vector Delivery

12–20 3 \times Tg-AD or C57BL/6J (wild type, WT) mice were intravenously injected with AAV-PHP.eB vector and used for behavioral tests in each group. 3 \times Tg+Prickle2 group was intravenously injected with AAV-PHP.eB-Syn-mScarlet-P2A-MCS-Prickle2 vectors, and the 3 \times Tg control groups were intracerebrally injected with empty AAV-PHP.eB vector. Intravenous injection was performed in a restrainer that positioned the tail in a heated groove. The tail was swabbed with alcohol and then injected intravenously with a viral concentration 1×10^{13} vg/mL according to the experimental setup in a total volume of 100 μ l of AAV-PHP.eB particles in PBS.

Cell Culture and Cell Transfection

The mouse neuroblastoma N2a cell line was obtained from the American Tissue Culture Collection (Manassas, VA, United States). The cells were cultured in Dulbecco's modified eagle medium (Sigma-Aldrich, Shanghai Trading Co. Ltd) containing 10% fetal bovine serum (FBS; Sigma-Aldrich, Shanghai Trading Co. Ltd) and 100 units penicillin-streptomycin (10,000 units/mL of penicillin and 10,000 μ g/mL of streptomycin) at 37°C in a humidified atmosphere with 5% (v/v) CO₂. N2a/APP695sw cell line was stably transfected with the human APP-695 Swedish mutation (K595N/M596L) and was maintained in a selective state by the antibiotic G-418 at a final concentration of 0.2 mg/ml in DMEM medium containing 10% FBS.

Cells were transfected using NeuroPorter™ Transfection Kit (Sigma-Aldrich, United States) according to the manufacturer's instructions. pcDNA3 (Vec), pSilencer 2.1-U6 Neo (shR-NC), pcDNA3-Prickle2 (Prickle2) and pSilencer-Prickle2 (shR-Prickle2) were constructed by GenePharma Co., Ltd. (Shanghai, China). Primers and oligonucleotides used for plasmid construction are listed in **Supplementary Table S1**.

Real-Time Quantitative PCR

The total RNAs were extracted from brain tissues using TRIzol reagent (Ambion) according to the manufacturer's instructions. Then, a NanoDrop ND-1000 spectrophotometer was used to quantify the isolated RNAs. After that, the cDNAs were prepared

using the Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, United States). RT-qPCR was performed using an SYBR Green Real-Time PCR Master Mix (Thermo Fisher Scientific, United States) on a Bio-Rad iQ5 system (Bio-Rad, Hercules, CA, United States). The relative expression levels of *Prickle2* were calculated by the $2^{-\Delta\Delta Ct}$ method as the endogenous controls. RT-qPCR primers are listed as follows (5'-3'): *Prickle2*: TGGAGAGAAGTTGCGAATCAAG (forward) and ACAAATAGCTCCTGTCATGGTG (reverse); *GAPDH*: CTGGGCTACACTGAGCACC (forward) and AAGTGGTCTGTTGAGGGCAATG (reverse).

Morris Water Maze

The behavioral test was performed as previously described using the Morris Water Maze (Jankowsky et al., 2007; Liu et al., 2017). Briefly, the navigation experiment was conducted on five consecutive days to record the time required for the mice to find the hidden platform. If the platform was not found, the mice were guided to the platform for 20 s, and the escape latency was recorded as 90 s. On day 6, the hidden platform was removed, and the mice were given a single 90 s to swim freely. The frequency of passing the hidden platform and the original angle were recorded.

Tissue Collection

After the behavioral test, the mice were euthanized and then perfused with phosphate-buffered saline. Their brains were removed immediately. Part of their brain tissue was fixed in 4% paraformaldehyde solution, and then dehydrated, waxed and sectioned. For the remainder of the brain tissue, the hippocampus was separated from the cortex on an ice table, and then both were frozen in liquid nitrogen.

Measurement of SOD, MDA and ROS

The hippocampus was separated from the cortex in the brain tissue, and then homogenized and centrifuged at $12000 \times g$ for 15 min to isolate the supernatants, respectively. A malondialdehyde (MDA) assay kit (TBA method), superoxide dismutase (SOD) typed assay kit (hydroxylamine method), and reactive oxygen species assay kit (Nanjing Jiancheng Bioengineering Institute, China) were used to measure the MDA level, SOD activity and ROS level according to the manufacturer's instructions, respectively.

Immunohistochemical Staining

Serial 10 μm coronal sections were rehydrated, and antigen retrieval was performed with 0.01 M citric acid buffer for 20 min. The sections were then incubated with 3% H_2O_2 solution for 10 min to eliminate the activity of endogenous enzymes. Next, slides were incubated with blocking solutions (5% normal goat serum) at room temperature for 15 min and then incubated with the dilution of primary antibodies. The following primary antibodies were used: PRICKLE2 polyclonal antibody (Cat # PA5-96903, 1:100, Thermo Fisher Scientific, United States), Anti-Tau (phospho S396) antibody (Cat # ab109390, 1:1000, Abcam, United States). After incubation overnight at 4°C , the sections were incubated with a biotin-labeled secondary antibody for

15 min. Following the development, the sections were incubated with streptavidin-biotin complex and DAB peroxidase substrate kit was used to detect the expression of each protein. As a negative control, the sections were processed in the same step but used rabbit serum instead of primary antibodies. For $\text{A}\beta$ immunofluorescence staining, brain paraffin section dewaxed in water, then the sections were incubated with $\text{A}\beta 4\text{G8}$ mouse monoclonal antibody (cat # 800703, 1:100, Biolegend, United States), followed by goat anti-mouse IgG H&L (Alexa Fluor[®] 488) (Cat # ab150113, 1:500, Abcam, United States). The tissue sections were also counterstained with DAPI, a nucleic acid-specific marker, to visualize the nuclei at a 633-nm excitation wavelength.

X-34 and Thioflavin S (Thios) Staining

X-34 staining was performed according to previously described procedures (Styren et al., 2000). The brain paraffin sections were dewaxed in water, then the sections were incubated with 100 μM X-34 solution (dissolved in DMSO) and DAPI for 10 min and then further processed for immunohistochemistry. ThioS staining was performed according to previously described procedures (Wang et al., 2017). The brain paraffin section dewaxed in water, then the sections were incubated with 1% thioflavin S solution (dissolved in distilled water containing 50% ethanol) for 5 min and then differentiated in 50% ethanol for 5–15 min. Fluorescence imaging was collected using an Olympus fluorescence microscope (Olympus Corporation, Japan).

ELISA

$\text{A}\beta$ ELISA was performed according to the methods reported previously (Wang et al., 2017). Briefly, the cortical and hippocampal tissues were thoroughly homogenized using an animal tissue homogeniser. Then, the homogenates containing a protease inhibitor cocktail (Roche) were mixed 1:1 with 0.4% diethylamine (DEA). The mixture was centrifuged at $13,000 \times g$ for 30 min at 4°C . The supernatants were isolated to measure soluble $\text{A}\beta$. In addition, the pellets were homogeneously mixed using 70% formic acid by sonication. Subsequently, the mixture was centrifuged at $13,000 \times g$ for 30 min at 4°C , and the supernatants were collected to detect insoluble $\text{A}\beta$. Human $\text{A}\beta 40$ or $\text{A}\beta 42$ ELISA kits (Invitrogen) and IL-1 β or IL-6 (Thermo Fisher Scientific) were used to measure the $\text{A}\beta$, IL-1 β and IL-6 concentrations according to the manufacturer's instructions.

Western Blotting

The isolated hippocampus or cortex tissues were, respectively, mixed with RIPA buffer to extract protein, and the BCA method was used for the measurement of the total protein concentration. The proteins were separated by SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% skimmed milk for one hour at room temperature and then incubated with one of the primary antibodies overnight at 4°C . The following primary antibodies were used: PRICKLE2 polyclonal antibody (Cat # PA5-96903, 1:1000, Thermo Fisher Scientific, United States), Tau (phospho Ser356) antibody (Cat# ab75603, 1:1000, Abcam, United States), Tau (phospho S396) antibody (Cat # ab109390, 1:5000, Abcam, United States), Tau

(phospho S202) antibody (Cat # ab108387, 1:5000, Abcam, United States), JNK antibody (Cat # ab179461, 1:500, Abcam, United States), JNK (phospho T183 + Y185) antibody (Cat# ab4821, 1:500, Abcam, United States), c-Jun antibody (Cat # 9165, 1:500, Cell Signaling Technology, United States), c-Jun (phospho Ser63) antibody (Cat # 9261, 1:500, Cell Signaling Technology, United States), GAPDH antibody (Cat # 5174, 1:1000, Cell Signaling Technology, United States). Next, the membranes were washed and incubated with HRP-conjugated secondary antibody for two hours. Then, the ECL detection was used to visualize the bands, which were then quantified using the ImageJ analysis tool.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism8 software. All values were reported as mean \pm SEM. A Student's *t* test or ANOVA, followed by Bonferroni's post hoc test, were used to analyze the differences between groups. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Prickle2 Was Dysregulated in AD

To investigate the role of Prickle2 in AD, we detected the transcription and expression of Prickle2 in a model of AD using 3 \times Tg mice by RT-qPCR, Western blot and IHC, respectively. The results showed that Prickle2 was significantly downregulated in the cortex and the hippocampus of 3 \times Tg mice compared with WT mice (Figures 1A–C). We then analyzed the expression of Prickle2 in 3 \times Tg mice during aging by Western blot. The results showed that Prickle2 expression gradually decreased in 3 \times Tg mice from when they were three months old to 9 months old (Figure 1D). Collectively, these results indicated that the downregulation of Prickle2 in 3 \times Tg-AD mouse model might be related to AD etiology.

Prickle2 Improved the Cognitive Deficits in 3 \times Tg-AD Mice

The downregulation of Prickle2 in 3 \times Tg mice led us to evaluate the role of Prickle2 by the tail intravenous injection of Prickle2 overexpressing AAV-PHP.eB vectors (AAV-PHP.eB-Syn-mScarlet-P2A-MCS-Prickle2) and control AAV-PHP.eB vectors (AAV-PHP.eB-Syn-mScarlet-P2A-MCS) into 6-month-old 3 \times Tg transgenic mice (Figure 2A). We then assessed the distribution of viruses in the brain at three weeks post-injection, using immunofluorescence to confirm that the delivery was effective. Moreover, the neurons of the cortex and the hippocampus in 3 \times Tg mice were infected by AAV-PHP.eB (Figure 2B). This confirmation indicated that AAV-PHP.eB vectors were able to effectively deliver in brain cells in mice after tail intravenous injection. Then, we tested the effect of Prickle2 on the learning and memory deficits in 3 \times Tg mice using the MWM. The escape latency test results suggested that treatment with AAV-PHP.eB-Syn-mScarlet-P2A-MCS-Prickle2, but not AAV-PHP.eB-Syn-mScarlet-P2A-MCS (control), exerted a markedly shorter escape latency on day 5 (Figures 2C,D). In addition, the

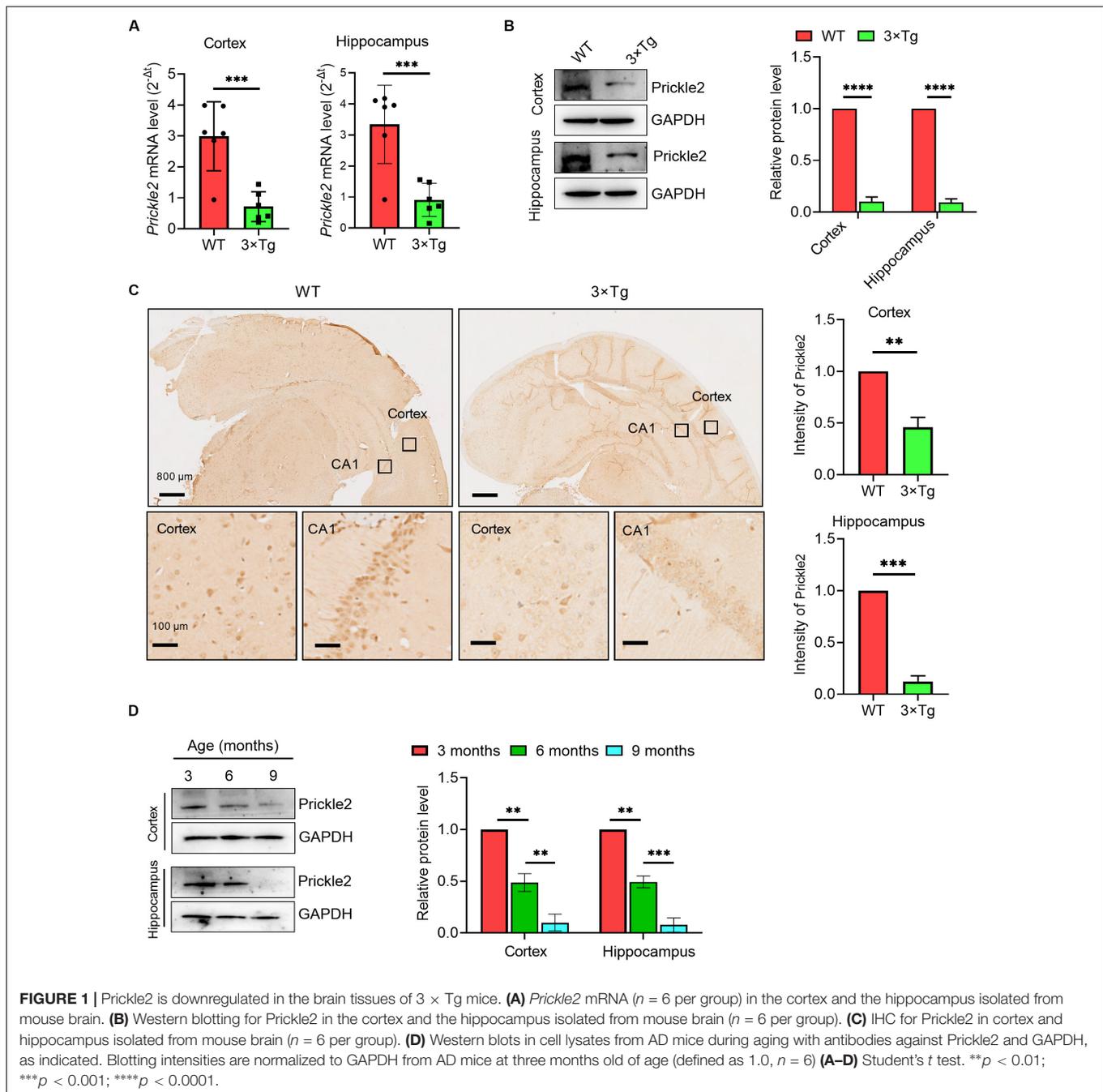
frequency passed the hidden platform for Prickle2-overexpressed 3 \times Tg mice was higher than 3 \times Tg mice on day 6 (Figure 2E). Finally, the spatial probe test (with no platform) results showed that Prickle2-overexpressed 3 \times Tg mice displayed an increased target quadrant occupation compared with the 3 \times Tg mice, indicating that the Prickle2-overexpressed 3 \times Tg mice had a good ability to find the platform compared with the 3 \times Tg mice (Figures 2F–I). The data demonstrated that overexpression of Prickle2 could improve cognitive deficits in 3 \times Tg mice.

Prickle2 Reduced Oxidative Stress and Inflammation in the Brains of 3 \times Tg Mice

In the pathogenesis of AD, neural oxidative stress and neuroinflammation could lead to the progressive impairment of cognition. Therefore, we measured the level of MDA, the activity of SOD and the level of ROS. The results showed that the levels of MDA and ROS were markedly enhanced and the activity of SOD was significantly reduced in the brains of 3 \times Tg mice compared with those of WT mice, however. Prickle2 overexpression markedly relieved the oxidative stress status (Figures 3A–C). Accordingly, we observed that two key neuroinflammatory markers, IL-1 β and IL-6, were noticeably enhanced in the brains of 3 \times Tg mice compared with those of WT mice (Figures 3D,E), whereas the upregulation of Prickle2 significantly inhibited the levels of IL-1 β and IL-6 in 3 \times Tg mice. It is well known that the activation of microglia or astrocytes is an important component in neuroinflammation mediated AD pathology. Herein, we examined whether the upregulation of Prickle2 could inhibit microglia activation in 3 \times Tg mice. Coronal sections of the brain tissues were stained with an antibody against Iba1, which is a biomarker of microglia activation. We showed that the upregulation of Prickle2 displayed less intense immunohistochemical Iba1 labeling in the cortex and hippocampus than did vehicle treated 3 \times Tg mice (Figure 3F). These results indicated that Prickle2 overexpression could repress oxidative stress and neuroinflammation in 3 \times Tg mice.

Prickle2 Reduced Amyloid Plaque Pathology in the Brains of 3 \times Tg Mice

First, we measured the A β profile to determine whether Prickle2 affected the deposition of A β in 3 \times Tg mice. The results of thioflavin S (ThioS) staining showed that Prickle2-overexpressed 3 \times Tg mice displayed fewer mature amyloid plaques than vehicle-treated 3 \times Tg mice (Figure 4A). Then, we found that the fibrillar amyloid burden was significantly reduced by Prickle2-overexpressed 3 \times Tg mice using X-34, which has been proven to label amyloid structures (Figure 4B). In addition, we used β -Amyloid (4G8) immunostaining for detection of total amyloid plaques, which showed a significant decrease in Prickle2-overexpressed 3 \times Tg mice compared with control 3 \times Tg mice (Figure 4C). Moreover, we performed ELISA to measure several A β isoforms in the cortex and the hippocampus. Both soluble and insoluble A β 40 and A β 42 levels in the brains of Prickle2-overexpressed 3 \times Tg mice were significantly decreased compared with vehicle-treated 3 \times Tg mice (Supplementary Figures S1A,B). These results indicated that the upregulation of

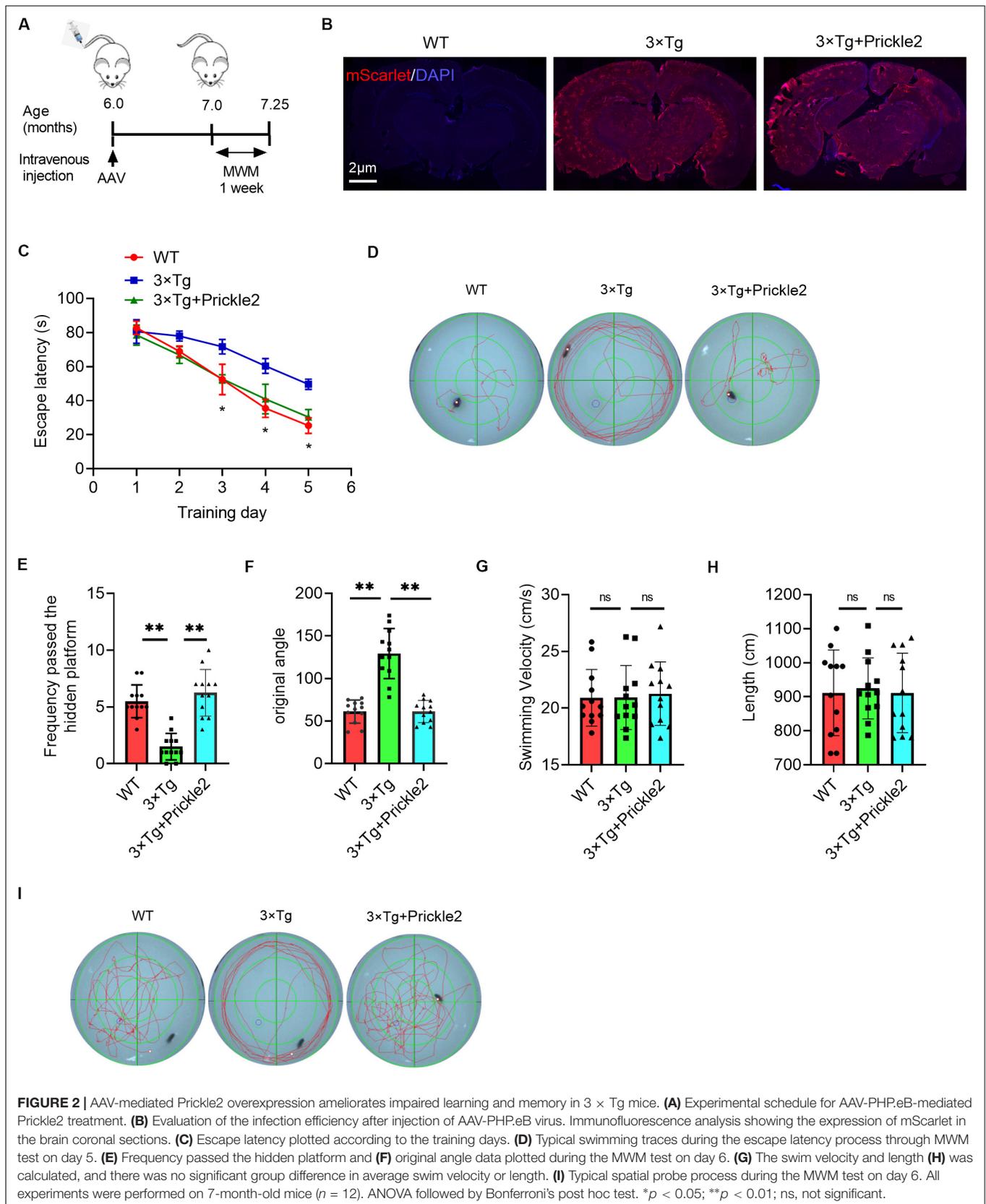


Prickle2 ameliorated amyloid plaque pathology in the brains of 3 × Tg mice.

Prickle2 Ameliorated Tau Phosphorylation in the Brains of 3 × Tg Mice

IHC staining of the cortex and the hippocampus was performed using antibodies against phospho-Tau S202 and S396 to confirm whether upregulation of Prickle2 could affect Tau pathology in 3 × Tg mice. The results of IHC staining showed that

phospho-Tau S202 and pS396 protein levels in the cortex and the hippocampus were markedly reduced in Prickle2-overexpressed 3 × Tg mice than in control 3 × Tg mice (**Figures 5A,B**). Consistently, Western blots results showed that the protein levels of Prickle2 in the cortex and hippocampus were markedly reduced in Prickle2-overexpressed 3 × Tg mice compared with control 3 × Tg mice. In addition, phospho-Tau S202 and phospho-Tau S396 protein levels in the cortex and hippocampus were markedly reduced in Prickle2-overexpressed 3 × Tg mice compared with control 3 × Tg mice (**Figures 5C–F**). These results suggested that Prickle2



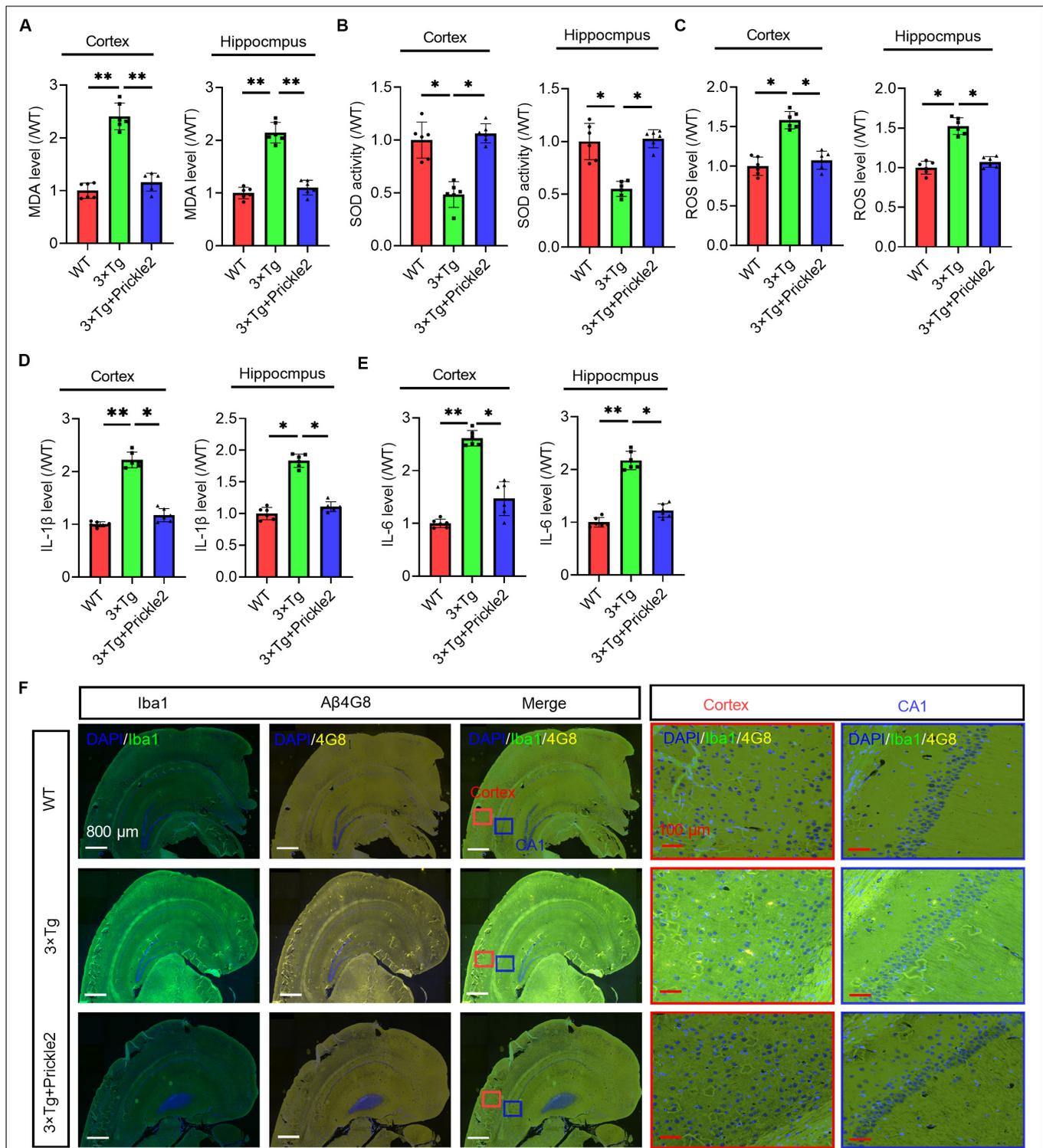


FIGURE 3 | Prickle2 overexpression ameliorates oxidative stress and neuroinflammation in 3 × Tg mice. **(A)** The level of MDA and **(B)** the activity of SOD was determined in the brain of WT, 3 × Tg and 3 × Tg/Prickle2 treatment mice ($n = 6$ per group). **(C)** The ROS level was measured in the supernatants of the brain homogenates of WT, 3 × Tg and 3 × Tg/Prickle2 treatment mice ($n = 6$ per group). **(D)** The levels of IL-1 β and **(E)** IL-6 were determined by ELISA in the brain of WT, 3 × Tg and 3 × Tg/Prickle2 treatment mice ($n = 6$ per group). **(F)** Brain sections of the cortex and hippocampus were stained with anti-Iba1 and anti-A β 4G8 antibodies. **(A–E)** ANOVA followed by Bonferroni's post hoc test. * $p < 0.05$; ** $p < 0.01$.

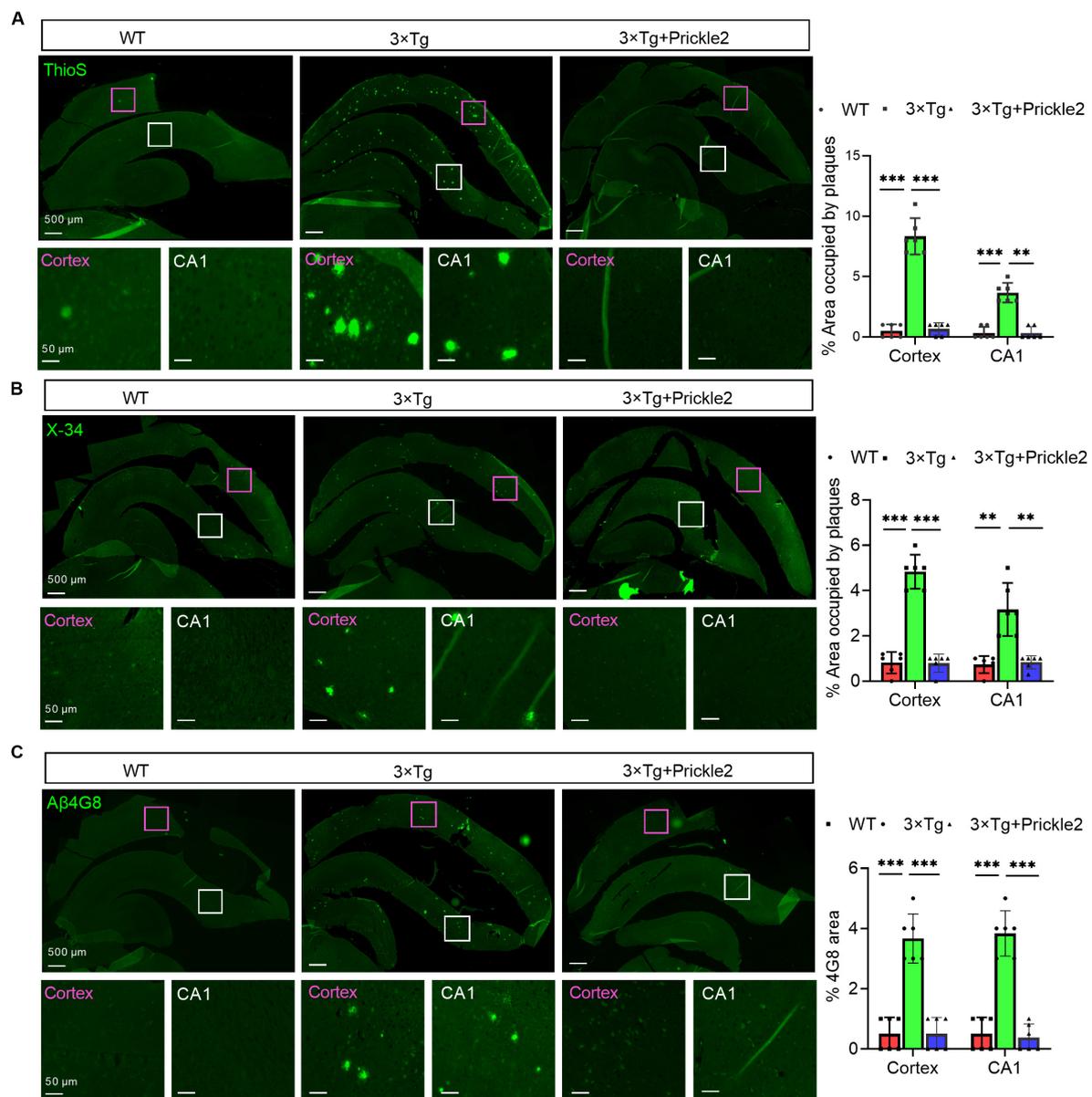


FIGURE 4 | Prickle2 treatment diminishes A β deposition and A β levels in 3 \times TG mice. **(A)** Representative thioflavin S (ThioS, A β plaques) immunofluorescence images in cortex and hippocampus of WT, 3 \times Tg and 3 \times Tg/Prickle2 treatment mice. The percentage of cortical and hippocampal area occupied by A β plaques ($n = 6$ per group). **(B)** Representative images of WT, 3 \times Tg and 3 \times Tg/Prickle2 treatment mice stained with X-34 and the percentage of cortical and hippocampal area stained with X-34 was quantified ($n = 6$ per group). **(C)** Representative immunofluorescence images stained by β -Amyloid 4G8 antibody. The percentage of cortical and hippocampal area stained with β -Amyloid 4G8 antibody was quantified ($n = 6$ per group). ANOVA followed by Bonferroni's post hoc test. ** $p < 0.01$; *** $p < 0.001$.

overexpression reduced Tau hyperphosphorylation in the brains of 3 \times Tg mice.

Prickle2 Ameliorated AD-Like Pathology via Inhibition of the Wnt/PCP Pathway

Prickle2 acts on a core component of the Wnt/PCP pathway, which has shown to transduce signals mainly via the JNK-mediated signaling pathway in AD (Killick et al., 2014). We

further investigated the potential mechanisms responsible for the effect of Prickle2 on AD etiology. We detected the protein levels of JNK, p-JNK (T183/Y185), c-Jun and p-c-Jun (S63) in brain tissues from WT, 3 \times Tg and 3 \times Tg/Prickle2-treated mice by Western blot. Results showed that Prickle2 overexpression markedly reduced the levels of p-JNK (T183/Y185) and p-c-Jun (S63) (Figures 6A,B). We further confirmed the mechanisms responsible for the effect of Prickle2 on the etiology of AD by transfection with an empty vector (Vec),

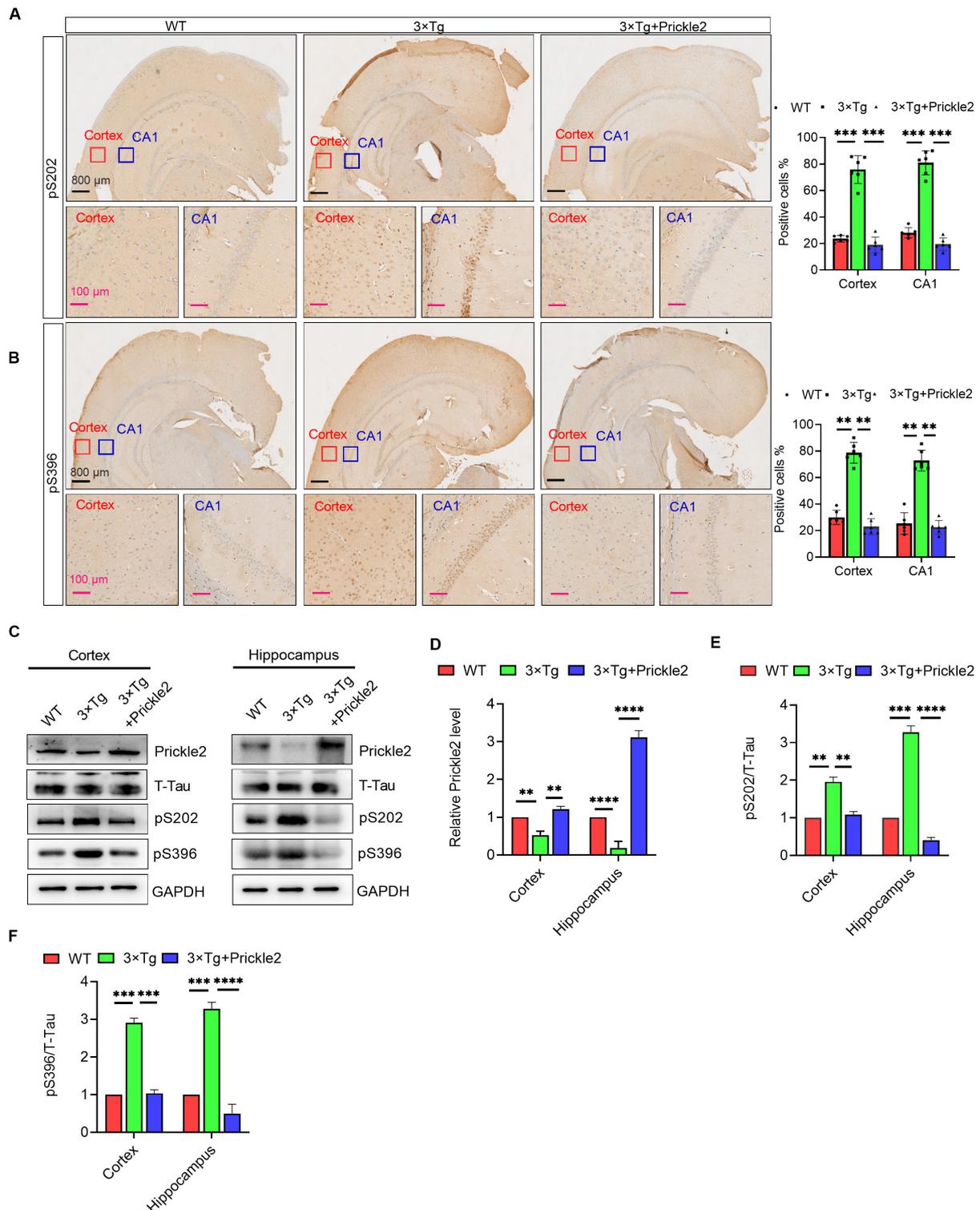
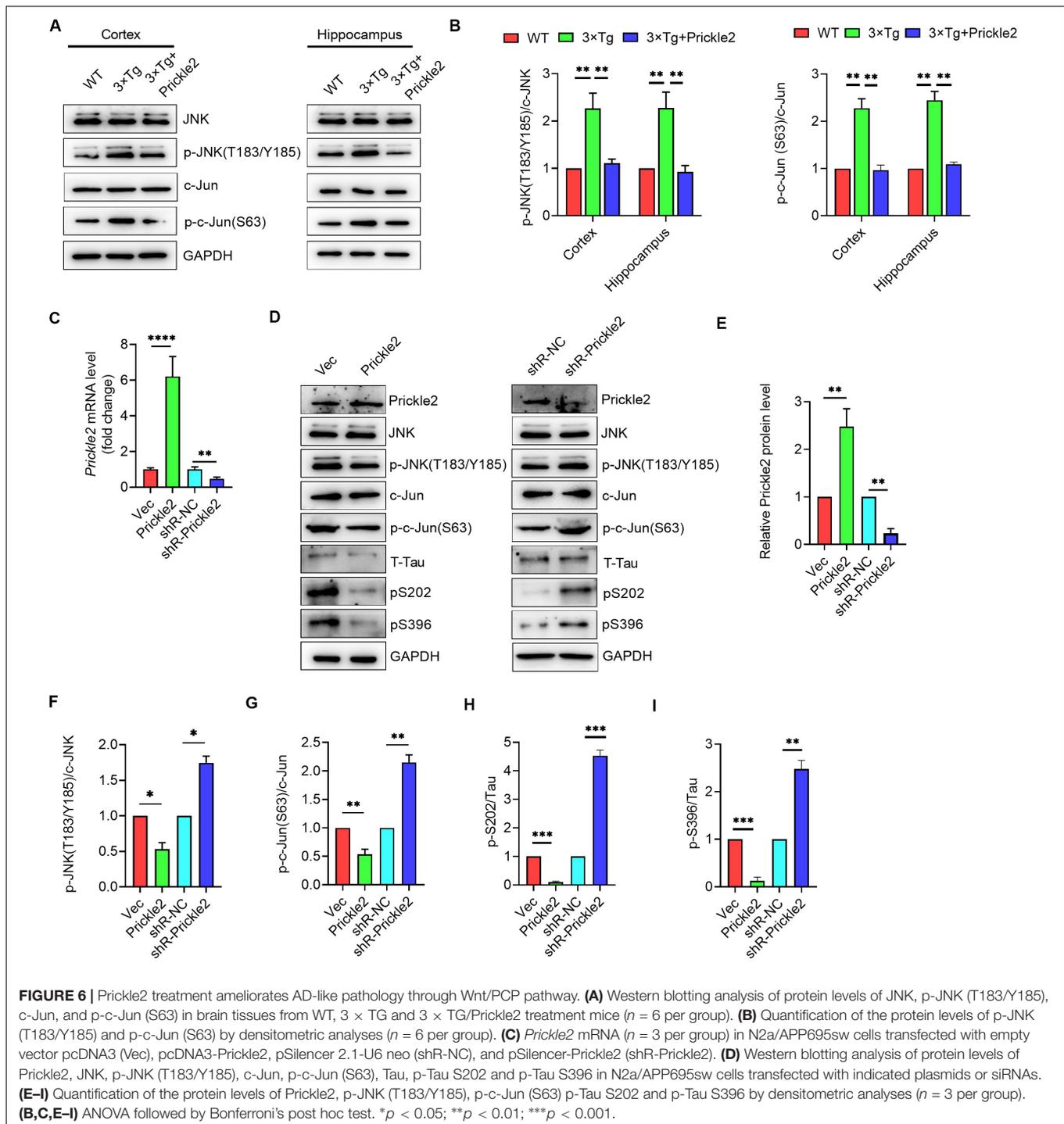


FIGURE 5 | Prickle2 treatment reduces Tau phosphorylation in 3 x Tg mice. **(A)** Representative IHC images of WT, 3 x Tg and 3 x Tg/Prickle2 treatment mice stained with anti-Tau (phospho S202) antibodies. The proportions of pS202-positive cells were calculated in cortical and hippocampal, respectively ($n = 6$ per group). **(B)** Representative IHC images of WT, 3 x Tg and 3 x Tg/Prickle2 treatment mice stained with anti-Tau (phospho S396) antibodies. The proportions of pS396-positive cells were calculated in cortical and hippocampal, respectively ($n = 6$ per group). **(C)** Western blots detected the phosphorylated Tau using pS202 and pS396 antibodies in the brain tissues from WT, 3 x Tg and 3 x Tg/Prickle2 mice. **(D–F)** Quantification of the protein levels of phosphorylated Tau by densitometric analyses ($n = 6$ per group). **(B–D)** ANOVA followed by Bonferroni's post hoc test. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.



pcDNA3/prickle2, si-NC and si-Prickle2 to a cell model of AD (N2a/APP695sw) by stably expressed human APP-695 Swedish mutation (K595N/M596L) in N2a cells. RT-qPCR was performed to detect the transcriptional level of *Prickle2*, and results showed that the level of *Prickle2* mRNA markedly increased after transfection with pcDNA3/Prickle2, whereas the level of *Prickle2* mRNA markedly decreased after transfection with si-Prickle2 in N2a/APP695sw cells (Figure 6C). In addition, we

detected the protein levels of Prickle2, JNK, p-JNK (T183/Y185), c-Jun, p-c-Jun (S63), phospho-Tau S202 and pS396 by Western blot. Results showed that Prickle2 overexpression markedly decreased the levels of p-JNK (T183/Y185), p-c-Jun (S63), phospho-Tau S202 and pS396, whereas the silence of Prickle2 markedly increased the levels of p-JNK (T183/Y185), p-c-Jun (S63), phospho-Tau S202 and pS396 in N2a/APP695sw cells (Figures 6D–I). Taken together, these results suggested that

Prickle2 overexpression alleviated AD-like neurodegeneration via inhibiting the Wnt/PCP pathway.

DISCUSSION

Alzheimer's disease is a complex and progressive cognitive dysfunction disease. Although many molecules have been proven to be involved in the pathogenesis of AD, the etiology of AD remains unclear. Recent studies have shown that the Wnt/PCP pathway contributed to the development and several human diseases (Yang, 2012; Zhang et al., 2017; Humphries and Mlodzik, 2018; Lopez-Escobar et al., 2018; Sellers et al., 2018). Therefore, further investigation of specific facets of the Wnt/PCP pathway might confer certain advantages to improve our understanding regarding the diagnosis and treatment of AD in the future.

To our best knowledge, Wnts are known as ligands for FZD receptors of PCP signaling (Bhanot et al., 1996; Wang et al., 2006). Recently, Wnt5a was demonstrated to interact genetically with Ltap/Vangl2 and functioned in the regulation of PCP in mice (Qian et al., 2007). Tang et al. reported that Wnt5a overexpression promoted A β -evoked inflammatory responses in AD-related neurodegeneration during AD pathogenesis (Li et al., 2011). In addition, APP was demonstrated to activate Wnt/PCP signaling by physical interaction with the Wnt co-receptor VANGL2 to promote synapse loss and A β production (Elliott et al., 2018). Moreover, Disheveled 1, a member of the PCP core components, has been demonstrated to increase sAPPa and reduce Tau phosphorylation via both JNK and protein kinase C (PKC)/mitogen-activated protein kinase signaling *in vitro* (Mudher et al., 2001). Taken together, several core components of Wnt/PCP signaling were demonstrated to be involved in the pathogenesis of AD.

Prickle2, the cytoplasmic regulator of Wnt/PCP signaling, has been reported to localize with Frizzled and Disheveled proteins (Katoh and Katoh, 2003). Okuda et al. (2007) have illustrated that the loss of Prickle2 could markedly reduce the neurite outgrowth levels in mouse N2a cells. Furthermore, Prickle2 was demonstrated to interact with PSD-95 and NMDA receptors and localize in the postsynaptic density in the mice brain (Hida et al., 2011). In addition, (Lopez-Escobar et al., 2018) have demonstrated that the Prickle2 deletion could cause autism-like behaviors and hippocampal synaptic dysfunction in mice. Moreover, (Sowers et al., 2013) found that morphological deficits caused by the Prickle2 deletion in hippocampal neurons could be rescued by the non-canonical Wnt ligand Wnt5a, which has been demonstrated to be involved in the pathogenesis of AD. Although several studies have demonstrated that Prickle2 could be related to the nervous system, the exact function of this gene in the etiology of AD was unreported.

Herein, we first reported that the *Prickle2* mRNA level was significantly downregulated in the cortex and the hippocampus of 3 \times Tg mice, indicating that Prickle2 might be related to the etiology of AD. Subsequently, we upregulated the expression level of Prickle2 in the brains of 3 \times Tg mice by intracerebral injection of Prickle2-overexpressing AAV9 vectors and found that Prickle2 could improve the cognitive deficits in 3 \times Tg

mice. In addition, we found that the upregulation of Prickle2 inhibited oxidative stress, neuroinflammation, amyloid plaque pathology and Tau hyperphosphorylation in the brains of 3 \times Tg mice. It was well known that A β accumulation increased the superoxide anion, MDA and ROS. However, it also inhibited the activity of catalase and SOD, aggravated oxidative damage and neuroinflammation in the brain with AD (Cai et al., 2014; Tonnies and Trushina, 2017). In our study, we showed that the contents of MDA and ROS were markedly enhanced, and the activity of SOD was greatly reduced in 3 \times Tg mice, whereas Prickle2 overexpression relieved the oxidative stress. IL-1 β and IL-6, two key proinflammatory factors that could also exacerbate AD pathology, have been reported to be increased by A β accumulation in 3 \times Tg mice (de Lemos et al., 2013; Hoeijmakers et al., 2017). Our study found that Prickle2 overexpression reduced the levels of IL-1 β and IL-6 in 3 \times Tg mice. Taken together, the above results indicated that Prickle2 could ameliorate AD-like pathology in 3 \times Tg mice.

We further investigated the potential mechanisms responsible for the effect of Prickle2 on the etiology of AD. We found that Prickle2 overexpression alleviated AD-like neurodegeneration via the inhibition of the Wnt/PCP/JNK pathway *in vivo* and *in vitro*. Activation of the JNK signaling pathway has been well known as a critical regulator for neurodegenerative diseases, plaque formation, neuroinflammation and maturation in the pathogenesis of AD (Yarza et al., 2015; Zhu et al., 2019). In addition to Wnt/PCP pathway, numerous studies have shown that a sustained loss of function of Wnt/ β -catenin signaling components underlies the onset and progression of the AD (De Ferrari et al., 2014). Activation of Wnt- β -catenin signaling could stabilize synapses and increase the formation of new ones, while Wnt-PCP signaling could destabilize synapses and enhance the loss of synaptic connections (Elliott et al., 2018; Palomer et al., 2019). Mattes et al. (2018) reported that Wnt/PCP signaling could control the spreading of Wnt/ β -catenin signals by cytonemes in vertebrates. In summary, a number of studies strongly suggests that restoring or boosting Wnt signaling could protect cells and synapses from A β toxicity and ameliorate AD pathology.

In summary, we first demonstrated that Prickle2 was downregulated in the cortex and the hippocampus of 3 \times Tg mice. The role of Prickle2 in the pathogenesis of AD was demonstrated by tail intravenous injection of Prickle2-overexpressing AAV-PHP.eB vectors. We found that Prickle2 could improve the cognitive deficits in 3 \times Tg mice by ameliorating the oxidative stress, neuroinflammation, amyloid plaque pathology and Tau hyperphosphorylation. Further investigation of the mechanism of Prickle2 in AD revealed that Prickle2 could inhibit the Wnt/PCP/JNK pathway *in vivo* and *in vitro*. Our results suggested that Prickle2 holds potential as a promising candidate for the diagnosis and treatment of AD.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/Supplementary Material.

ETHICS STATEMENT

The animal study was reviewed and approved by Animal Care and Use Committee of Tianjin Medical University. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

WL conceived the study. WL, FS, FJ, and WT designed all figures and wrote the manuscript. FS and FJ performed the experiments. NZ, HL, and WT performed the data analysis. All authors reviewed the manuscript and approved its final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcell.2020.565020/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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