The Role of TREM2 in Microglial Function and Alzheimer's Disease

by

Yi Lu

BS, Nanjing University, China, 2015

MSc, University of Britsol, UK, 2017

Submitted to the Graduate Faculty of the

Department of Environmental and Occupational Health

Graduate School of Public Health partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

This thesis was presented

by

Yi Lu

It was defended on

June 11, 2020

and approved by

Aaron Barchowsky, PhD
Professor
Department of Environmental and Occupational Health
Graduate School of Public Health
University of Pittsburgh

Bistra Iordanova, PhD
Assistant Professor
Department of Bioengineering
Swanson School of Engineering
University of Pittsburgh

Thesis Advisor:
Radosveta Koldamova, MD, PhD
Professor
Department of Environmental and Occupational Health
Graduate School of Public Health
University of Pittsburgh

Radosveta Koldamova, MD, PhD

The Role of TREM2 in Microglial Function and Alzheimer's Disease

Yi Lu, MS

University of Pittsburgh, 2020

Abstract

Alzheimer's disease (AD) is the leading cause of dementia and death in the United States. AD is characterized by extracellular amyloid plaques and intracellular neurofibrillary tangles. Patients are classified into early-onset AD (EOAD), which are developed before 65, and late-onset AD (LOAD), developed after 65. Three causative genes were identified for EOAD: amyloid precursor protein (APP), presenilin (PSEN) 1 and 2. However, there are no causative genes found for LOAD. Risk factors of AD are complicated, including aging, family histories, high cholesterol levels, high blood pressure, brain injuries, and genetic risk factors. Recent genome-wide association studies have identified a set of genetic risk factors for LOAD, including the triggering receptor expressed on myeloid cells 2 (TREM2) and apolipoprotein E (APOE). Studies have identified several rare variants of TREM2 to increase the risk of developing LOAD by 2-4-fold. In the central nervous system (CNS), microglia play an important role in responding to plaque accumulation during AD pathology. TREM2, as the receptor of microglia, was proposed to affect microglia functions, including survival, proliferation, chemotaxis, and phagocytosis. TREM2 was also found to impair the activation of homeostatic microglia and energy metabolism of microglia cells. This review summarized previous studies in the past 5 years and elucidated the current understanding of the role of TREM2 in microglia function and AD pathology, as well as

opportunities and challenges of targeting of TREM2 as a potential therapeutic strategy, which is of high public health significance.

Table of Contents

List of Figures

Acknowledgements

I joined Dr. Koldamova and Dr. Lefterov's lab from April 2019. I would like to thank them for giving me the chance to learn and work in their lab. Also, I want to thank all our lab members, especially Nick and Kyong for their kind help and support during my working in the lab, as well as their valuable comments on my MS thesis.

I would also like to thank Dr. Barchowsky, my MS advisor, for his guidance through each stage of the process. His office was always open whenever I had any questions or problems about my study or research. In addition, I would like to thank Dr. Iordanova, one of my thesis committee members, for the useful comments, remarks, and engagement through the process of thesis writing.

Finally, I must express my gratitude to all my family members for their support and encouragement throughout my study, research, and thesis writing.

1.0 Introduction

As the most common form of dementia, Alzheimer's Disease (AD) is officially identified as the sixth leading cause of death in the United State. In 2019, 5.8 million of US population are estimated to live with AD, with 5.6 million of them aged 65 and older [1]. The number is growing rapidly with the population getting older. It is reported that deaths caused by AD of all ages have increased 145% from 2000 to 2017 [2]. According to the age of disease onset, two types of AD are classified: early-onset AD (EOAD) which were developed before 65 and late-onset AD (LOAD) which were developed after 65, with LOAD representing about 95% of all the cases. There are two morphological hallmarks of AD: extracellular deposits of β-amyloid peptide (Aβ) plaques and intracellular neurofibrillary tangles of tau protein [3].

Similar to other neurodegenerative diseases, aging is believed to be one of the most important risk factor but not direct cause of AD [4]. It has been reported that of the total US population, 3% of the population aged 65-74, 17% of the population aged 75-84, and 32% of the population aged 85 and older developed AD [1]. Aβ plaques and neurofibrillary tangles are also observed in healthy-aging brains [5]. Other risk factors, such as environmental exposures, lifestyles, diet, traumatic brain injury, and other systemic diseases are also reported to be involved in AD [5]. EOAD, or familial AD are widely believed to be linked with gene mutations of amyloid precursor protein (*APP*) and presenilin (*PSEN*) 1 and 2 that directly or indirectly increase production of toxic Aβ peptides. However, there is no causative gene mutations identified for LOAD. According to genome-wide association studies (GWAS), more than 30 risk loci for LOAD are identified [6], half of which are associated with immune response, such as apolipoprotein E (*APOE*) and triggering receptor expressed on myeloid cells 2 (*TREM2*) [7].

APOE, mostly secreted by astrocytes, plays an important role in lipid metabolism, which is an essential part of immune response. There are three alleles of *APOE*: *APOEε2*, *APOEε3*, and *APOEε4*, accounting for about 10%, 70%, and 20%, respectively [8]. The three alleles have different amino acids at position 112 and 158: the ε2 allele has cysteine (Cys) at both positions, the ε3 allele has a Cys at position 112 and an arginine (Arg) at position 158, while the ε4 allele has Arg at the two positions. The ε2 allele was found to be linked with decreased risk of developing AD and later disease onset, while one copy of ε4 allele was associated with increasing AD risk by 3- to 4-fold. The ε4 allele was identified by previous studies to have reduced ability on Aβ binding and clearing compared to the ε2 and ε3 allele [8-10]. TREM2 is a cell surface receptor of the immunoglobulin superfamily that is expressed by microglia in the central nervous system (CNS). Several rare variants of TREM2 have been identified to be associated with increasing the risk of developing AD by 2- to 4-fold [10]. One of these variants, the most well-studied one, rs75932628 variant (Arg-to-His change at amino acid 47, or R47H) is reported to significantly increase the risk of developing AD in North American and European population [11, 12]. In contrast, in Chinese population, there is no association between R47H TREM2 and AD [13], but rs2234255 variant (His-to-Tyr change at amino acid 157, or H157Y) was identified to increase the risk of developing AD (odds ratio: 11.01, $p = 0.02$) [14]. However, no evidence found between H157Y and AD risk in Caucasian cohort [15], suggesting the effects of TREM2 rare variants might be population-specific. A case-control study, including 36,790 controls and 48,343 AD patients reported association between rs143332484 variant (Arg-to-His change at amino acid 62, or R62H) and AD risk (odds ratio 1.67, $p = 1.55 \times 10^{-14}$) [12]. R67H TREM2 was found to reduce microglial response of Aβ plaques and increase microglial accumulation of autophagosomes [16, 17]. AD related variants of TREM2, such as R47H and R62H, were found to change the secondary structure

and impair ligands binding [18]. However, there do exist other variants, like T96K, which are probably gain-of-function mutations according to the structural and binding studies [18].

This review focuses on the role of TREM2 on microglial function and AD pathology.

2.0 TREM2

TREM2, a member of the TERM family, is a cell surface transmembrane receptor with an extracellular Ig-like domain, a cytoplasmic tail, and a transmembrane domain [10]. In human, the *TREM2* gene is located on chromosome 6p21 near some other *TREM* and *TREM*-like genes and encodes 230 amino acids [19]. TREM2 is expressed on resident macrophages, infiltrating and inflammatory macrophages, cerebrospinal fluid (CSF) monocytes, and dendritic cells [20, 21]. In the central nervous system (CNS), TREM2 is entirely expressed by microglia; its expression is strong in the basal ganglia, corpus callosum, spinal cord, and medulla oblongata [10]. Retinoid X receptor (RXR), which binds upstream of TREM2 locus, is reported to regulate the expression [22, 23]. Treatment of bexarotene, an RXR agonist, enhanced the expression of TREM2 mRNA in the cortex of AD mice [23]. Expression of TREM2 is affected by inflammation; pro-inflammatory molecules such as lipopolysaccharide (LPS) downregulated TREM2 expression, but anti-inflammatory molecules upregulated TREM2 expression *in vitro* [24-26]. During pathological processes, such as AD, traumatic brain injury (TBI), Amyotrophic lateral sclerosis (ALS), and Parkinson's disease (PD), TREM2 was found upregulated [24, 27-29]. In addition, aging is also a regulator of TREM2 expression [30].

Exact ligands that bind and activate TREM2 remains unclear. The extracellular region of TREM2 with an immunoglobulin domain is suggested to bind microbial products, such as LPS [31, 32]. As a member of the TREM family, TREM2 can also bind lipids because of the potential phospholipid binding site [18]. The ability of lipid-binding allows TREM2 to recognize and bind phosphatidylserine on apoptotic cells and debris [31, 33]. Some previous studies found TREM2 is able to bind lipoproteins including high-density lipoproteins (HDL), low-density lipoproteins

(LDL), and apolipoproteins, such as APOE [9, 11, 16, 34, 35]. An *in vitro* study also reported that TREM2 can bind to Aβ oligomers directly. Evidence suggested that Aβ enhances TREM2-DAP12 interaction, and additionally, TREM2 deletion impaired Aβ degradation both *in vitro* and *in vivo* [36].

As shown in Figure 1, upon ligand binding, intracellular signals are conveyed through DNAX-activating protein of 12 kDa (DAP12), also known as TYROBP (tyrosine kinase-binding protein). The cytosolic immunoreceptor tyrosine-based activation motifs (ITAMs) on DAP12 will recruit the tyrosine protein kinase SKY, which leads to the activation of phosphoinositide 3-kinase (PI3K) - AKT pathway and phosphorylation of linker for activation of T-cells family member 1 (LAT) and/or LAT2, which recruit other signaling adaptors such as phospholipase Cγ (PLCγ), diacylglycerol (DAG), and protooncogene vav (VAV1). PLCγ further degrades phosphatidylinositol-3,4,5-trisphosphate (PIP3) to inositol trisphosphate (IP3), thus mobilizing Ca^{2+}; DAG activates the protein kinase Cθ (PKCθ); VAV1 induces actin remodeling that controls migration and adhesion [37-40]. All these signals and pathways help survival, proliferation, phagocytosis, and release of cytokines and chemokines [17, 30, 32, 41-43]. Full length TREM2 can be cleaved by a disintegrin and metalloproteinase domain-containing protein (ADAM10) and γ-secretase, thus releasing soluble TREM2 (sTREM2) [41, 44, 45].

The exact biological and pathological role of sTREM2 is not clear. sTREM2 was found in human CSF and the level increased in the CSF of LOAD patients [46, 47]. Some proposed it as a decoy receptor against TREM2 [43]. From cell culture, sTREM2 helped survival of bone marrow-derived macrophages [41]. Zhong et al. firstly suggested that sTREM2 plays an important role in promoting microglia survival and regulating inflammatory responses [43]. In addition, sTREM2 was found to reduce Aβ plaque load and improve spatial memory [48].

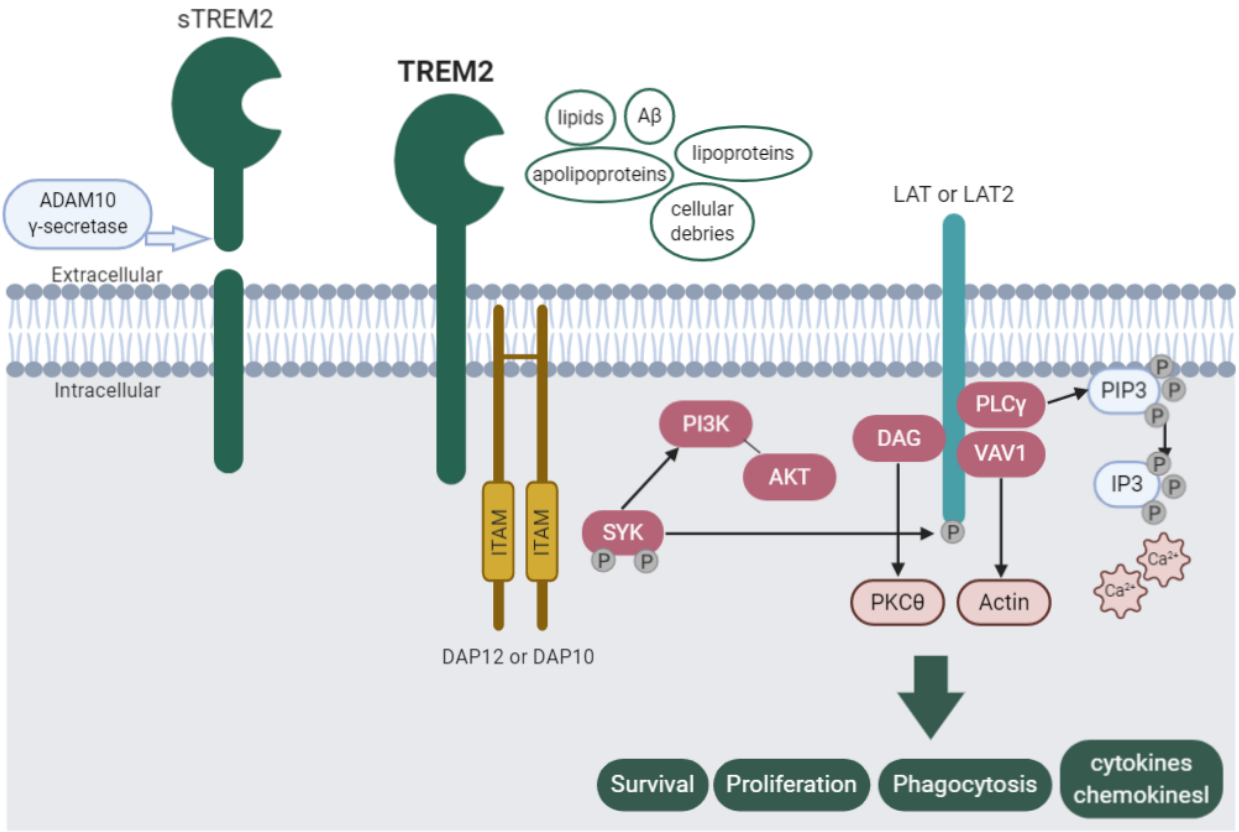

Figure 1: TREM2 ligands and signaling pathways.

3.0 TREM2 and Microglial Function

3.1 Proliferation

TREM2 expression and TREM2-induced signaling are essential in regulating microglia cell numbers, their survival, and proliferation. In primary microglial culture, cell numbers were reduced significantly when TREM2 was downregulated [25]. Wang et al. used a well-developed proliferation marker, Ki-67, with 5XFAD mouse model, and suggested that Aβ-induced microgliosis mostly depends on the proliferation of CNS-resident microglia, instead of peripheral blood monocytes. They also observed that the number of plaque-associated Ki-67^{+} microglia was decreased dramatically in case of *TREM2* knockout, indicating that proliferation of local microglia around the plaques were also impaired with TREM2 deficiency [20]. Another study assessed the loss of plaque-associated microglia at both early and late stages of AD pathology. They observed no significant change of proliferating microglia in 2-month-old mice, but significant reduction in 8-month-old *TREM2* knockout mice compared to wildtype mice. They divided the cells into $CD45^{high}$ and $CD45^{low}$ according to their expression level of CD45, and found that TREM2 loss of function affects the number of $CD45^{high}$ microglia throughout all AD progression, but affects $CD45^{low}$ cells only in the late stages of AD progression, suggesting the role of TREM2 in AD is disease-stage-dependent [49].

TREM2 also promotes microglial survival. Using *TREM2* knockout *in vivo* model and *TREM2* knockdown *in vitro* model, Zheng et al. demonstrated the relationship between TREM2/DAP12 signaling and Wnt/β-catenin signaling pathway and explained how TREM2 can promote the survival of microglia. β-catenin is an essential component of the Wnt signaling

pathway and important in maintaining a lot of biological activities, including cell survival. They proposed that in case of TREM2 deficiency, cell cycle is stopped at the G1/S transition and β-catenin becomes unstable, while TREM2 can inhibit the degradation of β-catenin through AKT/GSK3β signaling pathway, thus promoting microglial survival. Additionally, activation of Wnt/ β-catenin pathway by treating Wnt3a, LiCl, or TDZD-8 may rescue microglial survival in case of TREM2 loss-of-function [50]. With Aβ injection, microglial proliferation and caspase-3 activation were found to increase in wildtype (WT) mice but reduced in case of TREM2 deficiency [36]. Wang et al. investigated TREM2 deficiency and haploinsufficiency in 5XFAD mouse model and found the supporting role of TREM2 in microglial survival and proliferation together with colony stimulating factor-1 receptor (CSF-1R) signaling [32].

3.2 Chemotaxis

As the resident immune cells of CNS, microglia play an important role in responding to CNS injury, neuronal death, and certain diseases through rapid migration [51]. However, some recent studies have suggested that TREM2 loss of function may affect microglial chemotaxis. Mazaheri et al. analyzed the transcriptional profile of microglia from wildtype and *TREM2* knockout mice. They reported the downregulation of *Ccl2, Il1b, Tnf, Spp1*, and *Vegfa,* which are potential targets of TREM2 and involved in cell motility [52]. In an *ex vivo* study with brain slice, the migration distance of microglia was reduced [52]. Also, migration towards the defined chemo-attractants is limited with TREM2 deletion and rescued when TREM2 re-expressed [52]. Results from the *in vivo* study are consistent that with TREM2-deficiency,

microglial chemotaxis was reduced towards apoptotic neurons and extension of microglia processes was affected in TREM2 knockout mice [52]. Using high-resolution confocal and super-resolution microscopy, Yuan and their group observed that TREM2 or DAP12 deficiency impaired microglial function of polarizing, moving towards and compacting the plaques. Phosphorylated tyrosine, the marker of downstream kinase activation and indicator of microglial polarization was found reduced [53].

3.3 Phagocytosis

In CNS, TREM2 is expressed by microglia, which have a high phagocytic ability [54]. A lot of previous studies have demonstrated that TREM2 is involved in promoting microglial phagocytosis [22]. TREM2 loss of function have been associated with reduced phagocytosis of apoptotic neurons [55, 56]. Phagocytosis of Aβ is also impaired in both primary microglia and N9 microglial cell line with TREM2 deficiency [57]. Lee and their group developed transgenic mice (BAC-TREM2) with human TREM2 expressed in microglia and reported upregulation of some reactive microglial genes that are related to phagocytosis, like Lgals3 [58]. However, Wang et al. suggested that TREM2 does not have direct effects on phagocytosis of Aβ or apoptotic cells but can still help microglia phagocytosis indirectly through supporting survival of activated microglia [32]. Consistently, Yuan et al. observed that Aβ phagocytosis by microglia was not changed in $TREM2^{+/-}$ mice and proposed that fail-to-build the microglia barrier around the plaques could be the reason for increased Aβ accumulation and AD risks with *TREM2* mutations [53]. It is also possible that TREM2 interacts with other phagocytosis receptors, such as MerTK [59]. Collectively, it is confirmed by many studies that TRME2

plays an important role in microglia phagocytosis of Aβ and apoptotic neurons, but the mechanism of TREM2-dependent effects on such processes remains unclear.

3.4 Cytokine Release

Microglia are crucial mediators of inflammatory responses in CNS. In response to CNS injury or certain diseases, microglia regulate phagocytosis and secretion of inflammatory cytokines [60]. With Aβ exposure in wildtype microglia, Zhao and colleagues observed increased level of pro-inflammatory cytokines, like *interleukin 6 (IL-6)* and *macrophage inflammatory protein 1 α (CCL3)*, and decreased level of anti-inflammatory cytokines, like *Arg1*. However, these Aβ-associated cytokine releases were impaired in microglia with TREM2 deletion, suggesting that TREM2 regulates Aβ-associated cytokine expression [36]. Deficiency of TREM2 or DAP12 is observed to exacerbate lipopolysaccharides (LPS)-induced pro-inflammatory responses [61].

3.5 Cell-cell Communication

Microglia are found important in maintaining neural circuits during early stage of CNS development via phagocytosis of supernumerary synapses with receptor CX3CR1 or CR3 [62]. Previous studies have linked impaired synaptic pruning with weaker synaptic transmission and reduced functional connection. Microglia also regulate synaptic plasticity through brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) signaling

pathways [63]. In addition, Liddelow et al. proposed that activated microglia may induce A1 reactive astrocytes, which are toxic to the CNS, through cytokine release, including Il-1α, TNFα, and C1q. Formation of A1s impairs the normal function of promoting neuronal survival and phagocytosis, and induce death of oligodendrocytes and neurons [64].

Considering the vital role of microglia in synaptic pruning, some studies investigated the role of TREM2 in regulating such function and brain development. Recently, Filipello and colleague detected impaired defective synapse pruning and reduced number of microglia in the hippocampus in TREM2-deficient mice. They found that TREM2-deficient microglia in the hippocampus showed decreased internalization of synaptic markers in $CD68^{+}$ phagolysosome structures. In cultured neurons and TREM2-deficient microglia, synapse elimination was impaired [65].

Another study identified an endogenous cellular ligand on neuron (TREM2-L), which TREM2 can directly bind, and suggested a pathway for direct microglia-neuron communication. Expression of TREM2-L on apoptotic neurons were increased, thus enhancing phagocytosis by microglia [66].

4.0 TREM2 Importance in Activation States of Microglia

As the resident immune cells in the CNS, microglia are continually detecting the environment in CNS and responding rapidly through inflammatory reaction. Normally, microglia have a ramified shape in resting state, and transform to an ameboid or hypertrophic morphology upon activated [67]. Activated microglia are considered both protective and harmful to the CNS. They secrete protective neurotrophic factors assisting phagocytosis of damaged neurons or cellular debris. If over activated, they secrete toxic molecules, including reactive oxygen species and nitric oxide [38].

Previous studies have identified two sequential steps of microglial activation. The first stage includes the activation of Tyrobp, Apoe, and B2m, along with decreased expression of homeostatic factors, including Cx3cr1, and P2ry12/P2ry13. The second stage involves energy metabolism and phagocytosis, such as Lpl, Cst7, and CD9, which was found impaired in case of TREM2 loss-of-function, suggesting TREM2-dependent [68]. This mechanism explains the findings that deficiency of TREM2 in microglia at late stages of AD, rather than early stages of disease, worsen the disease progression [32].

In healthy brains, microglia have a specific homeostatic molecular signature, which is regulated by transforming growth factor β (TGFβ) signaling. While under disease conditions, microglia lose such homeostatic signatures and functions, and displayed specific disease-associated microglia (DAM) characteristics [16]. Holtman et al. demonstrated a disease-specific microglia molecular signature. (as shown in Figure 2) Primed microglia are associated with expression of cell surface markers, including *Itgax*, *Lgals3*, *Axl*, *Clec7a*, *MHC class 2*, and *Cxcr4*. [69] However, Zhou et al. demonstrated that signature of DAM in AD patients is different from

that in the 5xFAD model. In AD patients, some genes considered as homeostatic genes in mice were also upregulated, such as *P2RY12*, *CX3CR1*, and *TMEM119*. They also found upregulation of some genes, which are not part of DAM signature in mouse models, such as *SORL1*, *A2M*, and *CHI3L1* [70]. Krasemann and colleague proposed a mechanism that TREM2-APOE pathway regulates the functional phenotype of microglia in disease progression. Activation of TREM2-APOE pathway downregulates homeostatic phenotype of disease-associated microglia, thus unable to maintain homeostasis of CNS [16].

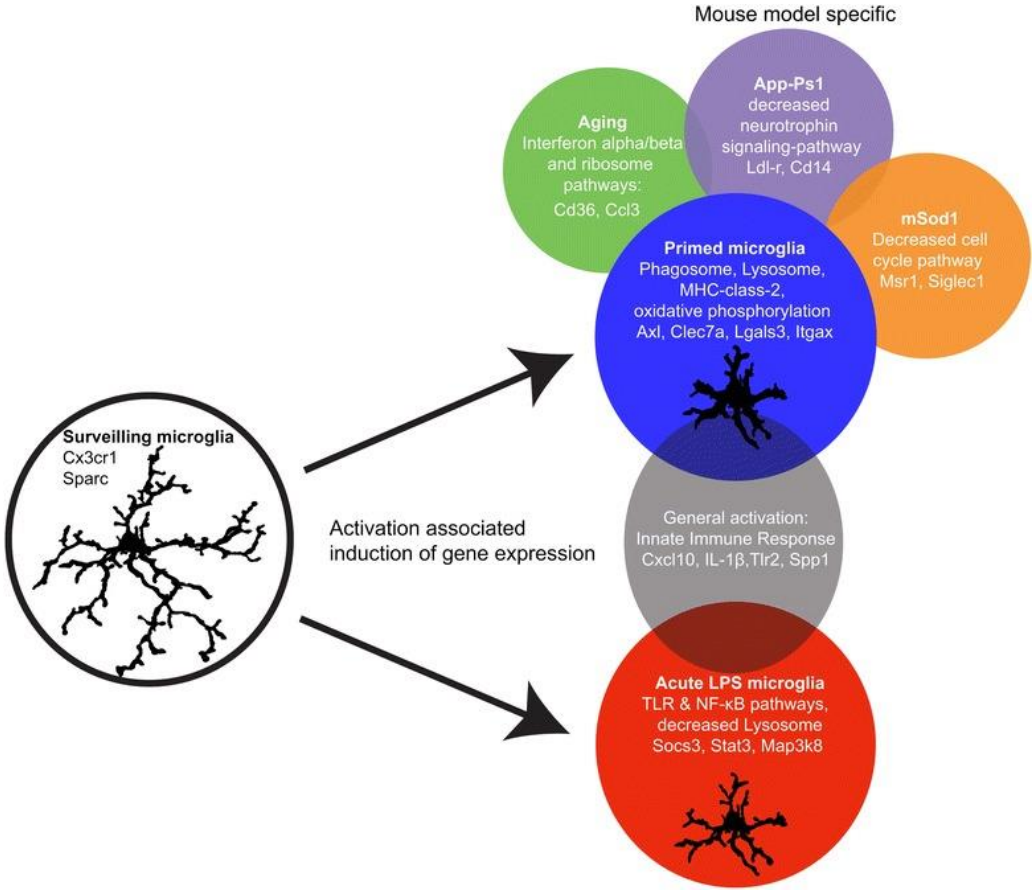

Figure 2: Specific gene expression networks with microglia activation by LPS or aging.

(Figure from Holtman et al. 2015) [69]

There are a lot of researches done to understand how TREM2 regulate microglia activation states. In primary microglia, LPS treatment reduced expression of TREM2, and modulation of TREM2 levels enhanced expression of LPS-induced pro-inflammatory cytokine genes [25].

Aβ treatment can also reduce expression of TREM2 in primary microglia [25]. It is also reported that microglia from APPPS1/*Trem2*$^{-/-}$ mice expressed increased number of *TGFbr1*, which is involved in maintaining resting microglia *in vivo*, suggesting that TREM2 loss of function might prevent the resting microglia from being activated [52]. Similarly, another study analyzed molecular signature of microglia using APPPS1/*Trem2*$^{-/-}$ mice and observed the suppression of AD-associated signature and significant upregulation of homeostatic mRNA signature [71]. Cantoni et al. studied how TREM2 regulates microglial activation in response to demyelination and found that compared to wild type mice, *TREM2* knockout microglia were less activated with downregulation of activation markers MHC II and more resting morphology observed [72].

Over and above the role as a receptor, TREM2 itself can activate microglia. As mentioned, TREM2 can be cleaved by ADAM10 and generate sTREM2, which enhance pro-inflammatory responses through nuclear factor (NF)-κB pathway and promote survival of microglia through PI3K pathway [43]. It is also proposed that sTREM2 act as an inhibitor of TREM2 through competitive ligands binding. Some suggested that sTREM2 could be a biomarker of AD because concentration of sTREM2 was found increased in CSF of AD patients [47, 73].

5.0 TREM2 Importance in Bioenergetic Regulation of Microglia

Recently, several studies reported the association between TREM2 and microglial metabolic state. Microglia respond rapidly to changes in the CNS. Along with microglial activation, energy metabolism will be switched from oxidative phosphorylation to glycolysis, which enables microglial plasticity [74].

Kleinberger et al. reported a significant decrease in cerebral blood flow and brain glucose metabolism in TREM2 T66M (a variant related to frontotemporal dementia) knock-in mice [75]. Increased number of autophagic vesicles were found in microglia with TREM2 deficiency in 5xFAD mice compared to microglia in 5xFAD mice (Figure 3 A and B) [17]. Nugent et al. demonstrated that loss-of TREM2 causes dysregulated cholesterol transport and metabolism in microglia. They found that TREM2 deficiency leads to failure of myelin cholesterol clearing and accumulation of cholesteryl ester, indicating impaired cholesterol transport in the brain system [76].

As for human patients, more autophagic vesicles were observed in microglia in AD patients with AD-associated TREM2 variants compared to other AD patients (Figure 3 C and D) [17]. Using AD patient iPSC-derived microglia with rare variants of TREM2, Piers et al. showed that human iPSC-microglia with rare TREM2 variants, such as the R47H risk variant, displayed significant metabolic deficits, including impaired mitochondrial respiratory function and failure of glycolytic immunometabolism switch. The defective PPARγ/p38MAPK signaling was identified responsible for this impairment [77].

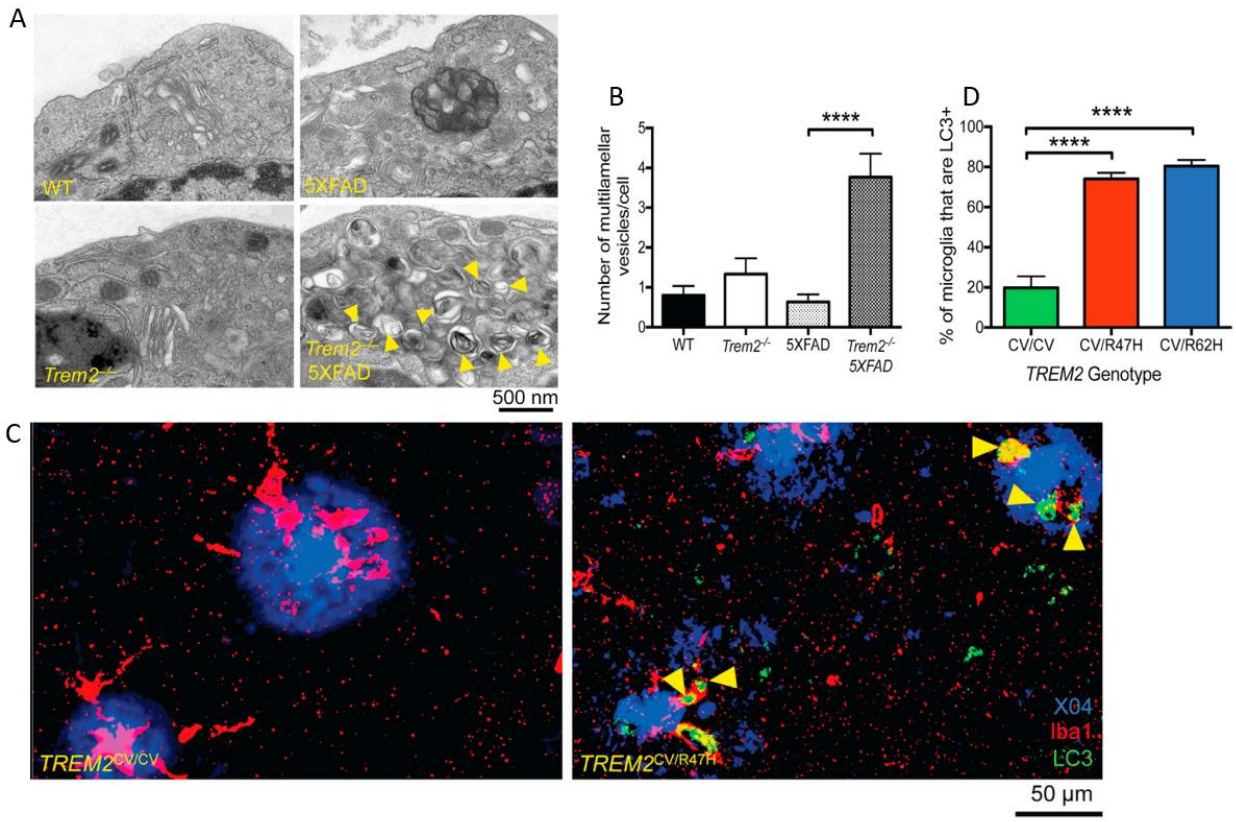

Figure 3: TREM2 deficiency increases autophagy in both 5xFAD mice and AD patients.

(A) Transmission electron microscopy images of microglia from 8-month-old wildtype, TREM2 KO, 5xFAD, and TREM2 KO 5xFAD mice. (B) Average number of multivesicular and multilamellar structures. (C) Confocal images of brain sections from R47H-AD patients and controls. (D) Percentage of LC3+ microglia from postmortem specimens of AD patients with R47H variant and R62H variant.

(Figures from Ulland et al. 2017) [17].

Ulland et al. reported decreased mammalian target of rapamycin (mTOR) signaling as well as markers for defective anabolic metabolism and energy production in microglia of *TREM2* knockout AD mice [17]. TREM2 was identified as the receptor for immune response that regulates metabolism of microglia during AD pathology through the mTOR signaling pathway, which supporting long-term cell growth, proliferation, and survival [17]. This mechanism provides an explanation for the impacts of TREM2 on microglial functions, including proliferation, migration

towards plaques, and phagocytosis of debris. The mTOR pathway also partially regulate autophagy, which is an intracellular recycling and degradation pathway for cellular components and energy homeostasis. Specifically, in response to extreme situations like cell starvation, autophagy helps maintaining cellular energy through breakdown and recycling of cellular components [78]. In case of mTOR impairment in TREM2-deficient microglia, autophagy is increased, and microglial clearance of Aβ would be enhanced in the short term. However, long-term impairment of mTOR signaling may still damage microglial function, including survival and proliferation [32]. So, although increased autophagy might help reduce inflammation and plaque for a short period, in the long-term, defective mTOR signaling can still impair microglial survival and function in response to plaque load. In addition, activation of Dectin-1, a cell surface receptor, or treatment with creatine analog 1-carboxymethyl-2-iminoimidazo-lidine (cyclocreatine) could rescue the metabolic impairment. Microglia survival and migration were found improved with regulation of dietary cyclocreatine during Aβ accumulation [17].

It is widely believed that microglia are able to detect any injuries or stressful situations in the CNS environment and react rapidly to those signals. These researches and findings regarding changes about bioenergetic regulation of TREM2-deficient microglia provide a potential answer to their dysfunction in responding to plaque stress in AD brains. Further studies are required to have a better understanding of microglial biology.

6.0 TREM2 and Other Neurodegenerative Diseases

TREM2 variants are risk factors for not only AD but also other neurodegenerative diseases, including polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL; or Nasu-Hakola Disease), frontotemporal dementia (FTD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS).

PLOSL is progressive disorder characterized by bone abnormalities, including pain and weakened ankles, and early-onset dementia. Although patients were reported all over the world, PLOSL is still a rare disease: 1 to 2 cases per million people in Finland and over 100 cases reported in Japan. Biallelic mutations in TREM2 or TYROBP (or DAP12) are considered as causes for PLOSL. According to the first report of TREM2 and PLOSL, DAP12 mutations were found in 79% patients and TREM2 mutations were reported in the other 21% patients [79]. A study in Italy has linked Q33X variant of TREM2 with amyloid accumulation in PLOSL [80]. Other rare variants of TREM2, such as E14X, W44X, W50C, W78X, G90VfsX99, A105RfsX84, V126G, and D134H, were also demonstrated as pathologic to PLOSL [81-84]. From immunohistochemistry study for PLOSL patients, amyloid plaques and neurofibrillary tangles were almost undetectable [85]. However, Kaivola et al. reported no significant differences of mental conditions between TYROBP deletion carriers and noncarriers in Finland cohort, indicating that heterozygous TYROBP deficiency is not strongly causative for dementia [86]. Similarly, another study investigated mutations in TYROBP in a Turkish cohort including 103 dementia patients and found no predicted mutations or variants, suggesting that TYROBP mutations are not a cause of cognitive decline in Turkish cohort [87].

FTD refers to a group of brain disorders, which mostly affect the frontal or temporal lobes of the CNS. They are much less frequent than AD and less understood. Causes of FTD are unknown, but family history is one of the risk factors. Some TREM2 rare variants were reported as pathologic to FTD, including Q33X, R47H, Y38C, R47C, T66M, W198X, c.391+1G>A, and c.482+1G>A, while some other variants were related with unclear pathogenicity, such as A28V, D39N, R62C, and D86V [12, 88-92].

TREM2 R47H was identified as risk factor for PD and ALS by some researches as well. A study investigated sporadic ALS among non-Hispanic white cohort and found that R47H variant was more frequent in ALS patients than healthy controls (odds ratio=2.40, $p=4.1\times10^{-3}$), indicating R47H may be a significant risk factor [93]. In a Caucasian cohort, TREM2 R47H was found significantly associated with increased risk of FTD (odds ratio=5.06, p=0.001) and PD (odds ratio=2.67, p=0.026) [94]. However, in European population, Lill and colleague failed to find consistent evidence for association between R47H variant and PD (odds ratio=1.36, p=0.0767) or ALS (odds ratio=1.41, p=0.198) [95]. Similarly, TREM2 R47H variant was not seen in sporadic ALS patients in a Chinese cohort [96].

Although there is no consistent conclusion about the relationship between TREM2 rare variants and risks of certain diseases, the role of TREM2 in CSN degeneration and microglial function has been established. Since some of these neurodegenerative diseases are very rare in the population, larger-scale studies in different population are difficult but still necessary and required for a better understanding.

7.0 Diagnostic and Therapeutic Targeting of TREM2: Opportunities and Challenges

To date, there is no treatment or medicine for preventing or slowing AD. As discussed above, TREM2 plays an important role in modulating microglia function, activation, lipid metabolism and immune response, thus leading to a lot of research on therapeutic targeting of TREM2. Some studies proposed that enhancing TREM2 levels or activities might be beneficial in AD treatment. It is reported that RXR can upregulate the expression of TREM2 and DAP12 [22, 23], although the mechanism remains unclear. Treatment of RXR agonist, such as bexarotene, was found to increase the expression of TREM2 mRNA in the cortex of AD mice [23]. An in vivo study reported that overexpression of TREM2 markedly improve cognition in the brain of APPPS1 mice (7-month), indicating increased TREM2 expression generates positive response to the disease progression. But this effect was abolished with overexpression of TREM2 in aged (18-month) APPPS1 mice [97, 98]. Brain section from AD patients with R47H variant had less microglia barrier around plaques, and it is proposed that TREM2-stimulating antibodies which can activate ERK and calcium pathway thus improving migration of microglia [53]. In addition to full-length TREM2, studies have demonstrated a protective role of sTREM2 to AD and as a potential biomarker of AD, while the mechanisms are not clear. Studies reported that sTREM2 promotes cell survival through activating extracellular signal-regulated kinases 1/2 and mitogen-activated protein kinase 14 [41]. Significant higher sTREM2 levels were observed in AD patients than case-matched controls. Positive correlation was found between sTREM2 levels in CSF and tau, but not Aβ, indicating the effects of sTREM2 depend on disease stages [46, 47].

However, there are still several concerns about TREM2 as a potential therapeutic target. Firstly, as reported, frequency of these TREM2 rare variants is less than 1% in the population,

while APOE4 accounts for about 20%. It is much less efficient to target TREM2 as a potential therapeutic strategy. For non-carriers, which are the majority of AD patients, there is no guarantee that TREM2-based therapeutic strategy will be effective for them. Secondly, the timing of TREM2-based treatment is important. It is ideal to activate TREM2 at early stage of the disease, along with the start of Aβ accumulation or even before, because normal or enhanced microglia activation would be beneficial to Aβ clearance, thus slowing down the disease progression. Thirdly, it remains unclear that how to target TREM2 in human. Further studies are required on TREM2-stimulting antibodies, because such activity might impair the ligands-binding of TREM2 [32]. Increasing TREM2 expression levels is proved to improve phagocytosis and reduce inflammation. However, overexpression of TREM2 through lentiviral approaches is impossible in human because of a high possibility of generating oncogenic transformation. In addition, expression of TREM2 outside the CNS is different and not only by microglia. We need to be aware of any unwanted or harmful side effects. Lastly, considering sTREM2 as a biomarker of the disease, only the CSF sTREM2 level is useful, but not plasma levels. Test of CSF biomarker levels is not suitable for primary care.

Collectively, targeting of TREM2 is a promising strategy for AD treatment or medicine. However, there still exist some challenges that need further study and test.

Bibliography

1. Association, A.s., *2019 Alzheimer's Disease Facts and Figures*, in *Alzheimers Dement*. 2019. p. 321-87.
2. U.S. Department of Health and Human Services, C.f.D.C.a.P., National Center for Health Statistics. *About Underlying Cause of Death, 1999-2017*. 2018 [cited 2020 Feb 10]; Available from: http://wonder.cdc.gov/ucd-icd10.html.
3. Ulrich, J.D., et al., *Elucidating the Role of TREM2 in Alzheimer's Disease.* Neuron, 2017. **94**(2): p. 237-248.
4. Trevisan, K., et al., *Theories of Aging and the Prevalence of Alzheimer's Disease.* Biomed Res Int, 2019. **2019**: p. 9171424.
5. R, A.A., *Risk factors for Alzheimer's disease.* Folia Neuropathol, 2019. **57**(2): p. 87-105.
6. Pimenova, A.A., T. Raj, and A.M. Goate, *Untangling Genetic Risk for Alzheimer's Disease.* Biol Psychiatry, 2018. **83**(4): p. 300-310.
7. Wes, P.D., et al., *Targeting microglia for the treatment of Alzheimer's Disease.* Glia, 2016. **64**(10): p. 1710-32.
8. Mahoney-Sanchez, L., et al., *The Complex Role of Apolipoprotein E in Alzheimer's Disease: an Overview and Update.* J Mol Neurosci, 2016. **60**(3): p. 325-335.
9. Jendresen, C., et al., *The Alzheimer's disease risk factors apolipoprotein E and TREM2 are linked in a receptor signaling pathway.* J Neuroinflammation, 2017. **14**(1): p. 59.
10. Wolfe, C.M., et al., *The Role of APOE and TREM2 in Alzheimer's Disease-Current Understanding and Perspectives.* Int J Mol Sci, 2018. **20**(1).
11. Song, W., et al., *Alzheimer's disease-associated TREM2 variants exhibit either decreased or increased ligand-dependent activation.* Alzheimers Dement, 2017. **13**(4): p. 381-387.
12. Sims, R., et al., *Rare coding variants in PLCG2, ABI3, and TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease.* Nat Genet, 2017. **49**(9): p. 1373-1384.
13. Bonham, L.W., et al., *Identification of a rare coding variant in TREM2 in a Chinese individual with Alzheimer's disease.* Neurocase, 2017. **23**(1): p. 65-69.
14. Jiang, T., et al., *A rare coding variant in TREM2 increases risk for Alzheimer's disease in Han Chinese.* Neurobiol Aging, 2016. **42**: p. 217 e1-3.
15. Ghani, M., et al., *Mutation analysis of the MS4A and TREM gene clusters in a case-control Alzheimer's disease data set.* Neurobiol Aging, 2016. **42**: p. 217 e7-217 e13.
16. Krasemann, S., et al., *The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases.* Immunity, 2017. **47**(3): p. 566-581 e9.
17. Ulland, T.K., et al., *TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease.* Cell, 2017. **170**(4): p. 649-663 e13.
18. Kober, D.L., et al., *Neurodegenerative disease mutations in TREM2 reveal a functional surface and distinct loss-of-function mechanisms.* Elife, 2016. **5**.
19. Del-Aguila, J.L., et al., *TREM2 brain transcript-specific studies in AD and TREM2 mutation carriers.* Mol Neurodegener, 2019. **14**(1): p. 18.
20. Wang, Y., et al., *TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques.* J Exp Med, 2016. **213**(5): p. 667-75.

21. Jay, T.R., et al., *TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer's disease mouse models.* J Exp Med, 2015. **212**(3): p. 287-95.
22. Jay, T.R., V.E. von Saucken, and G.E. Landreth, *TREM2 in Neurodegenerative Diseases.* Mol Neurodegener, 2017. **12**(1): p. 56.
23. Lefterov, I., et al., *RNA-sequencing reveals transcriptional up-regulation of Trem2 in response to bexarotene treatment.* Neurobiol Dis, 2015. **82**: p. 132-140.
24. Liu, G., et al., *Convergent Genetic and Expression Datasets Highlight TREM2 in Parkinson's Disease Susceptibility.* Mol Neurobiol, 2016. **53**(7): p. 4931-8.
25. Zheng, H., et al., *Opposing roles of the triggering receptor expressed on myeloid cells 2 and triggering receptor expressed on myeloid cells-like transcript 2 in microglia activation.* Neurobiol Aging, 2016. **42**: p. 132-41.
26. Bhattacharjee, S., et al., *microRNA-34a-Mediated Down-Regulation of the Microglial-Enriched Triggering Receptor and Phagocytosis-Sensor TREM2 in Age-Related Macular Degeneration.* PLoS One, 2016. **11**(3): p. e0150211.
27. Saber, M., et al., *Triggering Receptor Expressed on Myeloid Cells 2 Deficiency Alters Acute Macrophage Distribution and Improves Recovery after Traumatic Brain Injury.* J Neurotrauma, 2017. **34**(2): p. 423-435.
28. Celarain, N., et al., *TREM2 upregulation correlates with 5-hydroxymethycytosine enrichment in Alzheimer's disease hippocampus.* Clin Epigenetics, 2016. **8**: p. 37.
29. Perez, S.E., et al., *Neocortical and hippocampal TREM2 protein levels during the progression of Alzheimer's disease.* Neurobiol Aging, 2017. **54**: p. 133-143.
30. Raha, A.A., et al., *Neuroprotective Effect of TREM-2 in Aging and Alzheimer's Disease Model.* J Alzheimers Dis, 2017. **55**(1): p. 199-217.
31. Poliani, P.L., et al., *TREM2 sustains microglial expansion during aging and response to demyelination.* J Clin Invest, 2015. **125**(5): p. 2161-70.
32. Wang, Y., et al., *TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model.* Cell, 2015. **160**(6): p. 1061-71.
33. Painter, M.M., et al., *TREM2 in CNS homeostasis and neurodegenerative disease.* Mol Neurodegener, 2015. **10**: p. 43.
34. Lessard, C.B., et al., *High-affinity interactions and signal transduction between Abeta oligomers and TREM2.* EMBO Mol Med, 2018. **10**(11).
35. Yeh, F.L., et al., *TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia.* Neuron, 2016. **91**(2): p. 328-40.
36. Zhao, Y., et al., *TREM2 Is a Receptor for beta-Amyloid that Mediates Microglial Function.* Neuron, 2018. **97**(5): p. 1023-1031 e7.
37. Kobayashi, M., et al., *TREM2/DAP12 Signal Elicits Proinflammatory Response in Microglia and Exacerbates Neuropathic Pain.* J Neurosci, 2016. **36**(43): p. 11138-11150.
38. Konishi, H. and H. Kiyama, *Microglial TREM2/DAP12 Signaling: A Double-Edged Sword in Neural Diseases.* Front Cell Neurosci, 2018. **12**: p. 206.
39. Mecca, C., et al., *Microglia and Aging: The Role of the TREM2-DAP12 and CX3CL1-CX3CR1 Axes.* Int J Mol Sci, 2018. **19**(1).
40. Zhong, L., et al., *TREM2/DAP12 Complex Regulates Inflammatory Responses in Microglia via the JNK Signaling Pathway.* Front Aging Neurosci, 2017. **9**: p. 204.

41. Wu, K., et al., *TREM-2 promotes macrophage survival and lung disease after respiratory viral infection.* J Exp Med, 2015. **212**(5): p. 681-97.
42. Zheng, H., et al., *TREM2 in Alzheimer's Disease: Microglial Survival and Energy Metabolism.* Front Aging Neurosci, 2018. **10**: p. 395.
43. Zhong, L., et al., *Soluble TREM2 induces inflammatory responses and enhances microglial survival.* J Exp Med, 2017. **214**(3): p. 597-607.
44. Wunderlich, P., et al., *Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and gamma-secretase-dependent intramembranous cleavage.* J Biol Chem, 2013. **288**(46): p. 33027-36.
45. Thornton, P., et al., *TREM2 shedding by cleavage at the H157-S158 bond is accelerated for the Alzheimer's disease-associated H157Y variant.* EMBO Mol Med, 2017. **9**(10): p. 1366-1378.
46. Suarez-Calvet, M., et al., *sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers.* EMBO Mol Med, 2016. **8**(5): p. 466-76.
47. Heslegrave, A., et al., *Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease.* Mol Neurodegener, 2016. **11**: p. 3.
48. Zhong, L., et al., *Soluble TREM2 ameliorates pathological phenotypes by modulating microglial functions in an Alzheimer's disease model.* Nat Commun, 2019. **10**(1): p. 1365.
49. Jay, T.R., et al., *Disease Progression-Dependent Effects of TREM2 Deficiency in a Mouse Model of Alzheimer's Disease.* J Neurosci, 2017. **37**(3): p. 637-647.
50. Zheng, H., et al., *TREM2 Promotes Microglial Survival by Activating Wnt/beta-Catenin Pathway.* J Neurosci, 2017. **37**(7): p. 1772-1784.
51. Ransohoff, R.M., *A polarizing question: do M1 and M2 microglia exist?* Nat Neurosci, 2016. **19**(8): p. 987-91.
52. Mazaheri, F., et al., *TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury.* EMBO Rep, 2017. **18**(7): p. 1186-1198.
53. Yuan, P., et al., *TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy.* Neuron, 2016. **90**(4): p. 724-39.
54. Bisht, K., et al., *Dark microglia: A new phenotype predominantly associated with pathological states.* Glia, 2016. **64**(5): p. 826-39.
55. Atagi, Y., et al., *Apolipoprotein E Is a Ligand for Triggering Receptor Expressed on Myeloid Cells 2 (TREM2).* J Biol Chem, 2015. **290**(43): p. 26043-50.
56. Kawabori, M., et al., *Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke.* J Neurosci, 2015. **35**(8): p. 3384-96.
57. Xiang, X., et al., *TREM2 deficiency reduces the efficacy of immunotherapeutic amyloid clearance.* EMBO Mol Med, 2016. **8**(9): p. 992-1004.
58. Lee, C.Y.D., et al., *Elevated TREM2 Gene Dosage Reprograms Microglia Responsivity and Ameliorates Pathological Phenotypes in Alzheimer's Disease Models.* Neuron, 2018. **97**(5): p. 1032-1048 e5.
59. Savage, J.C., et al., *Nuclear receptors license phagocytosis by trem2+ myeloid cells in mouse models of Alzheimer's disease.* J Neurosci, 2015. **35**(16): p. 6532-43.

60. Ransohoff, R.M., *How neuroinflammation contributes to neurodegeneration.* Science, 2016. **353**(6301): p. 777-783.
61. Zhong, L., et al., *DAP12 Stabilizes the C-terminal Fragment of the Triggering Receptor Expressed on Myeloid Cells-2 (TREM2) and Protects against LPS-induced Pro-inflammatory Response.* Journal of Biological Chemistry, 2015. **290**(25): p. 15866-15877.
62. Paolicelli, R.C., et al., *Synaptic Pruning by Microglia Is Necessary for Normal Brain Development.* Science, 2011. **333**(6048): p. 1456.
63. Zhan, Y., et al., *Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior.* Nature Neuroscience, 2014. **17**(3): p. 400-406.
64. Liddelow, S.A., et al., *Neurotoxic reactive astrocytes are induced by activated microglia.* Nature, 2017. **541**(7638): p. 481-487.
65. Filipello, F., et al., *The Microglial Innate Immune Receptor TREM2 Is Required for Synapse Elimination and Normal Brain Connectivity.* Immunity, 2018. **48**(5): p. 979-991 e8.
66. Hsieh, C.L., et al., *A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia.* J Neurochem, 2009. **109**(4): p. 1144-56.
67. Fernandez-Arjona, M.D.M., et al., *Microglia Morphological Categorization in a Rat Model of Neuroinflammation by Hierarchical Cluster and Principal Components Analysis.* Front Cell Neurosci, 2017. **11**: p. 235.
68. Keren-Shaul, H., et al., *A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease.* Cell, 2017. **169**(7): p. 1276-1290 e17.
69. Holtman, I.R., et al., *Induction of a common microglia gene expression signature by aging and neurodegenerative conditions: a co-expression meta-analysis.* Acta Neuropathol Commun, 2015. **3**: p. 31.
70. Zhou, Y., et al., *Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease.* Nat Med, 2020. **26**(1): p. 131-142.
71. Gotzl, J.K., et al., *Opposite microglial activation stages upon loss of PGRN or TREM2 result in reduced cerebral glucose metabolism.* EMBO Mol Med, 2019. **11**(6).
72. Cantoni, C., et al., *TREM2 regulates microglial cell activation in response to demyelination in vivo.* Acta Neuropathologica, 2015. **129**(3): p. 429-447.
73. Piccio, L., et al., *Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status.* Acta Neuropathol, 2016. **131**(6): p. 925-33.
74. Kelly, B. and L.A. O'Neill, *Metabolic reprogramming in macrophages and dendritic cells in innate immunity.* Cell Res, 2015. **25**(7): p. 771-84.
75. Kleinberger, G., et al., *The FTD-like syndrome causing TREM2 T66M mutation impairs microglia function, brain perfusion, and glucose metabolism.* EMBO J, 2017. **36**(13): p. 1837-1853.
76. Nugent, A.A., et al., *TREM2 Regulates Microglial Cholesterol Metabolism upon Chronic Phagocytic Challenge.* Neuron, 2019.
77. Piers, T.M., et al., *A locked immunometabolic switch underlies TREM2 R47H loss of function in human iPSC-derived microglia.* FASEB J, 2020. **34**(2): p. 2436-2450.
78. Saxton, R.A. and D.M. Sabatini, *mTOR Signaling in Growth, Metabolism, and Disease.* Cell, 2017. **168**(6): p. 960-976.
79. Carmona, S., et al., *The role of TREM2 in Alzheimer's disease and other neurodegenerative disorders.* The Lancet Neurology, 2018. **17**(8): p. 721-730.

80. Ghezzi, L., et al., *Evidence of CNS β-amyloid deposition in Nasu-Hakola disease due to the TREM2 Q33X mutation.* Neurology, 2017. **89**(24): p. 2503-2505.
81. Klünemann, H.H., et al., *The genetic causes of basal ganglia calcification, dementia, and bone cysts: DAP12 and TREM2.* Neurology, 2005. **64**(9): p. 1502-1507.
82. Paloneva, J., et al., *DAP12/TREM2 deficiency results in impaired osteoclast differentiation and osteoporotic features.* J Exp Med, 2003. **198**(4): p. 669-75.
83. Paloneva, J., et al., *Mutations in Two Genes Encoding Different Subunits of a Receptor Signaling Complex Result in an Identical Disease Phenotype.* American journal of human genetics, 2002. **71**(3): p. 656–662.
84. Dardiotis, E., et al., *A novel mutation in TREM2 gene causing Nasu-Hakola disease and review of the literature.* Neurobiology of Aging, 2017. **53**: p. 194.e13-194.e22.
85. Satoh, J.I., et al., *Alzheimer's disease pathology in Nasu-Hakola disease brains.* Intractable Rare Dis Res, 2018. **7**(1): p. 32-36.
86. Kaivola, K., et al., *Heterozygous TYROBP deletion (PLOSLFIN) is not a strong risk factor for cognitive impairment.* Neurobiol Aging, 2018. **64**: p. 159 e1-159 e4.
87. Darwent, L., et al., *Mutations in TYROBP are not a common cause of dementia in a Turkish cohort.* Neurobiol Aging, 2017. **58**: p. 240 e1-240 e3.
88. Guerreiro, R.J., et al., *Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement.* JAMA Neurol, 2013. **70**(1): p. 78-84.
89. Chee, K.Y., et al., *A case of TREM2 mutation presenting with features of progressive non-fluent aphasia and without bone involvement.* Australian & New Zealand Journal of Psychiatry, 2017. **51**(11): p. 1157-1158.
90. Li, X., et al., *A novel homozygous mutation in TREM2 found in a Chinese early-onset dementia family with mild bone involvement.* Neurobiology of Aging, 2020. **86**: p. 201.e1-201.e7.
91. Cuyvers, E., et al., *Investigating the role of rare heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia.* Neurobiology of Aging, 2014. **35**(3): p. 726.e11-726.e19.
92. Ng, A.S.L., et al., *Targeted exome sequencing reveals homozygous TREM2 R47C mutation presenting with behavioral variant frontotemporal dementia without bone involvement.* Neurobiology of Aging, 2018. **68**: p. 160.e15-160.e19.
93. Cady, J., et al., *TREM2 variant p.R47H as a risk factor for sporadic amyotrophic lateral sclerosis.* JAMA Neurol, 2014. **71**(4): p. 449-53.
94. Rayaprolu, S., et al., *TREM2 in neurodegeneration: evidence for association of the p.R47H variant with frontotemporal dementia and Parkinson's disease.* Molecular Neurodegeneration, 2013. **8**(1): p. 19.
95. Lill, C.M., et al., *The role of TREM2 R47H as a risk factor for Alzheimer's disease, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Parkinson's disease.* Alzheimer's & Dementia, 2015. **11**(12): p. 1407-1416.
96. Chen, X., et al., *Assessment of TREM2 rs75932628 association with amyotrophic lateral sclerosis in a Chinese population.* Journal of the Neurological Sciences, 2015. **355**(1): p. 193-195.
97. Jiang, T., et al., *TREM2 Overexpression has No Improvement on Neuropathology and Cognitive Impairment in Aging APPswe/PS1dE9 Mice.* Mol Neurobiol, 2017. **54**(2): p. 855-865.

98. Jiang, T., et al., *Upregulation of TREM2 ameliorates neuropathology and rescues spatial cognitive impairment in a transgenic mouse model of Alzheimer's disease.* Neuropsychopharmacology, 2014. **39**(13): p. 2949-62.