Neuron
Review

Elucidating the Role of TREM2 in Alzheimer's Disease

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http://dx.doi.org/10.1016/j.neuron.2017.02.042

Alzheimer's disease (AD) is the sixth leading cause of death in the United States and the most common cause of dementia in the elderly. Genetic factors, such as rare variants in the microglial-expressed gene *TREM2*, strongly impact the lifetime risk of developing AD. Several recent studies have described dramatic TREM2-dependent phenotypes in mouse models of amyloidosis that point to an important role for TREM2 in regulating the response of the innate immune system to Aβ pathology. Furthermore, elevations in the CSF levels of soluble TREM2 fragments implicate changes in inflammatory pathways as occurring coincident with markers of neuronal damage and the onset of clinical dementia in AD. Here, we review the rapidly developing literature surrounding TREM2 in AD that may provide novel insight into the broader role of the innate immune system in neurodegenerative disease.

Alzheimer's Disease and Genetic Risk Factors

Alzheimer's disease (AD), a neurodegenerative disease that is the most common cause of dementia in the elderly, is distinguished by the presence of both amyloid plaques and neurofibrilary tangles (NFTs). Extracellular plaques deposited in the brain parenchyma consist predominantly of amyloid-β (Aβ) peptides derived from the amyloid precursor protein (APP), and NFTs consist of intracellular deposits of aggregated, hyperphosphorylated tau protein within neurons. Extensive biomarker and PET (positron emission tomography)-imaging studies indicate that Aβ plaques begin to form in the brain ~15–25 years prior to the onset of cognitive decline or the development of tau pathology (Bateman et al., 2012; Jack and Holtzman, 2013). Following this long, preclinical period, tau pathology, which is present in the medial temporal lobe in most people over 60, begins to spread first into the temporal and then other areas of the neocortex, which correlates with the onset of cognitive decline and brain atrophy. Changes in inflammatory signaling also accompany AD pathology, as detected by significant increases in activated astrocytes and microglia; however, the regulation and functional consequences of these glial changes in AD are still incompletely understood.

The risk for developing AD is strongly influenced by genetic factors. Variants in *APP* or in genes encoding components of the γ-secretase complex, presenilin-1 (*PSEN1*) and presenilin-2 (*PSEN2*), cause a rare form of autosomal dominant AD in which individuals develop dementia, usually in their 30s, 40s, or 50s. Autosomal dominant AD-associated variants usually increase the relative production of the highly fibrillogenic $A\beta_{42}$ and longer Aβ isoforms relative to shorter forms of Aβ, such as $A\beta_{40}$ (Musiek and Holtzman, 2015). Accordingly, individuals with autosomal dominant AD begin to develop Aβ plaques very early in life. The more common late-onset form of AD also has a strong genetic component, the most common and strongest risk factor being the ε4 allele of the apolipoprotein-E (*APOE*) gene (APOE4). A single copy of *APOE4* increases the risk of developing AD approximately 4-fold, and individuals with two copies of APOE4 face an approximately 12-fold increased risk of AD, relative to individuals carrying two copies of *APOE3*. *APOE4* carriers develop Aβ plaques earlier than non-carriers, and APOE4 appears to influence Aβ aggregation and the rate of Aβ clearance from the brain. Several other genetic variants that influence the risk for late-onset AD have been identified through genome-wide association studies (GWASs); however, the relative risk imparted by these variants appears to be far lower than that imparted by *APOE4* (Karch and Goate, 2015).

Whole-genome sequencing studies led to the discovery of rare variants in *TREM2* (Triggering Receptor Expressed on Myeloid Cells 2) that increase the risk of developing AD by ~2- to 3-fold (Guerreiro et al., 2013b; Jonsson et al., 2013). Several variants within *TREM2* appear to significantly increase the risk of developing AD (Jin et al., 2014; Song et al., 2016). The most common variant within *TREM2* that is now firmly established as increasing the risk for AD is rs75932628, an SNP that confers an Arg-to-His change at amino acid 47 (R47H) (Guerreiro et al., 2013b; Jonsson et al., 2013). In humans, the gene encoding TREM2 is located within a cluster of related *TREM* genes (*TREM1*, *TREM2*, *TREM4*, and *TREM5*) at chromosome 6p21.1. Two other TREM-like genes, *TREML1* and *TREML2*, are also located within the TREM gene cluster. Variants in other genes in the TREM gene cluster, such as rs6910730G in *TREM1*, may also be associated with increased risk of AD pathology (Replogle et al., 2015). Conversely, a missense variant in *TREML2* (S144G) may be protective against developing AD (Benitez et al., 2014). Interestingly, TREML2 and TREM2 may have opposing functions on

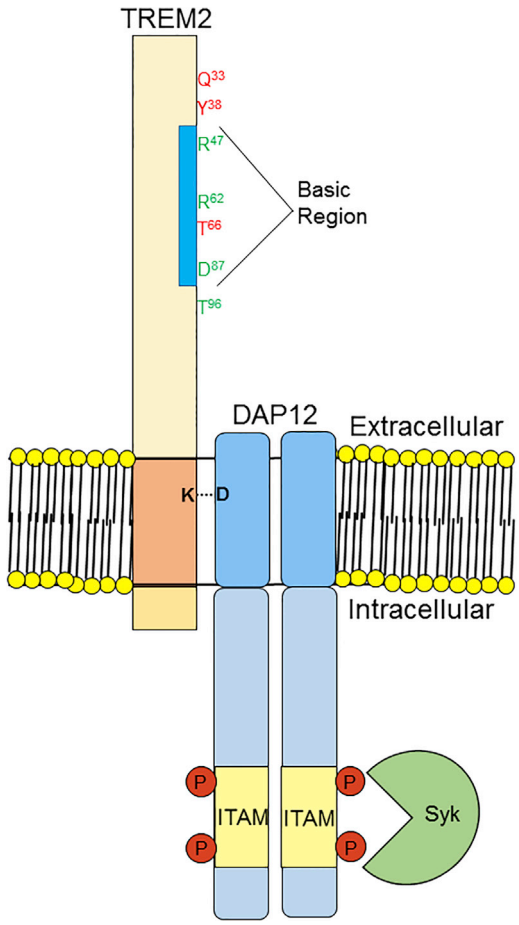

Figure 1. Schematic of TREM2 and DAP12
Variants in the extracellular region of TREM2 are associated with NHD (red) and AD (green). Several AD-associated variants lie within a region that forms a basic-patch on the surface of TREM2 (blue box) that is thought to be critical for association with anionic ligands, such as anionic phospholipids. The transmembrane regions of TREM2 and DAP12 associate through a lysine-aspartate electrostatic interaction. DAP12 contains an ITAM region that, upon phosphorylation, recruits Syk to mediate downstream signal transduction.

microglial proliferation and inflammatory gene expression (Zheng et al., 2016).

Functional Effects of TREM2 Variants

TREM2 is a V-type immunoglobulin (Ig) domain-containing transmembrane protein that is expressed in mononuclear phagocytes such as microglia, osteoclasts, and alveolar macrophages (Colonna and Wang, 2016). The precise ligand that activates TREM2 in vivo is still unclear. Lipids can activate TREM2 signaling either as components of a cellular membrane, in body fluids, or as components of a lipoprotein complex (Cannon et al., 2012; Poliani et al., 2015; Song et al., 2016; Wang et al., 2015). Given that APOE is the major apolipoprotein in the CNS, several studies have investigated whether APOE is a potential component of the TREM2 signaling pathway. Both lipidated APOE and non-lipidated APOE bind the extracellular region of TREM2 in vitro, although lipidated APOE bound with a significantly higher affinity than non-lipidated APOE (Atagi et al., 2015; Bailey et al., 2015; Yeh et al., 2016). Importantly, TREM2 appears to bind all isoforms of APOE similarly (Yeh et al., 2016). In addition to APOE, TREM2 also associates with other apolipoproteins, including APOA1, APOB, and APOJ, particularly in their lipidated state (Yeh et al., 2016). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) can also induce TREM2-dependent signaling presumably through their lipid moieties (Song et al., 2016; Yeh et al., 2016). Other proposed ligands for TREM2 include nucleotides and anionic species such as heparin sulfate proteoglycans or other negatively charged carbohydrates (Daws et al., 2003; Kawabori et al., 2015; Kober et al., 2016).

TREM2 associates with the signaling adaptor protein DAP12 through an electrostatic interaction between a conserved lysine in TREM2 and aspartic acid in DAP12 within the transmembrane region (Figure 1) (Bouchon et al., 2000). Loss-of-function mutations in *TREM2* or *TYROBP*, the gene that encodes DAP12, are associated with Nasu Hakola disease (NHD), an autosomal recessive disorder hallmarked by bone cysts, frequent bone fractures, and a presenile form of frontal-lobe dementia (Paloneva et al., 2001, 2002). Upon ligand binding to TREM2, DAP12 is phosphorylated on tyrosine residues within the immunoreceptor tyrosine-based activation motif (ITAM) region, leading to recruitment of spleen tyrosine kinase (Syk) (Figure 1). Syk activation initiates a number of signaling cascades, resulting in the activation of phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinases (MAPKs) and the elevation of intracellular Ca^{2+} ($[Ca^{2+}]_i$) through release of IP3-gated Ca^{2+} stores (Colonna and Wang, 2016). A multitude of cellular functions have been ascribed to TREM2, including regulation of phagocytosis, inhibition of inflammatory signaling, and promotion of cell survival (Colonna, 2003).

To understand how *TREM2* variants may impact the onset and progression of AD, it is important to first consider whether AD-associated *TREM2* variants enhance or impair functional TREM2 signaling. *TREM2* variants that cause NHD have dramatic detrimental effects on TREM2 function, and a single copy of NHD-associated *TREM2* variants, such as Q33X, increases the risk of developing AD (Guerreiro et al., 2013b; Song et al., 2016). This would strongly argue that loss of TREM2 function increases the risk of developing AD. One NHD-causative variant, Q33X, results in a premature truncation of the protein (Figure 1; Table 1) (Soragna et al., 2003). Other variants, such as T66M and Y38C, impair the trafficking of TREM2, resulting in reduced TREM2 surface expression (Figure 2; Table 1) (Kleinberger et al., 2014; Kober et al., 2016). The impairment in trafficking is likely explained by a recent structural study of the TREM2 ectodomain that found that the side chains T66 and Y38 are buried within the Ig fold, potentially indicating that mutations of these residues can severely disrupt the proper folding of TREM2, resulting in decreased protein stability (Kober et al., 2016).

By contrast, the AD-associated R47H variant does not appear to significantly affect the folding, trafficking, or stability of TREM2, nor does it appear to affect overall expression level (Table 1) (Kleinberger et al., 2014; Kober et al., 2016; Ma et al., 2016; Song et al., 2016). Rather, AD-associated *TREM2* variants appear to affect the efficiency of TREM2 signaling. Phospholipids and serum-derived lipoprotein particles activate TREM2 signaling in vitro, and TREM2-dependent signaling is impaired

Table 1. Summary of Observed Effects of TREM2 Variants

TREM2 Variant	Surface Expression	Ligand Affinity	Signaling Response	Phagocytosis/Cellular Uptake of Lipoprotein	Microgliosis
R47H	no change	reduced	reduced	reduced	reduced
	Kleinberger et al., 2014; Kober et al., 2016; Ma et al., 2016; Song et al., 2016	Atagi et al., 2015; Kober et al., 2016; Yeh et al., 2016	Song et al., 2016; Wang et al., 2015	Kleinberger et al., 2014; Yeh et al., 2016	Yuan et al., 2016
R62H	no change	reduced	reduced	reduced	N/A
	Song et al., 2016	Kober et al., 2016	Song et al., 2016	Yeh et al., 2016	
T66M	reduced	ablated	none	reduced	N/A
	Kleinberger et al., 2014; Kober et al., 2016	Kober et al., 2016; Yeh et al., 2016	Song et al., 2016	Kleinberger et al., 2014	
Y38C	reduced	ablated	N/A	reduced	N/A
	Kleinberger et al., 2014	Yeh et al., 2016		Kleinberger et al., 2014	
T96K	slight reduction/no change	increased	increased	N/A	N/A
	Song et al., 2016; Kober *et* al., 2016	Kober et al., 2016	Song et al., 2016		
D87N	no change	reduced	increased	slight reduction	N/A
	Kober et al., 2016; Song et al., 2016	Yeh et al., 2016	Song et al., 2016	Yeh et al., 2016	

N/A, not applicable.

in cells that express certain AD-associated TREM2 variants, including R47H and R62H (Figure 2; Table 1) (Song et al., 2016; Wang et al., 2015). Conversely, two other variants, D87N and T96K, which have been identified as potentially influencing AD risk, result in increased TREM2 activity relative to common variant TREM2 (Figure 2; Table 1) (Song et al., 2016). The rarity of these variants has, thus far, precluded a conclusive determination of their impact on AD risk; however, the ability of TREM2 variants to enhance or impair TREM2 signaling suggests that altered TREM2 homeostasis has serious consequences in regard to the development of AD. AD-associated TREM2 variants also appear to influence the affinity of TREM2 for putative ligands. The R47H, R62H, and D87N variants decrease the binding affinity between TREM2 and APOE in vitro, although the direct association with isolated phospholipids may not be altered (Figure 2; Table 1) (Atagi et al., 2015; Kober et al., 2016; Yeh et al., 2016). Interestingly, the binding affinity between TREM2 and proteoglycans on the surface of cells was decreased by R47H and R62H variants but increased by the T96K variant, consistent with the reported effects of TREM2 variants on signaling (Figure 2; Table 1) (Kober et al., 2016). Mapping of an electrostatic surface onto the crystal structure of the TREM2 ectodomain provides a potential structure-function mechanism for the differing effects of these AD-associated variants on ligand affinity and TREM2 activity. The R47H and R62H variants within TREM2 are part of a large basic region that is thought to be important for association with anionic ligands (Figure 1). Mutation of other arginine residues within this basic patch, such as R76 and R77, also decreased the binding affinity of TREM2 for proteoglycans on the surface of cells, further implicating the importance of the conserved arginine region for ligand recognition. Interestingly, T96 is located adjacent to the basic region, and the T96K variant is predicted to extend the size of the basic patch, which could, in turn, enhance TREM2 binding affinity for anionic ligands (Kober et al., 2016).

TREM2 is hypothesized to be important for phagocytosis, which could be particularly relevant in AD as a mechanism for clearing of pathogenic proteins, such as Aβ, or apoptotic cells. Heterologous expression of the NHD-associated TREM2 mutants Y38C and T66M strongly reduced phagocytic activity in HEK293 cells, and expression of the AD-associated R47H variant led to modest reductions in phagocytosis. Primary microglial cultures from post-natal day (P)5–P6 *Trem2* knockout (KO) mice, or BV2 cells lacking TREM2 expression, exhibited modest reductions in phagocytosis of bacteria, fluorescent beads, and $A\beta_{1-42}$ aggregates (Kleinberger et al., 2014). Bone-marrow-derived macrophages (BMDMs) from *Trem2* KO mice exhibited reduced ex vivo phagocytic clearance of Aβ plaques from brain slices of APPPS1–21 mice (Xiang et al., 2016). *Trem2* KO also decreased the potency and efficacy of antibody-mediated Aβ clearance (Xiang et al., 2016). Microglia isolated from P0–P2 *Trem2* KO mice similarly exhibited reduced uptake of APOJ and LDL (Yeh et al., 2016). The uptake and degradation of $A\beta_{1-42}$ aggregates that were complexed with APOJ or LDL were also reduced in microglia from *Trem2* KO mice, and monocyte-derived macrophages (MDMs) from human R62H variant carriers displayed reduced uptake of Aβ-LDL complexes (Figure 2; Table 1) (Yeh et al., 2016). Interestingly, in these culture systems, microglia or MDMs exhibited poor uptake of free Aβ aggregates compared to Aβ-lipoprotein complexes. Primary microglia isolated from adult *Trem2* KO mice and cultured in the presence of transforming growth factor β (TGF-β) exhibited no deficit in phagocytosis of either apoptotic cells or Aβ, suggesting that the activation state of microglia may influence potential

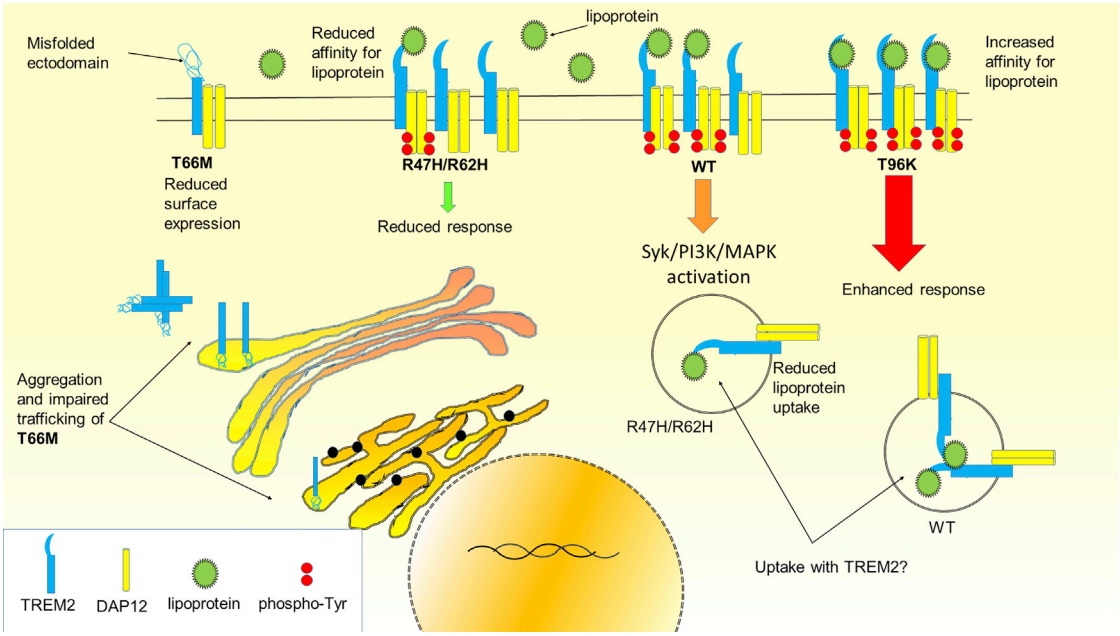

Figure 2. Functional Effects of TREM2 Variants

TREM2 variants associated with NHD or AD impact the expression, ligand binding affinity, and signaling efficacy of TREM2. TREM2 signaling via DAP12 can be activated by anionic phosopholipids, such as found in lipoprotein particles. Upon stimulation, Syk is recruited to DAP12 resulting in the downstream activation of PI3K, MAPKs, and elevations in intracellular Ca2+. The T66M TREM2 variant that is associated with NHD severely disrupts the proper folding of the TREM2 ectodomain. Heterologously expressed TREM2-T66M exhibits decreased surface expression, impaired trafficking, and intracellular aggregation. The AD-associated TREM2 variants, R47H and R62H, do not appear to alter the surface expression of TREM2. However, both variants decrease the affinity of TREM2 for lipid ligands and lipid-induced TREM2 activation. In contrast, the T96K variant exhibits increased affinity for lipids and an increased responsiveness to lipid stimulation. Interestingly, cells expressing TREM2-R47H and TREM2-R62H also exhibited reduced uptake of lipoprotein particles. Whether TREM2 directly mediates lipoprotein uptake has not yet been determined.

TREM2-dependent differences in phagocytosis (Butovsky et al., 2014; Wang et al., 2015).

TREM2 and Aβ Plaques

Microglial clustering around Aβ plaques is observed in both post-mortem AD brain tissue and mouse models of Aβ deposition, although the functional role of plaque-associated microglia is largely unknown. Microglia exhibit little competence to phagocytize fibrillar Aβ, and ablation of microglia through either colony-stimulating factor 1 (CSF1) inhibition or the ganciclovir administration in the CD11b-HSVTK model does not seem to affect total amyloid burden (Prokop et al., 2015; Spangenberg et al., 2016; Varvel et al., 2015). In contrast to these findings, genetic or pharmacological manipulation of microglial activation can alter amyloid burden in mouse models, suggesting that some aspect of microglial physiology can regulate amyloid deposition. For example, interleukin-10 (IL-10) deficiency reduced amyloid burden in APP/PS1 mice and increased microglial phagocytosis of Aβ (Guillot-Sestier et al., 2015). Conversely, overexpression of IL-10 increased amyloid deposition and inhibited phagocytosis of Aβ (Chakrabarty et al., 2015). The microglial-expressed protein CD33 appears to inhibit phagocytosis of Aβ, and a variant in *CD33* that is protective against AD reduces CD33 expression (Bradshaw et al., 2013; Griciuc et al., 2013). Accordingly, amyloid levels are reduced in *CD33*-deficient APP/PS1 mice (Griciuc et al., 2013). Administration of nuclear receptor agonists increases the expression of phagocytic receptors such as MerTK and Axl and leads to decreased amyloid burden in the APP/PS1 mice (Savage et al., 2015). In addition to effects on plaque burden, chronic microglial activation in response to amyloid pathology is hypothesized to lead to high levels of inflammatory cytokines and reactive oxygen species that may contribute to neurodegeneration (Heneka et al., 2014).

Given the clear relationship between microglia and amyloid pathology, several studies have investigated the functional role of TREM2 in the setting of Aβ pathology. TREM2 expression is increased in the brains of AD patients, although the R47H variant does not appear to significantly affect the expression level or processing of TREM2 in AD (Table 1) (Ma et al., 2016). TREM2 expression is also increased in mouse models of Aβ deposition, particularly within plaque-associated microglia, although the degree to which increased TREM2 expression detected at the tissue level reflects an increase in the number of microglia is unclear (Jay et al., 2017; Srinivasan et al., 2016; Yuan et al., 2016). A weighted gene co-expression network analysis of amyloid-bearing mice identified *TREM2* as a hub gene within a immune-gene-enriched module that contained other AD-risk loci, such as *CD33*, *Inpp5d*, and *Ms4a6d* (Matarin et al., 2015). A Bayesian network analysis of gene expression in late-onset AD patients identified *TYROBP* as strongly upregulated and a critical causal regulatory gene in a complement-gene-enriched module (Matarin et al., 2015; Zhang et al., 2013). These studies would

Table 2. Summary of the Effects of TREM2 in the Context of Amyloid Deposition

Source	Brain Region	Stage	Amyloid Burden	Amyloid Morphology	Plaque-Associated Microgliosis	Neuritic Dystrophy	Citations
5xFAD; TREM2$^{-/-}$	hippocampus	early	equivalent	less compact	decreased	increased	Wang et al., 2015; Wang et al., 2016; Yuan et al., 2016
		late	increased	less compact	decreased	increased	
	cortex	early	equivalent	less compact	decreased	increased	
		late	equivalent	less compact	decreased	increased	
APPPS1; TREM2$^{-/-}$	hippocampus	early	decreased	unknown	decreased	unknown	Jay et al., 2015; Jay et al., 2016
		late	equivalent	unknown	decreased	unknown	
	cortex	early	decreased	unknown	decreased	unknown	
		late	increased	larger	decreased	unknown	
Post-mortem human R47H AD patients	cortex	N/A	equivalent	increased filamentous plaques	decreased around filamentous and compact	increased	Yuan et al., 2016

N/A, not applicable.

suggest that TREM2-DAP12 expression is a critical component of the broader immune response to AD pathology marked by changes in inflammatory cytokines, phagocytosis, and complement signaling. The mechanism by which TREM2 expression is upregulated is not known; however, some studies suggest that RXR agonists can increase TREM2 and DAP12 expression and that RXR associates with the TREM2 promoter (Lefterov et al., 2015; Savage et al., 2015). Whether RXR is the physiological transcription factor that mediates TREM2 upregulation in the context of Aβ deposition remains to be determined.

Multiple studies from mouse models of Aβ deposition and post-mortem human brain sections have consistently found that TREM2 promotes microglial clustering around fibrillar Aβ plaques (Table 2) (Jay et al., 2015, 2017; Ulrich et al., 2014; Wang et al., 2015, 2016; Yuan et al., 2016). Aβ deposition model mice that were either haploinsufficient or completely lacking TREM2 expression had significant reductions in the number of plaque-associated microglia (Jay et al., 2015; Ulrich et al., 2014; Wang et al., 2015). A subsequent study found that AD patients who were R47H carriers exhibited a similar reduction in plaque-associated microgliosis compared to AD patients with the common variant of TREM2, particularly around plaques that were filamentous or compact in appearance (Yuan et al., 2016). The fact that decreased plaque-associated microgliosis is observed in both *Trem2* KO mouse models and in human R47H variant carriers supports the hypothesis that the R47H variant impairs TREM2 function. Similar reductions in plaque-associated microglia are seen in *Tyrobp* KO mice (Yuan et al., 2016). High-resolution confocal images revealed that the processes of plaque-associated microglia that are in contact with amyloid fibrils are highly enriched with TREM2, DAP12, and p-Tyr, possibly suggesting an enrichment of activated DAP12 signaling (Yuan et al., 2016). If so, this would suggest that TREM2 activation at the microglial-amyloid interface is necessary for sustaining or initiating microgliosis.

Microglial-mediated inflammation is a major facet of AD, and increases in inflammatory gene expression are evident in Aβ deposition model mice. Although in vitro data suggest that TREM2 signaling reduces inflammatory gene expression, *Trem2* KO mice, when crossed to Aβ deposition models, consistently exhibit reduced levels of inflammation-associated genes, such as *Il1b* and *Tnf* (Jay et al., 2017; Wang et al., 2015). Likely, this is a reflection of the reduced numbers of activated, plaque-associated microglia, rather than a specific regulation of inflammation by TREM2 (Srinivasan et al., 2016; Wang et al., 2015). Contrary to reduced inflammation observed in *Trem2* KO Aβ deposition mouse models, stimulation of cultured *Trem2* KO microglia with lipopolysaccharide (LPS) results in exaggerated inflammatory gene expression (Zheng et al., 2016). Whether, on a single-cell level, *Trem2* KO affects amyloid-induced inflammatory gene expression is unclear.

Whether TREM2 is important for resident microgliosis or infiltration of peripheral myeloid cells in the presence of Aβ pathology remains somewhat controversial. Since plaque-associated microglia express high levels of CD45 and low levels of P2Y12R, a phenotype commonly associated with peripheral myeloid cells, one potential function of TREM2 could be to facilitate the recruitment of peripheral immune cells to amyloid deposits in the brain (Jay et al., 2015). However, CD45 and P2Y12R expression can be dynamically regulated, depending on the activation state of microglia, which may explain the expression profile of these markers in plaque-associated microglia (Butovsky et al., 2015). Parabiosis experiments using CD45.2-expressing Aβ mouse models and CD45.1 B6 congenic mice failed to detect significant peripheral mononuclear cell brain infiltration or recruitment to Aβ plaques (Wang et al., 2016). Ablation of microglia in Aβ mouse models resulted in peripheral monocyte recruitment to the CNS; however, these cells failed to rapidly or robustly co-localize with Aβ plaques (Prokop et al., 2015; Varvel et al., 2015). While a role for peripheral myeloid cell recruitment to Aβ plaques cannot be excluded, these results would suggest that plaque-associated immune cells are likely derived predominantly from resident microglia. Fate-tracking experiments of myeloid cell populations may provide additional insight into the relative contribution of resident microglia and peripheral myeloid cells in TREM2-dependent microgliosis during Aβ pathology.

The molecular mechanism underlying the apparent dependence on TREM2 expression for plaque-associated microgliosis has not yet been described. Recent studies in mouse models of

Aβ deposition at early and later stages of pathology have described a reduction in the expansion of the microglial population as the mice age and pathology increases (Jay et al., 2017; Wang et al., 2016). In mouse models that exhibit aggressive amyloid deposition, reductions in plaque-associated and CD45^{high} microglia in *Trem2* KO mice are observable in young mice during the very early stages of pathology (Jay et al., 2017; Wang et al., 2016). Similar reductions in plaque-associated microglia are observed in older mice with more extensive plaque pathology, but there are also significant reductions in the total microglial population, both C45^{high} and CD45^{low}, which are typically associated with "resting" microglia (Jay et al., 2017; Wang et al., 2016). Appreciable microglial proliferation, particularly within CD45^{low} populations of microglia, is observed in older mice with Aβ pathology (Jay et al., 2017). Microglial proliferation is reduced in *Trem2* KO mice, which likely contributes to the reduced number of total microglia observed in older *Trem2* KO mice with Aβ pathology (Jay et al., 2017; Wang et al., 2016).

Trem2 KO mice also exhibit increased markers of apoptosis in plaque-associated microglia, suggesting that decreased microglial survival may also contribute to the lower microglial response to Aβ pathology (Wang et al., 2015). Microglial survival is dependent upon CSF1 receptor (CSF1R) signaling, which can be activated by either CSF1 or IL-34 (Elmore et al., 2014; Wang et al., 2012). In vitro studies indicate that TREM2 facilitates microglial survival in conditions of low CSF1 availability, as might occur in the localized setting of plaque-associated microgliosis (Wang et al., 2015). In support of this hypothesis, administration of a low-dose of PLX5622, a CSF1R antagonist, reduces microglial association with plaques in the 3xTg mouse model (Dagher et al., 2015). This may indicate that plaque-associated microglia are particularly sensitive to perturbations in CSF1 signaling. Interestingly, loss-of-function mutations in the CSF1R lead to an adult-onset leukoencephalopathy with axonal spheroids (ALSP), an autosomal dominantly inherited neurodegenerative disease marked by dementia, motor dysfunction, and epileptic phenotypes, similar to the degenerative neurological phenotype observed in NHD (Rademakers et al., 2011).

Trem2 KO mice exhibit remarkable changes in the overall structure of amyloid plaques (Table 2). Amyloid plaques in haploinsufficient or total *Trem2* KO;5xFAD mice exhibited a less compact morphology, as did plaques from *Tyrobp* KO;APPPS1-21 mice (Wang et al., 2016; Yuan et al., 2016). Ultrastructural analysis of amyloid plaques using stochastic optical reconstruction microscopy found that amyloid filaments were longer in mice with reduced or no TREM2 expression, suggesting that TREM2-dependent engagement of microglial processes with amyloid filaments may be critical for capping filament growth and compacting amyloid fibrils (Yuan et al., 2016). Whether the observed changes in amyloid structure are relevant to the role of TREM2 variants in AD is not clear, but R47H carriers exhibited an increase in relative amount of filamentous, poorly compacted plaques, potentially analogous to the less compact amyloid morphology found in mouse amyloidosis models with lower levels of TREM2 (Yuan et al., 2016).

The intimate arrangement of microglial processes and amyloid fibrils may limit the exposure of surrounding neurons to higher order, proto-fibrillar Aβ species that are hypothesized to be neurotoxic (Condello et al., 2015). Regions of Aβ plaques that exhibit the highest levels of $A\beta_{42}$ incorporation are juxtaposed with regions of Aβ plaques that exhibit high degrees of microglial coverage (Condello et al., 2015). Interestingly, these $A\beta_{42}$ "hotspots" on plaques are also associated with the highest levels of dystrophic axonal staining (Condello et al., 2015). In accordance with these observations, *Trem2* KO, or *Tyrobp* KO, results in higher levels of plaque-associated axonal dystrophy in amyloid-depositing mice (Wang et al., 2016; Yuan et al., 2016). Increased plaque-associated neuritic dystrophy was also observed in post-mortem brain sections from R47H variant carriers, consistent with the decreased microglial coverage of plaques in these individuals (Yuan et al., 2016). Thus, one potential effect of TREM2 deficiency may be the increased neuronal process damage due to greater neuronal exposure to neurotoxic species of Aβ due to decreased microglial coverage of amyloid fibrils. Another possibility is that TREM2 deficiency results in decreased phagocytic clearance or remodeling of damaged axons, as TREM2 is thought to be important for microglial clearance of damaged neurons (Takahashi et al., 2005). Regardless, the increases in plaque-associated neuritic dystrophy in the absence of TREM2 signaling provide a potential mechanism by which TREM2 variants could affect AD risk.

Although the consensus of the data strongly supports the conclusion that TREM2 is important for plaque-associated microgliosis, conflicting data have been reported regarding the effect of TREM2 on overall Aβ plaque deposition (Table 2). To date, the effect of TREM2 on Aβ deposition has only been characterized in two mouse models, APPPS1-21 and 5xFAD, both of which exhibit aggressive amyloid deposition that begins between 2 and 4 months of age (Oakley et al., 2006; Radde et al., 2006). Haploinsufficiency of TREM2, in 3-month-old or 7-month-old APPPS1-21 mice had no effect on cortical Aβ burden (Ulrich et al., 2014). However, total KO of TREM2 expression in APPPS1-21 mice appears to elicit age-dependent effects on Aβ deposition. In general, *Trem2* KO in APPPS1-21 mice was reported to reduce Aβ burden early in the appearance of pathology and increase Aβ plaque burden in later stages (Jay et al., 2017). *Trem2* KO may also differentially affect amyloid burden in the 5xFAD model, depending on the stage of pathology. In 4-month-old 5xFAD mice, *Trem2* deficiency did not significantly affect the levels of insoluble $A\beta_{40}$ or $A\beta_{42}$ (Wang et al., 2016). In 8-month-old 5xFAD mice, *Trem2* deficiency resulted in increased amyloid burden in the hippocampus but not in the cortex (Wang et al., 2015). Collectively, these data indicate that TREM2 exerts differential effects on detectable amyloidosis, depending on the stage of pathology and, possibly, the model. Given the aggressive nature of both the APPPS1-21 and 5xFAD models, it will be important to test whether *Trem2* deficiency affects amyloid burden in slower developing amyloid models that express physiological levels of APP, such as the NLF APP-KI (knockin) mouse (Saito et al., 2014).

TREM2 as a Biomarker

Changes in the levels of AD-related proteins in the cerebrospinal fluid (CSF), such as $A\beta_{42}$ and tau, reflect the presence and progression of AD pathology in the brain. One of the earliest detectable fluid biomarkers of AD pathology is a decrease in CSF levels

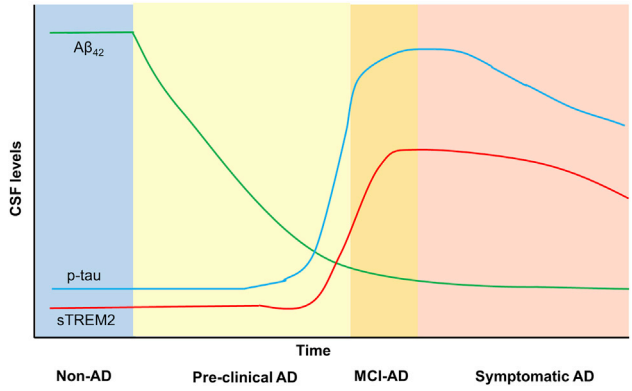

Figure 3. Schematic of Changes in CSF Levels of sTREM2, Aβ$_{42}$, and p-tau in AD

Amyloid plaques begin to deposit in the brain 15–25 years prior to the onset of cognitive symptoms. CSF levels of Aβ$_{42}$ decline during this preclinical period, likely due to sequestration of Aβ$_{42}$ into amyloid plaques. Subsequently, CSF levels of tau and p-tau increase, which likely reflects increased neuronal damage, and these increases coincide with the onset of MCI and dementia associated with AD. CSF levels of sTREM2 appear to correlate with the CSF levels of tau and p-tau as well as the onset of cognitive decline, but not with CSF levels of Aβ42. CSF sTREM2 levels may reflect significant levels of microgliosis and inflammation related to neurodegeneration due to AD pathology.

of Aβ$_{42}$ (Figure 3) (Fagan et al., 2014). This decrease is likely attributable to Aβ$_{42}$ sequestration into Aβ plaques within the brain parenchyma (Blennow et al., 2012). Although low CSF Aβ$_{42}$ levels are highly predictive of future cognitive impairment, individuals with low CSF Aβ$_{42}$ levels may be asymptomatic for many years (Fagan et al., 2014). Elevations in CSF tau and p-tau levels in AD occur due to increased neuronal toxicity and damage, likely due in large part to the spread of tau pathology throughout the medial temporal lobe and neocortex (Figure 3) (Brier et al., 2016). Elevations in CSF tau and p-tau are positively correlated with the onset of clinically detectable cognitive impairment (Brier et al., 2016). Subsequent to the identification of TREM2 variants that were associated with AD, several studies emerged that suggest that changes in CSF levels of TREM2 may reflect an inflammatory process in AD associated with the transition from pre-clinical to clinical AD. Proteolytic processing of TREM2 results in release of a soluble, ectodomain-containing TREM2 fragment (sTREM2) that is detectable in CSF and serum (Kleinberger et al., 2014; Piccio et al., 2008). Studies of late-onset AD and autosomal-dominant AD populations found that sTREM2 levels were increased in the CSF of AD patients and that sTREM2 levels were positively correlated with levels of tau and p-tau; however, no robust correlation between sTREM2 and Aβ42 levels was observed (Figure 3) (Heslegrave et al., 2016; Piccio et al., 2016; Suárez-Calvet et al., 2016a, 2016b). This suggests that sTREM2 levels increase in association with neuronal damage rather than with the appearance of Aβ plaques per se. A cross-sectional study of a late-onset AD population found that individuals with preclinical AD, defined by low CSF Aβ$_{42}$ with normal cognitive performance, did not exhibit a statistically significant elevation in CSF sTREM2 levels, whereas sTREM2 levels were significantly higher in individuals with mild cognitive impairment due to AD (MCI-AD) (Figure 3) (Suárez-Calvet et al., 2016b). Similarly, a cross-sectional study of the Dominantly Inherited Alzheimer's Disease Network (DIAN) AD population found elevations in CSF sTREM2 levels subsequent to Aβ deposition and CSF tau increases but coincident with changes in hippocampal volume, precuneus glucose uptake, and cognitive performance (Suárez-Calvet et al., 2016a). Accordingly, CSF sTREM2 levels were significantly elevated in groups with a clinical dementia rating (CDR) score of 0.5 or 1 but not in a cognitively normal group (CDR = 0) of dominantly inherited AD mutation carriers (Suárez-Calvet et al., 2016a). When taken together, these observations are consistent with a role for significant microglial activation and inflammation occurring after the deposition of Aβ plaques and neuronal injury but coincident with significant neurodegeneration and the onset of cognitive decline.

Given that several *TREM2* variants are associated with AD, and that CSF levels of sTREM2 are elevated in AD, an intuitive question is whether AD-associated *TREM2* variants alter CSF sTREM2 levels. An initial study reported that CSF levels sTREM2 were elevated in R47H carriers but unchanged in R62H carriers, relative to cognitively normal controls (Piccio et al., 2016). Whether these results indicate a TREM2 variant-dependent effect on sTREM2 levels in the CSF is difficult to determine, given the small group sizes. In addition, the *TREM2* variant carrier groups in this study were a mixture of individuals who were cognitively normal or had symptomatic AD (Piccio et al., 2016). AD-associated *TREM2* variants are rare, meaning that obtaining a sufficiently large cohort to determine the effect of *TREM2* variants on CSF sTREM2 levels in individuals with a similar disease status will be a challenging endeavor.

The possible relationship between sTREM2 levels and the onset of cognitive impairment in AD could provide both a useful tool for monitoring the progression of the disease and novel insight into a potential pivotal role for microglial function in the transition from preclinical AD to cognitive impairment. However, much work remains to both validate the relationship between sTREM2 and AD and understand the mechanism underlying a potential relationship between TREM2 and neuronal toxicity. Although several studies indicate that elevated sTREM2 levels may mark the transition from preclinical AD to symptomatic AD, some studies have reported contrary findings of either decreased or unchanged CSF sTREM2 levels in AD patient populations (Henjum et al., 2016; Kleinberger et al., 2014). These disparate findings may result from technical differences in sTREM2 quantification or differences based on the cohort of patients included in the study. One intriguing possibility is that sTREM2 levels may be increased early in clinical AD but decline in later stages of the disease (Suárez-Calvet et al., 2016b). Additional, well-powered studies accounting for differences in the core AD biomarkers Aβ$_{42}$, tau, and p-tau, as well as cognitive status, are needed to develop a more thorough picture of potential dynamic changes in sTREM2 levels during the progression of AD.

Currently, the preponderance of evidence would indicate a positive correlation between CSF levels of sTREM2 and tau, indicative of a relationship between a change in inflammation and neuronal toxicity in AD. Whether the inflammation occurs secondary to significant neurodegeneration or, in fact, is a driving force in initiating neurodegeneration (or both) is unclear.

Longitudinal biomarker studies, coupled with mechanistic experiments in disease models, are needed to investigate the potential relationship between changes in inflammatory phenotype and cognitive decline. Finally, the apparent increase in sTREM2 levels in AD raises some basic biological questions about TREM2 function. Do increased sTREM2 levels reflect regulated alterations in proteolytic processing of TREM2 or simply reflect increased TREM2 expression? Does sTREM2 play a physiological function in regulating inflammation in the CNS? Although a function for sTREM2 has not yet been described in vivo, a recent in vitro study found that application of recombinant $TREM2_{19–136}$ increased the survival of BMDMs grown under low-CSF1 conditions (Wu et al., 2015). Further experiments to investigate the regulation and potential function of sTREM2 could provide novel insight into how TREM2 impacts microglial function and inflammation.

Significant correlations between CSF sTREM2 levels and neurodegenerative disease are not limited to AD. Carriers of *TREM2* variants that cause NHD due to reduced TREM2 function or expression exhibit reduced sTREM2 levels in the CSF (Kleinberger et al., 2014). Multiple studies indicate that sTREM2 levels are positively correlated with age, which may be indicative of a general increase in proinflammatory signaling in aging (Piccio et al., 2016; Suárez-Calvet et al., 2016b). CSF levels of sTREM2 were elevated in individuals who fit the criteria for suspected non-Alzheimer pathophysiology (SNAP), which includes cognitively normal individuals with elevated CSF levels of tau and p-tau (Suárez-Calvet et al., 2016b). Prior to the identification of *TREM2* variants as a risk factor for AD, increases in sTREM2 were first described in the CSF of patients with inflammatory-related neurological diseases, particularly multiple sclerosis (MS) (Piccio et al., 2008). In a group of MS patients treated with natalizumab, a therapeutic antibody that prevents T cell infiltration into the CNS and reduces inflammation, CSF levels of sTREM2 were reduced to a level comparable to that of healthy controls, which was accompanied by a clinically significant improvement in the patients (Öhrfelt et al., 2016). A separate group of MS patients treated with mitoxantrone, an antineoplastic agent that reduces inflammatory cell proliferation and signaling, exhibited no change in CSF sTREM2 levels or in clinical status (Öhrfelt et al., 2016). These observations suggest that sTREM2 may be a dynamic biomarker that could be used to monitor the efficacy of therapeutics that target inflammation within the CNS.

TREM2 in Other Neurodegenerative Diseases

Although some initial reports suggested that the R47H variant may also increase the risk of developing other neurodegenerative diseases, such as frontotemporal dementia (FTD), amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD), additional studies and meta-analysis cast some doubt on the strength and significance of a link between TREM2 and risk for developing these diseases (Borroni et al., 2014; Cady et al., 2014; Guerreiro et al., 2013a; Lill et al., 2015; Rayaprolu et al., 2013). Regardless, studies in a variety of neurodegenerative animal models have yielded interesting TREM2-dependent phenotypes that can inform the role of TREM2 and microglia in the context of injury to the CNS. As mentioned in the preceding section, sTREM2 levels are elevated in the CSF of MS patients, suggesting a potential role for TREM2 in MS (Piccio et al., 2008). Animal models of MS, such as the experimental autoimmune encephalitis (EAE) and cuprizone models, exhibit increased expression of TREM2 (Cantoni et al., 2015; Piccio et al., 2007; Poliani et al., 2015). Inhibition of TREM2 in the EAE model resulted in a more severe clinical phenotype, suggesting a potential protective role for TREM2 (Piccio et al., 2007). *Trem2* KO mice treated with cuprizone, a demyelinating agent, exhibited reduced microglial proliferation in the corpus collosum and impaired phagocytic clearance of damaged myelin (Cantoni et al., 2015; Poliani et al., 2015). These observations corresponded with impaired upregulation of genes important for phagocytosis and lipid metabolism in microglia from cuprizone-treated *Trem2* KO mice (Poliani et al., 2015). Overall, *Trem2* KO mice also exhibited impaired motor function compared to WT mice following cuprizone treatment (Cantoni et al., 2015). Together, these studies would indicate that TREM2-dependent microglial functions are important for mitigating CNS injury in MS.

TREM2 upregulation and TREM2-dependent microgliosis occurs in mouse models of prion disease, stroke, and pain. *Trem2* KO mice inoculated with scrapie brain homogenate exhibited decreased microglial activation and proliferation, although the clinical course of the disease and expression level of inflammatory cytokines was unaffected (Zhu et al., 2015). TREM2 expression is upregulated in response to medial cerebral artery occlusion (MCAO), suggesting a role for TREM2 in stroke recovery (Sieber et al., 2013). *Trem2* KO mice subjected to MCAO exhibited reduced microgliosis, inflammatory gene expression, reduced resorption of damaged brain tissue, and poorer performance on functional tests following recovery (Kawabori et al., 2015; Sieber et al., 2013). These results would indicate a protective role for TREM2-dependent microglial functions in the context of neuronal injury following stroke. Potentially in contrast to the protective role of TREM2 in stroke, TREM2 deficiency in a mouse model of traumatic brain injury reduced injury-induced inflammation and hippocampal atrophy (Saber et al., 2017). Spinal nerve injury induces CSF1 expression by injured sensory neurons and upregulation of DAP12 and TREM2 expression (Guan et al., 2016; Kobayashi et al., 2016). DAP12 and CSF1 expression are required for mechanical allodynia and inflammatory gene expression following nerve injury, and *Tyrobp* KO mice exhibit a reduced number of microglial cells in the dorsal horn ipsilateral to injury (Guan et al., 2016; Kobayashi et al., 2016). Interestingly, administration of a TREM2 antibody purported to have agonist properties was sufficient to induce mechanical allodynia and inflammatory cytokine expression (Kobayashi et al., 2016). Overall, these studies in very diverse mouse models point to a critical role for a TREM2-DAP12 signaling pathway in facilitating microglial responses to pathology in response to CNS injury.

Future Perspective

Great strides have been made in our understanding of TREM2 in the context of AD in just a short time since the initial description of AD-associated TREM2 variants. Studies from amyloid mouse models and post-mortem human brain tissue suggest that

TREM2 is important for the microglial response to amyloid pathology and that impairment of TREM2 function may alter plaque morphology and increase plaque-associated toxicity. In vitro studies strongly suggest that at least the R47H variant impairs TREM2 signaling in response to stimulation by lipids, and initial studies of post-mortem human brain sections from R47H variant carriers with AD find that they recapitulate phenotypes observed in *Trem2* KO models that develop amyloidosis, such as reduced microgliosis and increased neuritic dystrophy. Whether these phenotypes are relevant to how TREM2 variants affect the risk of developing AD is not currently well defined. Conceivably, decreased plaque-associated microgliosis due to impaired TREM2 function could hasten the onset of cognitive symptoms following amyloid plaque deposition through increases in protofibrillar toxic Aβ species (Condello et al., 2015). Developing animal models that express human TREM2 containing AD-associated variants will be an important tool for investigating how TREM2 function affects different aspects of AD pathology. How reduced TREM2 expression or function would detrimentally affect microgliosis is not known, but it could provide important insight into the particular physiology of activated microglia in the context of amyloid pathology.

Another potential mechanism by which impaired TREM2 activity could influence AD risk is through affecting tau pathology. Although CSF levels of sTREM2 do not provide direct insight into specific physiological roles of TREM2 in AD, the fact that increases in sTREM2 correspond to the onset of cognitive decline, rather than amyloid deposition, suggests a role for TREM2 separate from affecting the rate or degree of Aβ plaque formation. Given the correlative relationship between CSF levels of sTREM2 and tau, and the observation that TREM2 variants are significantly associated with elevated CSF tau levels, TREM2 may play a critical role in the onset and progression of tau pathology in AD. Currently, whether TREM2 affects tau pathology or the microgliosis in the context of tau pathology is not known. The role of microglia in tau pathology is poorly defined. Microglial activation may precede the development of detectable tau pathology in mouse models of FTD (Yoshiyama et al., 2007). Furthermore, microglial activation is associated with increases in tau phosphorylation and the potential spread of tau pathology (Maphis et al., 2015). Alternatively, increases in plaque-associated neuritic dystrophy resulting from impaired TREM2 signaling could facilitate the development or spread of tau pathology (Li et al., 2016). Future studies examining the role of TREM2 in tauopathy may yield important insight into how TREM2 variants increase the risk of AD and in the role of microglia in tau pathology.

Evidence from biomarker studies suggesting that accumulation of amyloid pathology begins many years prior to the onset of clinical dementia, combined with negative clinical trials of some Aβ-targeting therapies, has led to the view that early therapeutic intervention will be the most effective treatment of AD. Identifying drug targets downstream of amyloid pathology—currently, the earliest detectable sign of pre-clinical AD—could be critical in developing effective therapies to slow or halt the conversion of preclinical to clinical AD. The association of sTREM2 with the onset of cognitive decline and the striking impact of TREM2 on microgliosis in response to pathology suggest that TREM2-dependent functions downstream of the appearance of amyloid pathology may be important in the transition from pre-clinical to clinical AD.

ACKNOWLEDGMENTS

This work was supported by grants from the Cure Alzheimer's Fund to J.D.U., M.C., and D.M.H. and from the JPB Foundation to D.M.H.

D.M.H. cofounded and is on the scientific advisory board of C2N Diagnostics, LLC, Neurophage and Denali and consults for Genentech, Eli Lilly, GlaxoSmithKline, and AbbVie.

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