Impact of TREM2R47H variant on tau pathology-induced gliosis and neurodegeneration

Maud Gratuze
*Washington University School of Medicine in St. Louis*

Cheryl Eg Leyns
*Washington University School of Medicine in St. Louis*

Andrew D Sauerbeck
*Washington University School of Medicine in St. Louis*

Marie-Kim St-Pierre
*University of Victoria*

Monica Xiong
*Washington University School of Medicine in St. Louis*

See next page for additional authors

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Authors
Maud Gratuze, Cheryl Eg Leyns, Andrew D Sauerbeck, Marie-Kim St-Pierre, Monica Xiong, Nayeon Kim, Javier Remolina Serrano, Marie-Ève Tremblay, Terrance T Kummer, Marco Colonna, Jason D Ulrich, and David M Holtzman
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Impact of TREM2<sup>R47H</sup> variant on tau pathology–induced gliosis and neurodegeneration

Maud Gratuze,1,2,3 Cheryl E.G. Leyns,1,2,3 Andrew D. Sauerbeck,1 Marie-Kim St-Pierre,4,5 Monica Xiong,1,2,3 Nayeon Kim,1,2,3 Javier Remolina Serrano,1,2,3 Marie-Ève Tremblay,4,5 Terrance T. Kummer,1 Marco Colonna,2,3,6 Jason D. Ulrich,1,2,3 and David M. Holtzman1,2,3

1Department of Neurology, 2Hope Center for Neurological Disorders, and 3Knight Alzheimer’s Disease Research Center, Washington University School of Medicine, St. Louis, Missouri, USA. 4Axe Neurosciences, Centre de Recherche, Centre Hospitalier Universitaire (CHU) de Québec-Université Laval, Québec City, Québec, Canada. 5Division of Medical Sciences, University of Victoria, Victoria, British Columbia, Canada. 6Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri, USA.

Alzheimer’s disease (AD) is characterized by plaques containing amyloid-β (Aβ) and neurofibrillary tangles composed of aggregated, hyperphosphorylated tau. Beyond tau and Aβ, evidence suggests that microglia play an important role in AD pathogenesis. Rare variants in the microglia-expressed triggering receptor expressed on myeloid cells 2 (TREM2) gene increase AD risk 2- to 4-fold. It is likely that these TREM2 variants increase AD risk by decreasing the response of microglia to Aβ and its local toxicity. However, neocortical Aβ pathology occurs many years before neocortical tau pathology in AD. Thus, it will be important to understand the role of TREM2 in the context of tauopathy. We investigated the impact of the AD-associated TREM2 variant (R47H) on tau-mediated neuropathology in the PS19 mouse model of tauopathy. We assessed PS19 mice expressing human TREM2<sup>CV</sup> (common variant) or human TREM2<sup>R47H</sup>. PS19-TREM2<sup>R47H</sup> mice had significantly attenuated brain atrophy and synapse loss versus PS19-TREM2<sup>CV</sup> mice. Gene expression analyses and CD68 immunostaining revealed attenuated microglial reactivity in PS19-TREM2<sup>R47H</sup> versus PS19-TREM2<sup>CV</sup> mice. There was also a decrease in phagocytosis of postsynaptic elements by microglia expressing TREM2<sup>R47H</sup> in the PS19 mice and in human AD brains. These findings suggest that impaired TREM2 signaling reduces microglia-mediated neurodegeneration in the setting of tauopathy.
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The TREM2 R47H variant results in decreased TREM2 signaling upon ligand-induced stimulation (14, 15). In order to better understand the role of TREM2 and AD-associated TREM2 variants in AD pathogenesis, several studies have used models of AD pathology. In vivo studies in mouse models of amyloid deposition indicate a critical function for TREM2 in the clustering of microglia around plaques, plaque compaction, microglial proliferation, and phagocytosis, and in decreasing Aβ-induced neuritic dystrophy and tau seeding/spreading. TREM2 knockout or expression of the TREM2 R47H variant have been shown to affect these phenotypes in the opposite direction (14–18). These studies suggest that TREM2 signaling is protective in decreasing Aβ-mediated local injury and in exacerbating the Aβ-induced spreading of tau pathology. However, in AD and in primary tauopathies, the accumulation of aggregated, hyperphosphorylated tau, and not Aβ, strongly correlates with local brain atrophy and neuronal death. In AD, the accumulation of pathological forms of tau in the neocortex associated with atrophy and cell death does not begin to occur until about 15–25 years after the onset of Aβ deposition (19). The role of TREM2 and TREM2 variants linked to AD in the setting of tauopathy has been much less studied. However, numerous clues in the literature suggest a link between tau pathology and TREM2 in AD. In the cerebrospinal fluid (CSF) of AD patients, soluble TREM2 has been shown to correlate with total and phosphorylated tau (p-tau, Thr181) levels (20, 21). Moreover, AD patients harboring the R47H variant of TREM2 display higher levels of both total tau and p-tau (Thr181) in CSF compared with noncarriers (22, 23). Importantly, levels of p-tau in the CSF correlate with tau pathology burden in the brain and with neuronal loss and cognitive decline in AD patients (24). Previously, we reported a decrease in brain atrophy as well as attenuated microgliosis in the brains of PS19 mice lacking TREM2 without a significant change in tau pathology (25). In another study, Sayed et al. also found that TREM2 knockout protected against tau-mediated microglial reactivity and atrophy (25), but that TREM2 haploinsufficiency was associated with elevated expression of proinflammatory markers, exacerbated atrophy, and increased tau pathology in PS19 mice (26). Finally, Bemiller et al. crossed TREM2 knockout mice with mice expressing human tau (hTau mice), a less aggressive mouse model of tauopathy, and identified a decrease in microgliosis in TREM2-deficient hTau mice (27), as observed in TREM2-deficient PS19 mice, but reported that complete deletion of TREM2 in hTau mice exacerbated tau pathology-related markers. These studies in primary tauopathy mouse models suggest that TREM2 may play a dual role depending on the disease stages; during early stages of tau pathology development in the absence of neurodegeneration (e.g., hTau mice), decreased TREM2 function might promote tau pathology, whereas the complete loss of TREM2 function in advanced stages (PS19 mice) of the disease seems to protect from neurodegeneration. However, no study to our knowledge has evaluated the impact of AD-associated TREM2 variants, such as TREM2 R47H, in the setting of a model of pure tauopathy, nor in a model that develops robust neurodegeneration as is seen in tau-related neurodegenerative diseases.

Given the emerging role for microglia in tau-mediated neuro-pathology, it is important to understand the role of AD-associated TREM2 variants that decrease, but do not eliminate, TREM2 signaling in the context of tauopathy and its associated neurodegeneration. Therefore, we investigated the impact of the most prominent AD-associated TREM2 variant (R47H) on tau pathology and tau-mediated brain damage in the PS19 mouse model of tauopathy. PS19 mice were crossed with mice expressing human TREM2 common variant, TREM2 CV (PS19-T2 CV mice), or human TREM2 R47H lacking the endogenous murine Trem2 gene (PS19-T2 R47H mice). We hypothesized that TREM2 R47H would result in a decreased microglial inflammatory response and would protect against tau-dependent neurodegeneration. We observed that PS19-T2 R47H mice have reduced p-tau staining compared with PS19-T2 CV mice. Moreover, PS19-T2 R47H mice have significantly attenuated brain atrophy and synapse loss compared with PS19-T2 CV mice. Gene expression analyses and CD68 immunostaining revealed attenuated microglial reactivity in PS19-T2 R47H versus PS19-T2 CV mice. Importantly, a decrease in phagocytosis of postsynaptic elements by microglia expressing TREM2 R47H was detected in PS19 mice and in postmortem human AD brains. Collectively, these findings suggest that reduced TREM2 signaling reduces microglial conversion to a more proinflammatory, phagocytic state and is protective against neurodegeneration in the setting of advanced tauopathy.

Results

Effects of TREM2 R47H on p-tau pathology. To determine whether the presence of the R47H variant of TREM2 affects tau pathology and tau-related inflammation and neurodegeneration, we used the PS19 tauopathy mouse model that overexpresses IN4R hTau containing the P301S mutation that causes a familial form of frontotemporal dementia. By 9 months of age, this model develops strong tau hyperphosphorylation and aggregation, neurofibrillary tangle deposition, and gliosis, as well as neuronal loss, brain atrophy, and loss of synaptic proteins in specific brain regions including the hippocampus, entorhinal cortex, piriform cortex, and amygdala (28, 29). We crossed PS19 mice on a mouse TREM2-KO background with bacterial artificial chromosome (BAC) transgenic mouse lines expressing either human TREM2 CV or the AD-associated human variant TREM2 R47H on a mouse TREM2-KO background (15). Importantly, TREM2 CV and TREM2 R47H mice express a similar level of TREM2 protein in the brain and expression of TREM2 CV, but not TREM2 R47H, rescues TREM2-dependent phenotypes such as plaque-associated microgliosis (15).

At 3 months of age, before the overt onset of tau pathology, we observed reduced staining with an antibody (AT8) specific for p-tau phosphorylated on serine 202 and threonine 205 (p.Ser202/p. Thr205), in the hippocampus (Supplemental Figure 1, B and D; supplemental material available online with this article; https://doi.org/10.1172/JCI138179DS1) and piriform cortex (Supplemental Figure 1, A and C) of PS19-T2 R47H mice compared with PS19-T2 CV mice, although the change was only statistically significant in the hippocampus. Staining with antibodies AT180 (p.Thr231) and PG5 (p.Ser409) did not reveal any significant differences in p-tau between PS19-T2 R47H mice and PS19-T2 CV mice in the piriform cortex (Supplemental Figure 1, E, G, I, and K) or hippocampus (Supplemental Figure 1, F, H, J, and L). To further examine tau and p-tau levels in these mice, we performed a biochemical extraction of hippocampal brain tissue and measured the concentration of...
Figure 1. R47H variant of TREM2 attenuates tau pathology in 9-month-old PS19 mice. Representative images of p-tau staining (AT8, AT180, and PG5) in the piriform cortex (A, E, and I) and hippocampus (B, F, and J) from 9-month-old PS19-T2CV and PS19-T2R47H mice. Scale bars: 1 mm. Quantification of the percentage area covered by p-tau staining (antibodies AT8, AT180, and PG5) in the piriform cortex (C, G, and K) and hippocampus (D, H, and L). Data are presented as the mean ± SEM. Significance was determined using an unpaired, 2-tailed Mann-Whitney test due to the nonparametric data set, except for PG5 staining in the hippocampus where an unpaired, 2-tailed t test with Welch’s correction was used due to significantly different variances. *P < 0.05 (PS19-T2CV, n = 18 and PS19-T2R47H, n = 15/16). (M–O) ELISA results showing concentrations of p-tau (p.Ser202/p.Thr205 and p.Thr181) and total tau and p-tau/total tau ratio in the hippocampus were quantified using a human tau–specific (hTau-specific) sandwich ELISA to measure p-tau (M), total tau (N), and the p-tau/total tau ratio (O). The levels of p-tau and total tau (M and N) were normalized to total protein concentration. Data are presented as mean ± SEM. Significance was determined by an unpaired, 2-tailed Student’s t test except for total tau where an unpaired, 2-tailed Mann-Whitney test was used due to the nonparametric data set. *P < 0.05 (PS19-T2CV, n = 17 and PS19-T2R47H, n = 15).
p-tau and total tau using a p-tau-specific (p.Ser202/p.Thr205 and p.Thr181) and hTau-specific ELISA. We observed no significant differences in p-tau (Supplemental Figure 1M) and tau levels (Supplemental Figure 1N) or in the p-tau/tau ratio (Supplemental Figure 1O) between PS19-T2TRE突变 mice and PS19-T2CV mice. At 9 months of age, when substantial tau pathology had developed, we again observed a significant decrease in hippocampal p-tau staining on serine 202/threonine 205 (Figure 1, B and D) as well as on serine 409 (Figure 1, J and L) between PS19-T2TRE突变 and PS19-T2CV mice. There was no significant difference observed on these p-tau epitopes in the piriform cortex (Figure 1, A, C, I, and K). Interestingly, staining p-tau on threonine 231 revealed less p-tau in the piriform cortex of PS19-T2TRE突变 mice compared with PS19-T2CV mice (Figure 1, F and H), but no difference in the hippocampus (Figure 1, E and G). Biochemical analysis of p-tau in hippocampal brain tissue samples by ELISA revealed no significant differences in p-tau level (Figure 1M) but increased total tau levels in PS19-T2TRE突变 mice compared with PS19-T2CV mice (Figure 1N), resulting in a slight but not statistically significant (P = 0.0815) decrease in the p-tau/tau ratio (Figure 1O) in PS19-T2TRE突变 mice compared with PS19-T2CV mice. These findings demonstrate that the R47H variant of TREM2 significantly attenuates decreases in p-tau on some tau sites mostly in the later stages of tau pathology in PS19 mice. Interestingly, we (25) and others (27) did not observe lower p-tau pathology in PS19 mice with TREM2 deleted compared with PS19 mice. New studies will be needed to better understand this difference, but we can hypothesize that the human TREM2R47H assessed in the present study has a slightly different impact on tau pathology than mouse TREM2 used in the previous studies.

Effects of TREM2R47H on brain atrophy. Because pathological tau is directly linked to neurodegeneration, we next evaluated the brain volume of these mice. As expected, no change in brain volume was detected between PS19-T2TRE突变 and PS19-T2CV mice at 3 months of age, which is before when neurodegeneration has been observed in this model (Supplemental Figure 2, A–E). At 9 months of age, which is before when neurodegeneration has been extensively reported in PS19 knockout for TREM2 (25), we found that glial fibrillary acidic protein (GFAP) gene expression, a marker of reactive astrocytes, was decreased in PS19 mice expressing TREM2R47H compared with PS19-T2CV mice. When we assessed microglial coverage in 9-month-old mice, we observed a marked decrease in Iba1 staining in the piriform cortex (Figure 3, B and D) and the hippocampus (Figure 3, C and E) of PS19-T2TRE突变 in comparison with PS19-T2CV mice. Consistent with these results, Iba1 gene expression was reduced by approximately 32% in the cortex of PS19-T2TRE突变 versus PS19-T2CV mice (Figure 3A). Importantly, reduced microgliosis induced by TREM2R47H in PS19 mice was qualitatively similar to previous observations when TREM2 was deleted in the same mouse model (25).

We next assessed microglial phenotypic and functional changes in the mice (Figure 4). CD68 (cluster of differentiation 68) labeling, a marker of phagolysosomal activity, was drastically reduced in the piriform cortex (Figure 4C) and the hippocampus (Figure 4D) of 9-month-old PS19-T2TRE突变 compared with PS19-T2CV mice. Strikingly, there was a decrease in CD68 staining of approximately 78% per Iba1+ cell volume detected in PS19-T2TRE突变 versus PS19-T2CV brains (Figure 4, E and F), confirming a reduced activation state of PS19-T2TRE突变 microglia. We also assessed the microglial gene expression profile in the cortex of 9-month-old PS19-T2TRE突变 and PS19-T2CV mice (Figure 4G). Consistent with lower CD68 staining in PS19-T2TRE突变 mice, all the DAM genes tested were downregulated in PS19-T2TRE突变 compared with PS19-T2CV mice such as Axl, Cst7, and Cd9. Interestingly, an increase in the expression of the homeostatic gene P2ry12 was observed in PS19-T2TRE突变 mice, suggesting a more homeostatic status of microglia compared with PS19-T2CV mice. On the other hand, Cx3cr1 gene expression was decreased in PS19 mice expressing TREM2R47H, which may be due to a lower number of microglia in these mice. Evaluation of several inflammatory cytokine genes revealed a significant decrease in proinflammatory mediators such as IL-1α, IL-1β, TNF-α, and TGF-β. IL-6 gene expression was not altered by the AD-associated TREM2 variant in PS19 mice, as previously reported in PS19 knockout for TREM2 (25).

Another brain cell type, astrocytes, also plays an essential role in neuroinflammation. It has been previously shown that reactive microglia can induce astrogliosis through the production of cytokines as IL-1α and TNF-α that can promote neuronal death (32, 33). As previously observed in PS19 mice lacking TREM2 (25), we found that glial fibrillary acidic protein (GFAP) gene expression, a marker of reactive astrocytes, was decreased in 9-month-old PS19-T2TRE突变 compared with PS19-T2CV mice (Supplemental Figure 4A). In addition, the GFAP signal was drastically reduced in the piriform cortex (Supplemental Figure 4, B and D) of PS19-T2TRE突变 compared with PS19-T2CV mice, but...
in synapses of PS19 mice and AD patients (34). Synaptic C1q accumulation correlated with augmented microglial engulfment of synapses in vivo and decline of synapse density in vitro. Because we previously showed that C1q gene expression can be modulated by the absence of TREM2 in PS19 mice, we assessed how TREM2 R47H affected protein and gene expression not in the hippocampus (Supplemental Figure 4, E and C). Taken together, these data suggest that the R47H variant of TREM2 strongly reduced microglial activation and the concomitant astrogliosis in the setting of tauopathy.

**Effects of TREM2 R47H on synapses.** A recent study by Dejanovic et al. reported a striking accumulation of complement C1q in synapses of PS19 mice and AD patients (34). Synaptic C1q accumulation correlated with augmented microglial engulfment of synapses in vivo and decline of synapse density in vitro. Because we previously showed that C1q gene expression can be modulated by the absence of TREM2 in PS19 mice, we assessed how TREM2 R47H affected protein and gene expression not in the hippocampus (Supplemental Figure 4, E and C). Taken together, these data suggest that the R47H variant of TREM2 strongly reduced microglial activation and the concomitant astrogliosis in the setting of tauopathy.

**Figure 2. R47H variant of TREM2 decreases neurodegeneration in 9-month-old PS19 mice.** (A) Representative images of PS19-T2C

and PS19-T2R47H brain sections stained with Sudan black at 9 months of age. Scale bars: 1 mm. (B–E) Quantification of the average volume of the hippocampus (B), ventricles (C), entorhinal and piriform cortices (D), and half brain minus ventricle (E). (F–I) Thickness of the pyramidal layer of the piriform cortex (F and H) and the granule cell layer of the dentate gyrus (DG) (G and I) in 9-month-old mice with cresyl violet staining. Data are presented as mean ± SEM. Significance was determined by an unpaired, 2-tailed Student’s t test. *P < 0.05, **P < 0.01 (9-month-old: PS19-T2C, n = 18/19 and PS19-T2R47H, n = 16).
levels of C1q, as well as C1q+ synapses in PS19 mice. First, we found that $C_{1q}$a gene expression was reduced by approximately 60% in the cortex of 9-month-old PS19-T2CV compared with the PS19-T2R47H mice (Figure 5C). Consistent with these findings, C1q staining and confocal analysis revealed reduced C1q staining in PS19-T2R47H mice (Figure 5B). To evaluate C1q accumulation at putative synapses, we then assessed the number of puncta labeled with pre- and postsynaptic markers that were positive for C1q. PSD-95+ puncta and synapsin+ puncta positive for C1q were significantly decreased in PS19-T2R47H versus PS19-T2CV mice (Figure 5, A, D, and E).

We next assessed whether the decrease in C1q-opsonized synaptic-marker puncta and attenuated brain atrophy would correspond to improved synaptic integrity in PS19-T2R47H mice. We therefore quantified presynaptic (synapsin) and postsynaptic (PSD-95) marker puncta in PS19-T2R47H and PS19-T2CV mice in the polymorphic layer of the piriform cortex (Figure 6A). This revealed significantly more pre- and postsynaptic puncta in PS19-T2R47H compared with PS19-T2CV mice (Figure 6, B and C). As many such puncta are nonsynaptic (35, 36), we turned to a more specific method of quantifying intact synaptic loci. Using SEQUIN analysis (synaptic evaluation and quantification by imaging of nanostructure) (36), a super-resolution imaging and image analysis platform for quantifying synaptic loci, we detected significantly more intact synaptic loci in PS19-T2R47H versus PS19-T2CV mice (Figure 6D). To better understand ultrastructural changes at synapses under these conditions, electron microscopy was undertaken in a small parallel cohort in the polymorphic layer of the piriform cortex. Synapses from PS19-T2CV mice exhibited several dystrophic features, including swollen presynaptic elements, accumulation of enlarged autophagosomes, and a reduced number of synaptic vesicles (Figure 6E). Moreover, mitochondria often appeared swollen, a feature also seen with hypoxia, and abundant dark dendritic spines, indicative of postsynaptic cellular stress, were visible (Figure 6E). In contrast, electron microscopic images from PS19-T2R47H mice revealed improved synaptic integrity with fewer autophagosomes, preserved mitochondrial ultrastructure, and less frequent dark dendrites/spines and axon terminals (Figure 6F). Importantly, these results demonstrate that reduced brain atrophy in PS19-T2R47H mice is associated with decreased synapses with dystrophic features and better preservation of pre- and postsynaptic elements.

**Effects of TREM2R47H on microglial phagocytosis of synaptic elements.** Regarding the role of TREM2 in microglial phagocytosis (37, 38) and the fact that we observed strong reduction of CD68+ phagolysosomes in microglia of PS19-T2R47H versus PS19-T2CV mice, we then hypothesized that impairment of microglial phagocytosis in 9-month-old PS19-T2R47H mice is responsible for the decreased synapse loss. A previous study by Filipello et al. (39) demonstrating that microglial TREM2 is required for synapse elimination during the early stages of brain development strongly support this hypothesis. Costaining and confocal analysis for the microglial phagolysosomal marker CD68 and the postsynaptic marker PSD-95 revealed significantly fewer PSD-95 puncta in CD68+ vesicles per microglia in the polymorphic layer of the piriform cortex of 9-month-old PS19-T2R47H compared with PS19-T2CV mice (Figure 7, A and B). Consistent with the higher content of synapses in 9-month-old PS19-T2R47H mice, these data show an important role for TREM2 in synapse loss in PS19 mice that is decreased by TREM2R47H in a pure tauopathy model.

Given these results, to assess the relevance of these findings in the setting of tauopathy in human AD, we assessed whether the brains of individuals who were TREM2-variant carriers who had AD exhibited similar reductions of postsynaptic puncta engulfed in CD68+ phagolysosomes compared with AD cases expressing TREM2C. We stained prefrontal cortex tissue from late-onset AD TREM2R47H and TREM2R62H individuals and their case-matched controls for Iba1, CD68, and PSD-95. R62H is another TREM2 variant associated with an increased risk for AD (40, 41). Consistent with our mouse data, we detected significantly fewer PSD-95 puncta engulfed in CD68+ vesicles per microglia in AD TREM2R47H and TREM2R62H brains compared with AD brains with TREM2C (Figure 7, C and D). The detection of reduced synaptic phagocytosis by microglia expressing R47H or R62H variants of TREM2 in AD cases confirms the defective synapse elimination by microglia with TREM2 loss of function. Taken together, these data suggest that microglia expressing TREM2R47H phagocytose fewer synapses due to lower C1q accumulation in synapses, resulting in neuroprotection in the setting of tauopathy.

**Discussion**

TREM2R47H has been associated with higher risk of developing AD. Data from animal models and humans suggest that the
Figure 4. Reduced microglial activation and inflammatory gene expression in 9-month-old PS19-T2<sup>CV</sup> versus PS19-T2<sup>R47H</sup> mice. (A and B) Representative images of CD68 staining from 9-month-old PS19-T2<sup>CV</sup> (A) and PS19-T2<sup>R47H</sup> (B) brains. (C and D) Quantification of the percentage area covered by CD68 staining in the piriform cortex (C) and hippocampus (D) (PS19-T2<sup>CV</sup>, n = 18 and PS19-T2<sup>R47H</sup>, n = 18). (E) Confocal images and 3D reconstruction of CD68+ structures within Iba1+ microglia in PS19-T2<sup>CV</sup> and PS19-T2<sup>R47H</sup>. Scale bars: 10 μm. (F) Quantification of CD68+ structures per Iba1 volume (PS19-T2<sup>CV</sup>, n = 19 and PS19-T2<sup>R47H</sup>, n = 15). Data are presented as mean ± SEM. Significance was determined using an unpaired, 2-tailed Mann-Whitney test due to the nonparametric data set. ** P < 0.01, *** P < 0.001, **** P < 0.0001. (G) Heatmap analysis of microglial gene expression in 9-month-old PS19-T2<sup>CV</sup> and PS19-T2<sup>R47H</sup> piriform cortex generated by hierarchical gene clustering based on genotypes. DAM genes, disease-associated microglia genes. For IL-1α, P2ry12, and TNF-α, significance was determined by an unpaired, 2-tailed Student’s t test. For Apoe, Cst7, Il-1β, and Il-6, significance was determined using an unpaired, 2-tailed Mann-Whitney test due to the nonparametric data set. For all other analyses, significance was determined by an unpaired, 2-tailed t test with Welch’s correction due to significantly different variances. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 (PS19-T2<sup>CV</sup>, n = 11 and PS19-T2<sup>R47H</sup>, n = 10).
due to lower C1q accumulation in synapses, resulting in neuro-protection in the setting of tauopathy. Although the TREM2 R47H variant has been associated with a higher risk of developing AD, we observed delayed progression of p-tau pathology and neurodegeneration in the hippocampus of PS19-T2R47H mice. Surprisingly, in 2 previous studies of PS19 mice with complete absence of TREM2, no change in tau pathology has been reported (25, 26). We do not know the answer to why TREM2R47H decreases some p-tau species at 9 months of age compared with TREM2CV in PS19 mice, whereas TREM2 deletion did not have this effect compared with mice expressing murine TREM2 (25). However, we can speculate that this dissimilarity results from the slightly different age of the mice used between the 2 studies or because of the difference between mouse and human TREM2. Indeed, in the Leyns et al. study (25), the PS19 mice expressed mouse TREM2, which may have a slightly different overall effect compared with human TREM2CV used in this study. Unlike studies on PS19 mice, complete deletion of TREM2 in hTau mice exacerbated tau phosphorylation and insolubility (27). Despite the apparent confusion regarding the impact of TREM2 on tau pathology, we hypothesized that reduced tau pathology in PS19-T2R47H mice results from decreased microglial activation. Indeed, in a mouse model of tau propagation using an injection of the AAV2/6-SYN1 promoter driving the expression of a human P301L tau mutant into the brain, Asai et al. report that depleting microglia dramatically suppressed tau propagation (44). Moreover, microglial activation has been shown to correlate with a deficit in spatial memory and the spread of tau pathology in hTau mice (45). However, during the time course of AD, Aβ aggregation and accumulation in the neocortex occurs over a 15- to 25-year period before symptom onset. Tau aggregation and pathology development in the neocortex does not occur until just a few years before the onset of cognitive decline. Thus, while TREM2 signaling may protect against Aβ-related changes in the brain, the role of TREM2 signaling during the neocortical tau phase (symptomatic phase) of AD and in primary tauopathies may not be the same. This is critical to understand, as therapeutic targeting of TREM2 may differ depending on the stage of the disease. Our study unveils a role of TREM2 in the control of tau-mediated synaptic elimination and neurodegeneration in the setting of pure tauopathy that may also be relevant to the neocortical tau phase of AD. Surprisingly, we demonstrated that the AD-associated TREM2^{R47H} variant strongly reduces the development of brain atrophy, as well as synapse loss in PS19 mice. Importantly, in the presence of TREM2^{R47H}, tau phosphorylation was decreased, suggesting that p-tau pathogenesis might be influenced by TREM2 function. Further analysis revealed decreased microglial activation and reduced expression of several DAM genes in PS19-T2^{R47H} mice. These results indicate that TREM2 contributes to the microglial response to tau pathology or tau-mediated impairment. Importantly, a decrease in phagocytosis of postsynaptic elements by microglia expressing TREM2^{R47H} was detected in PS19 mice and AD patients and can, at least in part, explain the decrease in synaptic damage observed in PS19-T2^{R47H} mice. Finally, we observed that microglia expressing TREM2^{R47H} phagocyte fewer synapses due to lower C1q accumulation in synapses, resulting in neuro-protection in the setting of tauopathy. Although the TREM2^{R47H} variant has been associated with a higher risk of developing AD, we observed delayed progression of p-tau pathology and neurodegeneration in the hippocampus of PS19-T2^{R47H} mice. Surprisingly, in 2 previous studies of PS19 mice with complete absence of TREM2, no change in tau pathology has been reported (25, 26). We do not know the answer to why TREM2^{R47H} decreases some p-tau species at 9 months of age compared with TREM2CV in PS19 mice, whereas TREM2 deletion did not have this effect compared with mice expressing murine TREM2 (25). However, we can speculate that this dissimilarity results from the slightly different age of the mice used between the 2 studies or because of the difference between mouse and human TREM2. Indeed, in the Leyns et al. study (25), the PS19 mice expressed mouse TREM2, which may have a slightly different overall effect compared with human TREM2CV used in this study. Unlike studies on PS19 mice, complete deletion of TREM2 in hTau mice exacerbated tau phosphorylation and insolubility (27). Despite the apparent confusion regarding the impact of TREM2 on tau pathology, we hypothesized that reduced tau pathology in PS19-T2^{R47H} mice results from decreased microglial activation. Indeed, in a mouse model of tau propagation using an injection of the AAV2/6-SYN1 promoter driving the expression of a human P301L tau mutant into the brain, Asai et al. report that depleting microglia dramatically suppressed tau propagation (44). Moreover, microglial activation has been shown to correlate with a deficit in spatial memory and the spread of tau pathology in hTau mice (45). Recently, our lab and others demonstrated that the progression of p-tau pathology is also driven by microglia in PS19 mice (28, 30). Moreover, the same study by Shi et al. (28) demonstrated that microglia-mediated damage was the leading force driving neurodegeneration in a pure-tauopathy mouse model. Similarly, Leyns et al. have shown that microglia expressing TREM2^{R47H} phagocyte fewer synapses due to lower C1q accumulation in synapses, resulting in neuro-protection in the setting of tauopathy.

likely reason for this effect is that decreased TREM2 function leads to a decreased microglial response to Aβ deposition, increased Aβ-associated local neurotoxicity, and an exacerbation of Aβ-inducedtau seeding and spreading (15, 42, 43). However, during the time course of AD, Aβ aggregation and accumulation in the neocortex occurs over a 15- to 25-year period before symptom onset. Tau aggregation and pathology development in the neocortex does not occur until just a few years before the onset of cognitive decline. Thus, while TREM2 signaling may protect against Aβ-related changes in the brain, the role of TREM2 signaling during the neocortical tau phase (symptomatic phase) of AD and in primary tauopathies may not be the same. This is critical to understand, as therapeutic targeting of TREM2 may differ depending on the stage of the disease. Our study unveils a role of TREM2 in the control of tau-mediated synaptic elimination and neurodegeneration in the setting of pure tauopathy that may also be relevant to the neocortical tau phase of AD. Surprisingly, we demonstrated that the AD-associated TREM2^{R47H} variant strongly reduces the development of brain atrophy, as well as synapse loss in PS19 mice. Importantly, in the presence of TREM2^{R47H}, tau phosphorylation was decreased, suggesting that p-tau pathogenesis might be influenced by TREM2 function. Further analysis revealed decreased microglial activation and reduced expression of several DAM genes in PS19-T2^{R47H} mice. These results indicate that TREM2 contributes to the microglial response to tau pathology or tau-mediated impairment. Importantly, a decrease in phagocytosis of postsynaptic elements by microglia expressing TREM2^{R47H} was detected in PS19 mice and AD patients and can, at least in part, explain the decrease in synaptic damage observed in PS19-T2^{R47H} mice. Finally, we observed that microglia expressing TREM2^{R47H} phagocyte fewer synapses due to lower C1q accumulation in synapses, resulting in neuro-protection in the setting of tauopathy.
Figure 6. R47H variant of TREM2 reduces synapse loss in 9-month-old PS19 mice. (A) Representative images from confocal analysis of presynaptic (synapsin in green) and postsynaptic (PSD-95 in red) markers of 9-month-old PS19-T2CV and PS19-T2R47H piriform cortex. Scale bars: 3 μm. (B–D) Quantification of puncta numbers from PSD-95 (B) and synapsin (C) staining, and SEQUIN quantification of synaptic loci (D). Data are presented as mean ± SEM. A Mann-Whitney test was used to determine statistical significance for PSD-95 puncta due to the nonparametric data set. For all other graphs, significance was determined by an unpaired, 2-tailed t test with Welch’s correction due to significantly different variances. **P < 0.01 (PS19-T2CV, n = 20 and PS19-T2R47H, n = 17). (E and F) Electron microscopic images of the polymorphic layer of the piriform cortex from (E) a PS19-T2CV mouse and (F) a PS19-T2R47H mouse. Images were obtained at ×9300 and ×6800 magnification, respectively, using an FEI Tecan Spirit G2 transmission electron microscope. Purple = healthy terminal axons, light blue = healthy dendritic spines, light pink = healthy mitochondria, yellow = dystrophic terminal axons, orange = altered mitochondria, red arrow = dark dendritic spines, green arrow = dark dendrite. Asterisks indicate autophagosomes.
attenuation in PS19 mice observed in this study. Because synapse loss is well characterized in mouse models of tauopathies (29, 46–48), and TREM2 has been shown to play a key role in synaptic pruning during brain development (39), we then hypothesized that TREM2 loss of function leads to lower brain atrophy in PS19 mice by inhibiting synaptic phagocytosis by microglia. Indeed, our lab previously reported higher levels of PSD-95, a postsynaptic marker, in PS19-TREM2-KO compared with PS19 mice (25). We confirmed that PS19-T2 R47H mice have strikingly more pre- and postsynaptic markers, as well as better-maintained pre- and postsynaptic interactions compared with PS19-T2CV mice.

Although the TREM2 R47H protective effect against tau-mediated brain atrophy and synapse loss seems clear in this study, the possible mechanisms underlying this phenomenon are multiple. First, chronic immune activation is known to result in high levels of inflammation and inflammatory cytokines. Importantly, even though we observed decreased tau pathology in PS19-T2 R47H mice, the neuroprotective effect by TREM2R47H characterized in this study is likely attributable to reduced microglia-dependent neuronal damage rather than a direct effect on p-tau pathology per se. Indeed, decreased tau pathology in the piriform cortex has been observed only with p.Thr231, while preserved brain volume, pyramidal layer thickness, and synaptic integrity is reported in the piriform cortex of PS19-T2 R47H mice as well as a strong reduction of microgliosis. Moreover, Leyns et al. previously reported a neuroprotection induced by TREM2 deletion in PS19 mice without a change in tau pathology but with lower microgliosis (25). Thus, diminished microgliosis resulting from the R47H variant of TREM2 seems to explain the brain atrophy attenuation in PS19 mice observed in this study. Because synapse loss is well characterized in mouse models of tauopathies (29, 46–48), and TREM2 has been shown to play a key role in synaptic pruning during brain development (39), we then hypothesized that TREM2 loss of function leads to lower brain atrophy in PS19 mice by inhibiting synaptic phagocytosis by microglia. Indeed, our lab previously reported higher levels of PSD-95, a postsynaptic marker, in PS19-TREM2-KO compared with PS19 mice (25). We confirmed that PS19-T2 R47H mice have strikingly more pre- and postsynaptic markers, as well as better-maintained pre- and postsynaptic interactions compared with PS19-T2CV mice.

Although the TREM2 R47H protective effect against tau-mediated brain atrophy and synapse loss seems clear in this study, the possible mechanisms underlying this phenomenon are multiple. First, chronic immune activation is known to result in high levels
of several proinflammatory cytokines such as TNF-α or IL-1β that can induce synaptic excitotoxicity (49–52), and we show that PS19-T2R47H mice have a robust reduction in the expression of TNF-α and IL-1β, as well as several other proinflammatory cytokines compared with PS19-T2CV. Second, as previously mentioned, TREM2 is well known for its functions in promoting microglial cell survival (53) and in cellular phagocytosis (37, 54, 55). Filipello et al. also determined that TREM2 is essential for microglia-mediated synaptic refinement during the early stages of brain development and that TREM2 deletion resulted in impaired synapse elimination (39). Moreover, a recent study by Sheng at al. reported that TREM2 deficiency ameliorates synaptic deficits in 8-month-old APP/PS1 mice, an amyloid mouse model (56). In PS19-T2R47H mice, we confirmed the reduction of microglial phagocytic properties with fewer CD68+ phagolysosomes within microglia. In addition, a decrease in number of postsynaptic puncta engulfed in CD68+ vesicles was observed not only in PS19-T2R47H mice, but also in AD patients harboring either the R47H mutation or the R62H mutation, another AD-associated TREM2 variant (41, 57, 58). These data suggest that partial loss of function induced by AD-associated TREM2 variants might protect against aberrant synaptic phagocytosis by microglia due to chronic inflammation. The impaired phagocytosis of synapses by microglia might also be due to lower C1q accumulation in synapses. C1q, the initiating protein of the classical complement cascade, is increased and associated with synapses before overt amyloid plaque deposition in AD and inhibition of C1q reduces the number of phagocytic microglia, as well as the extent of early synaptic loss in familial AD-mutant hAPP (J20) transgenic mice (59). More recently, Dejanovic et al. (34) reported a striking accumulation of complement C1q in synapses of PS19 mice and AD patients. Moreover, they demonstrated an association of synaptic C1q accumulation with increased microglial engulfment of synapses and a decrease in synapse density in vitro. Interestingly, we reported that despite more synapses in PS19-T2R47H mice, there was dramatically less C1q-opsonized synapsin and fewer PSD-95 puncta detected in the brain of these mice in comparison with PS19-T2CV mice, which confirms the reduction of microglial phagocytic properties with fewer CD68+ phagolysosomes within microglia. In addition, a decrease in number of postsynaptic puncta engulfed in CD68+ vesicles was observed not only in PS19-T2R47H mice, but also in AD patients harboring either the R47H mutation or the R62H mutation, another AD-associated TREM2 variant (41, 57, 58). These data suggest that partial loss of function induced by AD-associated TREM2 variants might protect against aberrant synaptic phagocytosis by microglia due to chronic inflammation. The impaired phagocytosis of synapses by microglia might also be due to lower C1q accumulation in synapses. C1q, the initiating protein of the classical complement cascade, is increased and associated with synapses before overt amyloid plaque deposition in AD and inhibition of C1q reduces the number of phagocytic microglia, as well as the extent of early synaptic loss in familial AD-mutant hAPP (J20) transgenic mice (59). More recently, Dejanovic et al. (34) reported a striking accumulation of complement C1q in synapses of PS19 mice and AD patients. Moreover, they demonstrated an association of synaptic C1q accumulation with increased microglial engulfment of synapses and a decrease in synapse density in vitro. Interestingly, we reported that despite more synapses in PS19-T2R47H mice, there was dramatically less C1q-opsonized synapsin and fewer PSD-95 puncta detected in the brain of these mice in comparison with PS19-T2CV mice. This is consistent with lower C1q accumulation in synapses of PS19-T2R47H mice compromising the signal for microglia to engulfed them, preserving synaptic density. Importantly, a combination of all these mechanisms resulting from the inhibition of a chronic inflammatory vicious circle remains conceivable here.

Obviously, an evident question emerges from this study: how does TREM2847H increase the risk of developing AD, but appear protective against tau-mediated synapse loss? The answer might be amyloid, tau, and time dependent. Recently, we have found that TREM2 deficiency increases amyloid-induced tau spreading (42), which is a phase of AD pathogenesis that precedes the tau-linked neurodegeneration phase of AD. In this study (42), we showed that TREM2-KO or the AD-associated TREM2847H variant reduces microgliosis around Aβ plaques. Importantly, TREM2-KO and the AD-associated TREM2847H variant strongly promoted tau seeding and spreading in the form of neuritic plaque tau aggregates. In addition, decreased plaque-associated microgliosis in these mice corresponded with higher periplaque Aβ42 accumulation and formation of neuritic dystrophy around plaques. These findings demonstrate a critical role for TREM2 in microglia in inhibiting a key element of Aβ-facilitated tau pathology. Therefore, we hypothesize that AD-associated TREM2 variants increase AD risk by promoting Aβ-mediated local toxicity and facilitate tau seeding and spreading in the early pathological stages of the disease. However, in the later stages of the disease (i.e., symptomatic stage) and in primary tauopathies, when substantial tau pathology has developed, AD-associated TREM2 variants might slow down tau-mediated synapse loss in the brain through reduction of synapse phagocytosis by microglia. It is important to note that complete loss of function of TREM2 in humans results in Nasu-Hakola disease characterized by cerebral degeneration and white matter damage (60, 61). However, partial loss of function of TREM2 as seen with the TREM2847H mutation in the heterozygous or homozygous condition is associated with increased risk for AD (not with Nasu-Hakola disease) (9, 10, 62). Thus, it may be that decreasing but not ablating TREM2 function during the tau phase of neurodegeneration results in neuroprotection not only in mouse models but also in humans.

Taken together, our data show that TREM2 plays a key role in neurodegeneration in the context of tau pathology. Surprisingly, the AD-associated TREM2847H variant was strongly neuroprotective, probably because of decreased chronic inflammation and decreased synaptic phagocytosis. While research on microglia and TREM2 in AD has largely focused on TREM2 interactions with Aβ pathology, this study suggests that TREM2 can directly affect tau pathology and tau-mediated neurodegeneration in the brain. This study raises new important questions about targeting TREM2 as a therapeutic approach to treat AD. Indeed, stimulating the TREM2 pathway using antibodies or drugs is thought to be a promising therapeutic strategy to treat or slow down AD progression. However, this study suggests that TREM2 exacerbates tau-mediated neurodegeneration in the later stages of the disease when tau pathology is prominent. This study highlights the complexity of using TREM2 as a target to treat AD or a primary tauopathy. Moving forward, it will be important to identify and establish the appropriate therapeutic window using close monitoring of neurodegenerative stage and inflammation-related AD biomarkers to either stimulate or inhibit the TREM2 pathway based on the disease and its stage. This should be possible with the use of fluid- and imaging-based biomarkers. Importantly, this study revealed for the first time to our knowledge that the AD-associated TREM2847H variant is protective against tau pathology and tau-mediated neurodegeneration.

Methods

Animals. PS19 transgenic mice expressing human P301S 1N4R mutated tau driven by the PrP promoter were purchased from The Jackson Laboratory (stock 008169). PS19 mice were backcrossed and maintained on a mouse TREM2-KO background (T2KO). PS19-T2KO were crossed with mice that had been engineered using BAC technology to express either human TREM2CV or the AD-associated TREM2847H on a mouse T2KO background (15). These BAC transgenic mice carrying human TREM2 (common variant or R47H variant), TREML1, and TREML2 were backcrossed with T2KO mice to yield mice that express either TREM2CV or TREM2847H in the absence of mouse TREM2. The use of BAC TREM2 models prohibited the ability to compare littermates with different genotypes in this study. Because of the fact that male PS19 mice at different ages
have greater tau pathology and neurodegeneration, and less variability than female mice (63, 64), only males were used for analysis in this study. All mice were on the C57BL/6 background.

**Sample collection.** At time of death, mice were anesthetized with i.p. pentobarbital (200 mg/kg). Blood samples were collected in EDTA-treated tubes before cardiac perfusion with with 3 U/mL heparin in cold Dulbecco’s PBS. Blood samples were spun down (10 minutes, 2000 g, 4°C), and blood plasma was collected. Brains were carefully extracted and cut to 2 hemispheres. The left hemisphere was collected for immunostaining and fixed in 4% paraformaldehyde overnight before being transferred to 30% sucrose and stored at 4°C until they were sectioned. Brains were cut coronally into 50-μm sections on a freezing sliding microtome (Leica, SM1020R) and stored in cryoprotectant solution (0.2 M PBS, 15% sucrose, 33% ethylene glycol) at −20°C until use. The right hemisphere was dissected to isolate the hippocampus and the cortex for biochemical analysis, and the tissue was kept at −80°C until analyzed.

For electron microscopy, 3 mice per group were anesthetized with i.p. pentobarbital (200 mg/kg) and perfused with 0.1% glutaraldehyde in 4% paraformaldehyde (65). Fifty-micrometer-thick coronal sections of the brain were cut in sodium phosphate buffer using a vibratome (Vibratome series 1000 sectioning system) and stored at −20°C in cryoprotectant until further processing (66).

**Volumetric analysis of brains sections.** Every sixth brain section (300 μm between sections) starting rostrally at bregma −1.23 mm to the dorsal end of the hippocampus at bregma −2.73 mm were mounted on slides and allowed to dry overnight for each mouse. The following day, mounted sections were stained with 0.1% Sudan black in 70% ethanol at room temperature for 20 minutes, then washed in 70% ethanol 3 times for 1 minute. The sections were washed in Milli-Q water and covered with Fluoromount (Southern Biotech). Slides were imaged with the NanoZoomer 2.0-HT system (Hamamatsu Photonics) and areas of interest measured using the NDP viewer software (Hamamatsu Photonics). The volume for each region of interest was calculated using the following formula: volume = (sum of areas) × 0.3 mm.

**Neuronal layer thickness measurement.** Three sections (bregma −2.0, −2.4, and −2.8 mm) per mouse were mounted and allowed to dry overnight. The following day, sections were stained in cresyl violet for 6 minutes at room temperature. The slides were then sequentially dehydrated in increasing ethanol concentrations followed by xylene and coverslipped with Fluoromount (Southern Biotech). Slides were imaged with the NanoZoomer 2.0-HT system. Images were further processed and quantified with the use of Fiji software version 1.51. All areas were quantified in 2 to 3 sections (300 μm apart from each other) per mouse.

**Brain extraction and tau and p-tau ELISA.** Half of the hippocampus was weighed and homogenized using a pestle with 20 μL buffer/mg tissue (10 mM Tris–HCl pH 7.4, 0.8 M NaCl, 1 mM EDTA, 2 μM DTT, Complete and PhosStop [both Roche], and 10% sucrose). Samples were centrifuged for 10 minutes at 10,000 g and 4°C. Supernatant was removed and kept on ice while the pellet was re-homogenized in the same volume of buffer with a sonicator at 30% amplitude, 1 second on/1 second off pulse, for 1 minute, and centrifuged for 10 minutes at 10,000 g and 4°C. The 2 supernatants were pooled together and frozen until used. The concentration of tau and p-tau was quantified by sandwich ELISA as previously described (42) using Tau-5 (in-house antibody) as the coating antibody and human-specific biotinylated HT7 for detection for tau ELISA and using HJ14.5 (in-house pThr181-tau antibody) as the coating antibody and human-specific biotinylated AT8 for detection for p-tau ELISA. Briefly, 96-well half-area plates were coated with 20 μg/mL of either HJ14.5 or Tau-5 antibody and incubated at 4°C overnight. The next day, the plate was blocked in 3% BSA (RPI Corp.) in PBS for 1 hour at 37°C. Next, peptide standards and samples were diluted in sample buffer (0.25% BSA/PBS, 1x protease inhibitor, 300 mM Tris pH 7.4, PBS), loaded onto the plate, and incubated at 4°C overnight. On the third day, 0.3 μg/mL of biotinylated AT8 for p-tau ELISA (Thermo Fisher Scientific, MN1020B) or biotinylated HT7 for tau ELISA (Thermo Fisher Scientific, MN1000B) was applied to the plate for 1.5 hours at 37°C, and then Streptavidin-poly-HRP-40 (1:10,000 for tau and 1:6,000 for p-tau) (Fitzgerald) was applied for 1.5 hours at room temperature. TMB Superslow Substrate solution (MilliporeSigma) was added and the plates were read at 650 nm on a BioTek plate reader after developing for 30 minutes at room temperature. All samples were run in duplicate.

**Immunohistochemistry.** For staining microglia (Iba1, rabbit polyclonal, 1:5,000; Wako, 019-19741) and p-tau (AT8, mouse monoclonal, 1:500; Thermo Fisher Scientific, MN1020B — AT180, mouse monoclonal, 1:500; Thermo Fisher Scientific, MN1040 — PG5, mouse monoclonal, 1:500 [gift from Peter Davies, Feinstein Institutes for Medical Research, Northwell Health, Manhasset, New York, USA]), sections were washed 3 times in TBS for 5 minutes and blocked in 0.3% hydrogen peroxide for 10 minutes. After washing, sections were blocked in 3% milk in TBS with 0.25% Triton X-100 (TBSX) for 30 minutes. Primary antibody was diluted in 3% milk/TBSX, and the sections were incubated in the primary antibody overnight at 4°C. The next day, sections were washed and incubated with secondary antibody diluted in 3% milk/TBSX for 1 hour. For AT8 staining, after washing, sections were incubated in ABC Elite solution (VectaStain, PK-6100) for 1 hour, prepared following the manufacturer’s instructions, followed by another washing step. For Iba1, AT180, and PG5 staining, after washing, sections were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature (1:500; Jackson Laboratory, 111-035-003). Sections were developed in DAB solution (Vector Laboratories, SK4103), washed, and mounted on slides. After drying overnight, the slides were dehydrated in increasing ethanol concentrations followed by xylene and coverslipped with Cytoseal 60 (Thermo Fisher Scientific, 8310). Slides were scanned on the NanoZoomer 2.0-HT system. Images were further processed and quantified with the use of Fiji software version 1.51. All areas were quantified in 2 to 3 sections (300 μm apart from each other) per mouse.

**Immunofluorescence.** Immunofluorescence staining was performed as previously described (36). Briefly, free-floating tissue was placed in 12-well plates with Netwell inserts (Corning) containing PBS. Blocking solution and primary and secondary antibody mixtures were centrifuged at 17,000 g for 5 minutes just before use. Tissue was rinsed 3 times, 5 minutes each, in PBS followed by blocking in 20% normal goat serum (NGS) in PBS for 1 hour at room temperature. Tissue was then incubated overnight at room temperature with primary antibodies (CD68, rat monoclonal, 1:500; Abb SeroTec, MCA1957), Iba1 (goat polyclonal, 1:5,000; Abcam, ab5076), PSD-95 (rabbit polyclonal, 1:200; Thermo Fisher Scientific, 51-6900), synapsin-1/2 (guinea pig polyclonal, 1:500; Synaptic Systems, 106004),
and C1q (mouse monoclonal, 1:50; Abcam, ab71940) in 10% NGS containing 0.3% Triton X-100 in PBS. The following day, sections were rinsed 3 times, 5 minutes each, in PBS followed by incubation in secondary antibodies (Thermo Fisher Scientific; 1:500) in 10% NGS containing 0.3% Triton X-100 in PBS for 4 hours at room temperature. Sections were mounted, dried at room temperature, briefly rinsed in distilled water, and then coverslipped. Mounting medium (-150 μL/slide) was prepared the day of use by mixing Tris-MWL 4:88 (Electron Microscopy Sciences, 17977-150) with AF300 (Electron Microscopy Sciences, 17977-25) at a 9:1 ratio.

**Image acquisition for immunofluorescence.** Images were acquired on an LSM 880 microscope with AiryScan detector (Zeiss). Quantification was performed using a semiautomated pipeline based on MATLAB (MathWorks) and Imaris 9.3.1 software (Bitplane) as previously described (36). Spots were detected for each channel using an x-y size of 0.2 μm, a z size of 0.6 μm, and automated background subtraction. A 0.1-μm x-y and 0.3-μm z grid was applied to exclude spots intersecting the edge of the image volume. Synaptic loci were identified using custom MATLAB scripts from Sauerbeck (36) to find the nearest neighbor based on the x-y-z centroid of the top 20% brightest puncta. A cutoff of 270 nm pre-to-postsynaptic separation was used to quantify synaptic loci for Figure 6 from the frequency distribution of pre-to-postsynaptic separations. Complement labeling of synaptic loci was performed with Imaris 9.3.1 software by creating a surface of C1q staining based on a threshold applied to all images and by counting the number of pre- and postsynaptic spots positive for this surface. Three images per mouse were obtained in the polymorphic layer of the piriform cortex for this analysis.

Quantification of confocal images for Iba1 and CD68 was performed on a semiautomated platform using MATLAB and Imaris 9.3.1 software to create surfaces of each stain based on a threshold applied to all images and colocalize Iba1 and CD68 surfaces to evaluate the volume of CD68 vesicles within Iba1 cells. For quantification of the number of PSD-95 puncta engulfed in CD68 phagolysosomes, the PSD-95 puncta previously detected were automatically counted within the CD68 surface per microglia using Imaris 9.3.1 software. Three to 5 microglia were imaged for each mouse or subject.

**Immunofluorescence of human tissue.** Paraffin-embedded 8-μm sections were dewaxed in xylene for 6 minutes twice, followed by rehydration descending ethanol to water (100%, 95%, 90%, 70%, 30%, and 10% ethanol, followed by 100% water [twice]). For antigen retrieval, slides were incubated 20 minutes in a thiocarbohydrazide solution of 1% hydrogen peroxide and 2% hydrosoluble thiocarbonbide in phosphate buffer (PB, 0.1 M, pH 7.4). Sections were washed 3 times, 5 minutes each in ddH2O and dehydrated by rehydration descending ethanol to water (100% [twice], 95%, 70%, 30%, and 10% ethanol, followed by 100% water [twice]). For antigen retrieval, slides were incubated 20 minutes in a thiocarbohydrazide solution and washed again 3 times, 5 minutes each in double-distilled water (ddH2O). A 30-minute incubation of 2% osmium tetroxide (from 4% stock, diluted in ddH2O) was then performed. The brain sections were washed 3 times, 5 minutes each in ddH2O and dehydrated with an increasing amount of ethanol (5-minute washes each): 35% (twice), 50%, 70%, 80%, 90%, 100% (thrice). After dehydration in ethanol, the sections were washed 3 times, 5 minutes each, in propylene oxide and were emerged overnight in Durcupan resin. The following day, tissue sections were placed between 2 ACLAR sheets (Electron Microscopy Sciences) covered with a thin layer of resin and incubated 3 days at 55°C to polymerize.

**Ultramicrotomy and transmission electron microscopy.** The polymorphic layer of the piriform cortex was excised from the ACLAR sheet with a razor blade and glued onto a resin block. The tissues were then cut using an ultramicrotome (Leica Ultracut UC7), first with a thickness of 300 nm to confirm the region of interest using toluidine blue, and finally at a thickness of 70 nm. The ultrathin sections were collected onto copper-mesh grids. The polymorphic layer was imaged at 80 kV using a transmission electron microscope (FEI Tecnai Spirit G2) equipped with a Hamamatsu ORCA-ER digital camera (10 MP). The user was blinded to the experimental conditions during imaging and ultrastructural interpretation. Images of the synaptic neuropil were randomly acquired throughout the region of interest so as to avoid bias. Sixteen to 20 nonoverlapping images at a magnification between ×6,800 and ×13,500 were obtained per animal. Overall, between 575 μm² and 729 μm² of the synaptic neuropil were imaged per animal.

Images were analyzed using the software ImageJ (NIH). For the qualitative analysis of synaptic ultrastructure, the following parameters were considered: the presence and size of autophagosomes; the presence of altered mitochondria; the presence of dark dendrites, dendritic spines, or axon terminals; and the presence of dystrophic synapses. Dark cells (e.g., microglia, neurons) are characterized by ultrastructural signs of oxidative stress (endoplasmic reticulum dilation, condensation of the nucleoplasm and cytoplasm, altered mitochondria) (68, experiments. Human samples were obtained from the Alzheimer’s Disease Research Center at Washington University. TREM2Δ11 and TREM2Δ2222 AD cases were paired with TREM2Δ11 AD cases matching age, sex, and APOE genotype (Supplemental Table 1).

**Gene expression.** We extracted total RNA from mouse cortex with the RNeasy Mini Kit (QIAGEN) and prepared cDNA with the High-Capacity RNA-to-cDNA kit (Applied Biosystems), following manufacturers’ instructions. Gene expression analysis was performed using microarray in collaboration with the Genome Technology Access Core at Washington University. Using TaqMan probes, the relative gene expression was quantitatively measured using Fluidigm Biomark HD with integrated fluidic circuits.

**Tissue preparation for electron microscopy.** Three PS19-T2Δ5 mice and 2 PS19-T2Δ4Δ5 mice were used for electron microscopy. In each animal, brain sections containing the polymorphic layer of the piriform cortex (bregma –1.67 mm to –1.91mm) were selected based on the stereotaxic atlas of Paxinos and Franklin (67). The sections were washed 3 times, 10 minutes each, with PBS, pH 7.4, and then incubated 1 hour in a solution of equal volume of 3% potassium ferrocyanide in phosphate buffer (PB, 0.1 M, pH 7.4) and 4% aqueous osmium tetroxide. Following washing 3 times, 5 minutes each in PBS, tissues were incubated 20 minutes in a thio carbocarbohydrate solution and washed again 3 times, 5 minutes each in double-distilled water (ddH2O). A 30-minute incubation of 2% osmium tetroxide (from 4% stock, diluted in ddH2O) was then performed. The brain sections were washed 3 times, 5 minutes each in ddH2O and dehydrated with an increasing amount of ethanol (5-minute washes each): 35% (twice), 50%, 70%, 80%, 90%, 100% (thrice). After dehydration in ethanol, the sections were washed 3 times, 5 minutes each, in propylene oxide and were emerged overnight in Durcupan resin. The following day, tissue sections were placed between 2 ACLAR sheets (Electron Microscopy Sciences) covered with a thin layer of resin and incubated 3 days at 55°C to polymerize.
69) and have been seen previously in pathological conditions, such as AD (70). Axon terminals were recognized by their inclusion of multiple synaptic vesicles. Dendritic spines were recognized and differentiated from dendrites by their size and presence of a postsynaptic density (71). Dark synaptic elements were recognized by their electron-dense cytoplasmin (72). Altered mitochondria were distinguished from normal ones by their large increase in size and/or a disruption of their cristae and/or outer membrane structure (73). Dystrophic synapses were defined by the presence of autophagosomes, a reduced number of synaptic vesicles, and a swollen shape (seen as an increase in size and/or electronlucent content). Autophagosomes were recognized by their circular shape and delineating double membrane (74).

Statistics. Unless otherwise stated, all data are presented as mean ± SEM. GraphPad Prism 8.0.0 was used to perform statistical analyses. Gaussian distribution was evaluated using the D’Agostino-Pearson normality test. Statistical analysis was performed using 2-tailed Student’s unpaired t test under normal distribution. In case of unequal variances, Welch’s correction was used with the Student’s unpaired t test. If samples deviated from normal distribution, statistical analysis was performed using a Mann-Whitney test. For human data, significance was determined by paired 2-tailed t test. P less than 0.05 was considered significant: *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 versus PS19-T2CV.

Study approval. All animal experimental protocols were approved by the Animal Studies Committee at Washington University, St. Louis, Missouri, USA.

Author contributions
MG, MC, JDU, and DMH designed the study. MG, CEGL, ADS, MKSP, MX, NK, JRS, TTK, and MET performed the experiments and analyzed the data. MG, JDU, and DMH wrote the manuscript. All authors discussed the results and commented on the manuscript.

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Address correspondence to: David M. Holtzman, Washington University School of Medicine, 660 South Euclid Avenue, Box 8111, St. Louis, Missouri 63110, USA. Phone: 314.747.0644; Email: holtzman@wustl.edu.


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