The effects of Alzheimer's disease genetic risk factors APOE and TREM2 on tau pathology

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disease characterized by protein aggregation of amyloid-beta fragments in the brain. This is followed by hyperphosphorylation of tau which accumulates to form neurofibrillary tangles (NFTs). Clinical symptoms of AD are related to cognitive decline and memory issues caused by the loss of synapses and the death of neurons. Triggering Receptor Expressed on Myeloid Cells (TREM2) is a transmembrane protein expressed in microglia and is involved in immune responses. Certain genetic variants of TREM2 are significant risk factors for AD. Apolipoprotein E (APOE) is another protein that aids in lipid transport and other metabolic and immunomodulatory functions and its genetic variants also predispose individuals to AD. A great deal of research has been done to understand how TREM2 and APOE variants affect amyloid-beta deposition but only recently has their effects on tau pathology in AD been investigated. This paper aims to provide a guide on how TREM2 and APOE genes affect tau pathology.

1 Introduction:

Alzheimer's disease (AD) is a neurodegenerative disorder that was first observed in a 51-year-old female showing symptoms of cognitive decline and behavioral abnormalities [ea95]. Observed and named after the neuropathologist and psychiatrist Alois Alzheimer who diagnosed her in 1906, the disorder is believed to be caused by a variety of different factors from genetics to exposure to stress to age. It is characterized by cognition-related symptoms and memory decline. The two types of AD are familial AD (FAD), caused by genetic mutations passed down between generations, and late-onset AD (LOAD), which represents the majority of AD cases and is multifactorial, developing at a later stage than FAD. AD is the leading form of dementia, affecting over 55 million people worldwide, and is predicted to double in number in the next 20 years [ea15i]. Despite this, there is no certain drug or therapy that seems to work for all AD patients.

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This is mainly because the mechanisms that govern the disease, including the different risk factors and how they affect AD progression, are not properly understood. As a result, the mechanisms of AD must first be understood before viable therapies and significant progress can be made.

The basic pathological hallmarks of AD have been established as the deposition of amyloid- (A) in plaques followed by the formation of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein [ea18f] [ea18k] [ea17d]. This makes AD a primary proteinopathy of A and a secondary tauopathy. A is a peptide derived from Amyloid Precursor Protein (APP), a transmembrane protein highly expressed in the brain. Mutations in genes controlling A expression and processing or mutations in the APP gene can cause A levels to drastically increase and change proportions of different A forms, leading to A deposition. This triggers a cascade of downstream events (extensively described by Hass and Selkoe) [ea22i], driving AD progression and propagating the hyperphosphorylation of tau to form NFTs, as in line with the amyloid cascade hypothesis [ea92]. Tau is a microtubule-associated protein that plays a role in the structure of neurons and in axonal transport [ea23h]. In cases of AD, tau is hyperphosphorylated due to a range of factors, including developing A pathology, and becomes insoluble, causing it to accumulate in the brain. This aggregation of tau leads to neuronal dysfunction, decreased synaptic integrity and ultimately neuronal death. Recent studies have found a closer correlation of tau to the rapid decline in cognition and memory typical of AD, suggesting tau, rather than A, is the primary cause of cognitive impairment, making it a prime target for drug therapies [ea20c] [ea13d].

Neuroinflammation is an imbalance of pro-inflammatory responses in the brain and is also a hallmark of all neurodegenerative diseases, including AD. Recent literature in the past decades strongly indicates an influence of neuroinflammation on the development of AD pathology and the loss of neurons [ea18g] [ea18e] [ea15g] [ea14a]. Microglial cells are the resident immune cells of the central nervous system (CNS). They are the main culprits of neuroinflammation in AD. It has been found that they, directly and indirectly, react to A deposition and tau accumulation by changing their physiological functions as well as releasing proinflammatory cytokines and other immune factors, driving AD progression. The importance of neuroinflammation is supported by the fact that several genetic risk factors of AD are immune-related genes, as seen in multiple genome-wide association studies (GWAS) [ea18h] [ea15d] [ea22a]. Out of all these genes, it was found that the Apolipoprotein E (APOE) gene and Triggering Receptor Expressed on Myeloid Cells (TREM2) genes, related to immune responses and expressed mainly in glial cells, are significant risk factors for AD, with APOE being the most recurrent risk factor among AD patients. TREM2 is a transmembrane receptor protein expressed by microglia that plays a role in many proinflammatory functions and pathways [ea22f]. APOE, on the other hand, is a protein produced primarily by astrocytes though neurons and microglia can adapt to produce it as a response to stress. It mainly functions as a lipid and cholesterol transporter and is integral in the immune response and overall health of the brain, particularly during AD progression and pathol-

ogy [ea23b].

Significant advancements have been made in understanding the risk factors of AD, especially TREM2 and APOE, in the last few decades but research has been limited to their effects on A deposition and earlier stages of AD. Fewer studies have been done on the later stages of AD, which include the effects of tau pathology [ea11]. Only recently have significant breakthroughs been made on the effect of TREM and APOE on tau, and so this paper will provide an overall guide and understanding of these discoveries.

2 Tau pathology in AD:

Under normal physiological conditions, microtubule-associated protein tau (MAPT) regulates the assembly of axonal microtubules (MTs). It is typically highly soluble and binds with tubulin to maintain MT stability, which is important for axonal transport and outgrowth [ea21h]. Its structure is made up of four repeating subunits or domain regions called R1, R2, R3, and R4. Tau occurs in six isoforms that are formed by post-transcriptional modifications (PTMs) of the MAPT gene. These isoforms differ in the number of N-terminal inserts and repeating subunits [ea21a]. The structure of each isoform is integral to its function [ea23i].

Normal isoforms of MAPT are already phosphorylated to some extent so that they may bind to tubulin [Cle23]. This phosphorylation is mainly regulated by kinase/phosphatase signaling pathways, including glycogen synthase kinase-3 (GSK3-), cyclin dependent kinase-5 (CDK-5), protein kinase A (PKA), microtubule affinity regulating kinase (MARK) and Fyn kinase, as described by many researchers [ea21i]. Additionally, the activities of phosphatases, particularly protein phosphatase 2A (PP2A), are important in tau phosphorylation [ea21j]. In the event of AD and other tauopathies, however, tau is aberrantly hyperphosphorylated to be insoluble, altering its shape and preventing it from carrying out its physiological functions. Excessive phosphorylation due to disrupted kinase signaling is thus a major way by which the structure of tau is altered. These structural disruptions can lead to clumping, preventing the degradation of tau, eventually resulting in the accumulation and formation of NFTs, as seen in AD.

What causes this altered kinase signaling and aberrant tau hyperphosphorylation and aggregation, however, remains to be fully elucidated but a variety of factors such as structural deformities [Tra23], inflammation, A deposition, and impaired glucose metabolism are believed to play a role [ea08a]. Understanding these factors can also explain the complex interlinked role of tau in the brain, which is still being studied [ea08b]. Genetic risk factors, which will be explored, are one of these factors that underlie pathological changes in tau.

One important aspect of tau pathology includes the spreading of tau aggregates in a "prion" like manner, as noted by Devos et al. [ea18b] It occurs along anatomically connected networks and originates in areas with vulnerable cell populations. Cell vulnerability is derived from a range of different factors, including genetic, physiological, and neurochemical ones [ea18d]. Those more

vulnerable to early tau are typically large pyramidal neurons in the entorhinal cortex (EC). This is where the Braak stages of tau pathology begin, a method used by scientists to classify the spreading of tau between different brain regions [ea22k]. Braak stages I and II indicate NFTs confined mainly to the entorhinal region of the brain, Braak stages III and IV indicate involvement of limbic regions such as the hippocampus, and Braak stages V and VI indicate moderate to severe cortical involvement. Diagnosis of these stages is done through imaging technology, and the opinion of a neuropathologist [ea06a].

Tau is released and spread between these regions as both free tau and via extracellular vesicles [ea23g]. Microglia also play a role in tau propagation, especially through the secretion of tau-containing exosomes [ea15a]. Trans neuron and trans-synaptic tau propagation has also been found to occur, with an increase in neuron stimulation and activity increasing tau pathology [ea16c]. Neurons can also uptake tau at the axon terminals and be transported anterogradely but this depends on the conformation and size of tau aggregates [ea13e].

It is to be noted that restriction of NFTs and prevention of further phosphorylation is very important in this stage. Microglia sense and carry out responses to injury and stress in the brain, including phagocytosis of debris, tau aggregates, and other elements. As a result, their function is very significant in this stage and its further progression as they restrict tau seeding [ea21b].

Once tau seeding has occurred, tau-induced apoptosis of neurons begins to occur. This is the part of the pathology that causes the onset of the clinical symptoms and cognitive impairment seen in AD [Ram04].

3 Mouse models of AD:

The findings reviewed in this paper deal with many different AD and other neurodegenerative mice models.

P301S or PS19 [ea23j] is one significant mice model as it is a pure tauopathy that expresses transgenic mutant human tau and has no A plaque formation. NFT-like aggregations are seen in various brain regions from hippocampus to amygdala and spinal cord. Seeding occurs at around 1.5 months of age with mice developing neuronal loss and brain atrophy at 8 months, in the hippocampus and other regions such the neocortex and entorhinal cortex. P301S mice also display signs of cognitive impairment, as well as motor and memory deficits, including spatial learning problems. P301L [ea05] is another mice model, similar to P301S, but is caused by a different mutation and is less widespread in its pathology.

APP/PS1 or APP/PS1-21 [ea06c] is another AD mouse model that will be reviewed. Primarily, it expresses A pathology with signs of induced tau pathology. APP/PS1 mice contain two human transgenes, the APP Swedish mutation and PSEN1 L166P mutation. Expression of APP transgene is expressed in much higher amounts than endogenous murine APP. Human A42 is also preferentially generated over A40 but concentrations of both increase with age in the mice, and plaque deposition is seen at around six weeks of age, starting from the neocortex. Plaque then appears in other regions at 3 to 5 months of age. CSF

concentrations of total tau also increase in these animals, starting at six months and reaching a 5-fold increase by 18 months of age. Cognitive impairment and impacted learning is seen around 7 to 8 months.

5XFAD [ea06b] is also an AD mice model that is reviewed but expresses only A pathology. It expresses human APP and PSEN1 transgenes with a total of five AD-linked mutations and has a very early and aggressive presentation of pathology. Amyloid plaques, along with gliosis and other injury response mechanisms, are seen in mice as young as two months of age. Neuron loss occurs in several brain regions, beginning at about 6 months in the areas with the most amyloidosis. Mice display a range of cognitive and motor deficits, beginning at 4 to 5 months of age.

Other mice models were also used and they will be described along with the findings. It must be noted that mice models, despite expressing human transgenes, have their limitations [ea23k]. Most mice only develop human-like tau pathology when transgenes are inserted. However, recent studies have shown that insertion of transgenes disrupt the coding sequence of endogenous genes [ea19c]. The impact of this on tau pathology is unknown but it could lead to less accurate models of neurodegeneration. Injecting purified tau in models to induce pathology could be an alternate option to transgenes but it is a highly manipulated form of inducing tau pathology that does not always reflect the reality. Moreover, different lines of mice show different pathological features, making standardization of symptoms difficult throughout models [ea23l]. Hence, these limitations must be taken when reviewing AD mice models.

4 TREM2 in tau pathology:

TREM2 is a single-pass innate immune receptor that belongs to the Immunoglobulin superfamily (Ig-SF) [For08]. It is expressed in all tissue macrophages, including microglia, and serves as the means for many major immune pathways in the brain. Its substrates include glycolipids, phospholipids, lipidated particles, and lipoproteins including High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and APOE [ea17j]. TREM2 also plays a role in the phagocytic functions of microglia [ea15h].

The TREM2 genetic variant that is related to its normal function is the TREM2 Common Variant (T2CV). Other variants of TREM2 include R47H, R62H, T66M and more. Of these, R47H is perhaps the most noticeable as it causes a loss of function (LOF) phenotype in TREM2 and is the one of the more studied variants of TREM2 due to its association with risk of LOAD [ea13b]. It is also associated with other neurodegenerative diseases such as Nasu-Hokola disease (NHD) [ea17c] and frontotemporal dementia (FTD) [ea13c]. As a result, studying the general roles that TREM2 plays in AD can open doors to many different fields and treatment options.

The effect of TREM2 on the tauopathy stages of AD has been found to be context and A-dependent. In this paper, the differential effects of TREM2 on tau are explored using the presence and absence of A.

4.1 The effect of TREM2 on tau pathology in the presence of A:

In the presence of A it has been observed that TREM2 restrains AD progression, while LOF variants such as R47H result in more aggressive AD progression. This is hypothesized to be a result of the complex and interlinked roles of TREM2, A and tau. Microglia have long been shown to cluster around A plaques and these plaque-associated microglia express a higher number of their disease-associated microglia (DAM) genes, reflecting a more activated state of the cells [ea17a] [ea17k]. TREM2 was found to be significant in this microglial association with plaques. Studies showed that ablation or LOF variant of TREM2 reduced plaque associated microglia [ea14b] [ea15f] [ea18a]. TREM2 deficient A mice also developed more aggressive and enlarged plaques [ea16b], suggesting that TREM2 activates microglia to perform phagocytic and trimming functions on A plaques. TREM2 also plays a role in the transition of microglia between its homeostatic and active signatures [ea21f]. This means that TREM2 could be the regulatory factor that decreases the expression of DAM genes in microglia, further showcasing its control over A pathology. This control is significant in the context of tau as A pathology has been shown to drive tau pathology through the amyloid cascade hypothesis with numerous studies highlighting this link [ea21k] [ea19e] [ea19b].

Leyns et al. [ea19d], which investigated how the germline knockout of TREM2 and the TREM2 R47H variant affects tau pathology in APP/PS1 mice, highlighted how ablation or loss of TREM2 function reduces microgliosis near A plaques and allows tau seeding and spreading of neuritic plaques to progress, as studied 3 months post injection of tau aggregates purified from AD patients into the A-containing mice. This supports the fact that the presence of TREM2 mediates the effect of A on tau pathology. Lee et al. [ea21e] confirmed these findings by crossing TREM2 deficient mice with a line expressing P301L mutant protein, alone and in combination with PS2APP, a transgene which drives A production. This means that they used two models: one with only tau (P301L) and one with tau and A (TauPS2APP). Their results from single-cell RNA sequencing showcase that microglia transition into their DAM state in a TREM2 and amyloid-dependent manner. TREM-2 deletion, in the presence of A, further exacerbated tau accumulation and spreading, promoting brain atrophy, while in the condition without A pathology, TREM2 deletion did not affect these processes. As a result, TREM2 could possibly slow AD progression and reduce tau-driven neurodegeneration by restricting the degree to which A facilitates the spreading of pathogenic tau.

In a recent study by Gratuze et al. [ea21d] it was also found, by studying 5XFAD mice models which were unilaterally injected at 6 months of age with AD-tau then analyzed 3 months later, that both TREM2 knockout (KO) and microglial ablation (as done before injection of AD-tau) dramatically enhance tau seeding and spreading around A plaques. It was also noted in the study that microglia repopulated to cluster around plaques but had a reduction in DAM gene expression, with the elevation in tau seeding. This suggests that A-induced

tau propagation is delayed specifically by TREM2-dependent activation of the DAM phenotype.

4.2 The effect of TREM2 on tau pathology in the absence of A:

The role of TREM2 has been described to have different outcomes in the absence of A. This was reflected by Leyns et al. [ea17f] and Gratuze et al. [ea20b], both of which investigated the P301S human transgenic mouse model. Leyns et al. found that TREM2 deficiency reduces inflammation, astrogliosis and microgliosis, resulting in a higher brain mass. Notably, tau levels remained the same but activated microglia were decreased in TREM2 deficient mice with a decrease in microglial proliferation and fitness along with decreased inflammatory gene expression in the piriform cortex and hippocampus. Gratuze et al. supported the idea of decreased microgliosis as a cause for attenuated AD progression by highlighting how the TREM2 R47H variant caused lesser activated microglia and hence reduced phagocytosis, resulting in reduced synapse loss and brain atrophy, as compared to mice with the common variant of TREM2. As a result, in models of pure tau pathology, TREM2 deficiency alleviates AD related symptoms of brain atrophy and loss of neurons. This suggests that in the absence of A, TREM2 no longer impacts the seeding and spreading of tau, but in turn modulates the immune responses of microglia towards tau accumulation, leading to the phagocytosis of synapsis and viable neurons decreasing synaptic integrity and causing cognitive impairment. TREM2 also seems to increase inflammatory responses in the absence of A driving AD progression.

Studies investigating tau pathology that used the hemizygous or missense variant of TREM2 have found some unique findings, however. Sayed et al. [ea18i] used in vivo imaging to illustrate that microglia from aged TREM2-haploinsufficient P301S mice show a greater impairment in their injury response and ability to surround tau tangles compared with microglia from aged TREM2-KO mice. In transgenic mice expressing mutant human tau, TREM2 haploinsufficiency, but not complete loss of TREM2, thus increased tau pathology. They also showed that TREM2 haploinsufficiency, at late stages of tau, elevated the expression of proinflammatory markers and exacerbated brain atrophy. This means that TREM2 could play a direct role in preventing tau seeding and so further exploration of the linked role of A, tau and TREM2 must be done to fully understand the effects of TREM2 on tau pathology.

5 APOE and its alleles in tau pathology:

APOE is the main transporter of lipids and cholesterol in the brain and plays a role in the redistribution of cholesterol and the metabolism of lipoproteins. In the CNS, it is primarily produced by astrocytes but microglia and neurons can also produce it during stress. APOE plays a role in many immune pathways and so is significant in AD pathology [ea23c].

APOE in humans generally occurs in three isoforms - E2, E3 and E4 [ea23d]. APOE3 is the most common APOE isoform, with most people having at least one copy of the E3 allele. APOE4 is the next most common isoform and is the one associated with the most increased risk of AD. In fact, APOE4 confers the highest AD risk than any other genetic factor, including TREM2 LOF variants [ea17g]. The least common APOE isoform is APOE2 and is associated with the least risk for AD as it seems to be protective in nature [ea17h].

This review will explore how and why APOE4 is such a prominent risk factor for AD and comment on the other variants and alleles of APOE and its effects on tau pathology.

5.1 APOE4 in tau pathology:

Shi et al. [ea17i] studied the role of APOE in P301S mice. They found that APOE4 drives tau progressions the most of all APOE alleles and that complete KO of APOE is the most protective after APOE2. They observed that APOE4 leads to greater neuronal loss and greater loss of brain mass, with increased inflammation (seen by increased release of pro-inflammatory cytokines) and increased activated microglia in the presence of tau. E4 also had impaired autophagy, increasing tau levels, especially insoluble tau. Phosphorylated tau was also elevated and increased astrocytic activation also released more neurotoxic factors and is perhaps the cause of increased tau-related neurodegeneration observed in E4. Zhao et al. [ea20e] supported these observations using cerebral organoid models made by induced pluripotent stem cells (iPSCs). Results found that organoids from AD patients homozygous for the E4 allele had lower synaptic integrity and increased rates of neuronal death. Additionally, tau levels were elevated in organoid models from all patients harvesting the E4 allele. The isogenic conversion of the E4 allele to E3 improved these effects, emphasizing APOE4 as an effective therapy target.

However, as there may be discrepancies between the effects of APOE in transgenic models of tau versus actual human patients, studies using human participants were also reviewed. They provided similar findings to the studies described above with Dincer et al. [ea22c] using imaging and CSF data to show that APOE4 as associated with increased tau burden in regions with high APOE mRNA expression. The results also suggest that the severity of brain pathology might be driven by gene expression, further highlighting the potential of APOE4 as a therapy target. Benson et al. [ea22b] also analyzed human participants among a cohort of mildly cognitively impaired (MCI) individuals and found that APOE4 is associated with abnormal levels of p-tau and A42 in CSF, which are both associated with higher AD risk, further supporting the detrimental role of APOE4 in AD pathology. Young et al. [ea23m] also supported these results using cognitively unimpaired (CU) and MCI participants, finding that APOE2 was associated with lower amyloid positivity rates while APOE4 was associated with higher amyloid positivity rates. Among A-positive CU individuals, E2 and E4 were also associated with reduced and greater continuous amyloid burden, respectively. APOE2 was also associated with reduced regional tau in all regions,

whereas APOE4 was associated with greater regional tau. These differences were confirmed by contrasting APOE3 homozygous mice with mice that have one allele of APOE3 one APOE 2 and mice that have one allele of APOE 3 one of APOE4. The magnitude of protective E2 effects on regional tau was consistent across brain regions, whereas detrimental E4 effects were greatest in the medial temporal lobe. These patterns were further confirmed in A positive MCI participants.

Hence there is a general consensus that APOE plays a significant and recurrent role in AD and its tau stages.

5.2 Other APOE variants alleles in tau pathology:

The role of APOE in tau pathology can be further evidenced by the other variants and alleles of APOE. This is seen in the already described protective role of APOE2 but can also be observed in the effect of the Christchurch variant of APOE3.

This variant, also called R136S, is caused by a rare genetic mutation that alters the structure of APOE. It has been found to play a protective role in AD pathology by delaying the onset of clinical symptoms. The case report, as described by Quiroz [ea19a], studied a woman who, although having unusually high levels of A and tau tangles and a family history of FAD, showcased only clinical symptoms in her seventies, much later than predicted. This case study hypothesized that the reason for this could be her APOE status, which was homozygous for the APOE3 Christchurch variant. This protective nature of R136S was further studied by Sepulveda-Falla et al. [ea22j], who also used a participant with FAD and both alleles of Christchurch APOE3 but mainly focused on tau pathology. The results of their study also showed that the Christchurch APOE3 variant is protective and delays the effects and severity of tau pathology as found using in vivo follow-up PET imaging and postmortem findings. They identified a distinct anatomical pattern of tau pathology with atypical accumulation and unusual postmortem regional distribution characterized by sparing in the frontal cortex and severe pathology in the occipital cortex. Thus, the Christchurch variant may impact the distribution of tau pathology, and modulate age at onset, severity, progression, and clinical presentation of AD, further suggesting APOE as a possible therapeutic strategy for the tau stages of AD.

5.3 Why APOE affects tau pathology:

Why APOE affects tau pathology so extensively can lie in a number of different reasons. One reason could be its effect on tau phosphorylation. A study by Zhou et al. [ea16a], which used the E4FAD mice model, compared mice that expressed APOE4 with mice that expressed APOE3 (E3FAD). E4FAD model [ea12] is an AD model derived from 5XFAD crossed with APOE4 targeted replacement mice. E3FAD and E2FAD can also be created by crossing with APOE3 and APOE 2 respectively. In the study, tau phosphorylation and pathology were observed to be relatively more elevated in E4 and more extensive neuronal damage was

observed. Furthermore, CDK5, its subunits, and calpain were also increased in E4FAD mice. Evidence suggests that disrupted kinase signaling, mainly of the CDK-5-calpain pathway, leads to tau hyperphosphorylation [ea10]. Calpain, a calcium-dependent protease, cleaves the N-terminal form of p35 to give rise to p25, and the activation of CDK5. p35 and calpain are all highly elevated during AD, with an increase in CDK5 activity. This suggests that APOE4 influences the CDK5-calpain pathway to increase tau phosphorylation and hence could possibly explain the role of APOE4 as a risk factor.

Furthermore, Saroja et al. [ea22g] explored how APOE4 could interact with other factors, in this case, glypican-4 (GPC4), a heparan sulfate proteoglycan (HSPG), to drive tau phosphorylation. HSPGs, namely GCP4, are one of the required elements for tau aggregation, along with APP [ea22h]. Sarjo et al. found that in the absence of GPC4, APOE4-induced tau hyperphosphorylation was largely reduced in human-iPSC-derived astrocytes and in an in vivo P301S mouse model. They also showed that APOE4-mediated surface trafficking of APOE LDL receptor-related protein 1 through GPC4 can be a means for tau spreading. This means that APOE4-induced tau hyperphosphorylation is directly mediated by GPC4. The role of HSPGs is also seen in a study by Marion et al. [ea23f] which hypothesized that the APOE3 Christchurch variant is protective in nature particularly due to its reduced pathological interactions with HPSGs. Their results proved this by showcasing that one of its antibodies 7C11 disrupted APOE4 and HSPG interactions, resulting in reduced tau pathology in P301S mice.

The effect of APOE4 on tau pathology also lies in its role in tau-mediated neurodegeneration. A study by Shi et al. [ea22l] proved that it is mainly the microglial response to tau pathology that drives neurodegeneration. They also showed that APOE4 is the strongest risk factor for AD primarily because it affects microglial response by affecting microglial activation and subtypes, although they do acknowledge that minor changes to tau structure and hyperphosphorylation by APOE4 could also play a secondary role. They proved this by showcasing that ablation of microglia stops neurodegeneration and that suppression of APOE4 keeps microglia in its homeostatic state. The study also highlighted that it is the microglia state/subtype that affects neurodegeneration, not microglia proliferation. Hence it proved that as APOE4 majorly affects microglial subtypes, which play a significant role in tau-related neurodegeneration, the link between microglia activation and APOE4 could be the key to understanding the critical role of APOE4 in AD.

6 APOE-TREM2 axis in tau pathology:

The different signatures expressed by microglia are very important in AD progression. In the past, active microglia were divided into only two subtypes - M1 and M2. M1 microglia were pro-inflammatory and neurotoxic while M2 microglia were anti-inflammatory and neuroprotective [ea22e]. However, extensive amounts of research [ea15e] [ea13a] in the past decades have indicated that

microglia subtypes are not so clear-cut. Many signatures that were found could not be classified in the traditional M1/M2 subsets and so new subsets of microglia signatures were developed. As mentioned before, DAM [ea17b] remains one of the most relevant microglial signatures as it is highly prevalent in the context of neurodegeneration, including cases of AD pathology, and expresses a phenotype called microglial neurodegenerative (MGnD) [ea18j]. This is characterized by the downregulation of homeostatic genes and upregulation of genes involved in inflammation, phagocytosis, cell survival, lysosome function, and lipid metabolism.

Other reactive microglial subsets were also identified beyond DAM such as the IFN-R subtype, characterized by high expression of IFN-I-response (IFN-R) genes, and the MHC II subtype, characterized by abnormal expression of MHC class II (MHC II) genes [ea17l]. Additional subtypes like Cycling microglia were also identified [ea20a]. Many studies have corroborated this vast range of microglia subtypes, albeit with differing names. They were all observed in different models of A accumulation, tauopathy, and other neurodegenerative models, and were also confirmed by a recent meta-analysis of human AD cohorts [ea18c].

These different signatures have varied impacts on neurodegeneration. Some, like DAM, may be useful in the phagocytosis of plaques and tangles [ea09]. Others, like INF-R, which increase neuroinflammation and progress AD pathology, may be detrimental [ea20d]. All these subtypes can coexist together and so their overall net effect regulates the effect of microglia on AD progression.

APOE TREM2 have both been found to play a role in this transition of microglial signatures, especially through its combined APOE-TREM2 axis. As microglia signatures are so important, the effects of this axis could thus be the key to understanding why the two genes are such significant risk factors for AD.

The APOE-TREM2 axis refers to the interaction of TREM2 and APOE to modulate each other, as seen in the direct association and binding of APOE to TREM2 [ea15c]. Krasemman et al. [ea17e], using the various mice models of amyotrophic lateral sclerosis (ALS), AD and multiple sclerosis (MS), explored the role of this axis in neurodegeneration by investigating its effects on microglial phenotypes. They highlighted that a specific APOE-dependent molecular signature occurs in microglia during neurodegeneration with the APOE pathway itself mediating a switch from a homeostatic to a neurodegenerative microglia phenotype after phagocytosis of apoptotic neurons. Moreover, TREM2 is what induced this APOE signaling, as evidenced by how targeting the TREM2-APOE pathway restored the homeostatic signature of microglia. This prevented neuronal loss in an acute model of neurodegeneration and so their work identifies the TREM2-APOE pathway as a major regulator of microglial functional phenotype in neurodegenerative diseases.

Chakrabarty et al. [ea21c] theorized the effect of the APOE-TREM2 axis on tau pathology by using this role of the axis in microglial signatures. The study uses an experiment by Wang et al. [ea21g] to do so. In the experiment, a drug that conditionally knocked down APOE4 was introduced in P301S tau mice models that had either astrocytic APOE3 or APOE4 alleles. 4 months post

APOE suppression, results were compared with mice before APOE suppression. A significant reduction in p-tau pathology and its effects was observed, with findings indicating lesser tau-induced synaptic loss and microglial phagocytosis of synaptic elements. Importantly, RNA sequencing analysis revealed decreased disease-associated gene expression in all glial cells, including microglia, and neurons. Chakrabarty et al. thus hypothesize, using reasonable evidence, that this change in microglial gene expression is responsible for the changes observed in pathology and that the APOE-TREM2 axis is in turn responsible for the changes in microglia gene expression/subtype.

Atagi et al. [ea15b] also explored the role of the APOE-TREM2 axis. Their work, using a biochemical assay, showed APOE has a high affinity to bind with human TREM2. They also showed that increasing the phagocytic activity of APOE-bound apoptotic neurons by microglia occurs in a TREM2 dependent manner. Additionally, the TREM2 R47H variant showed significant reduction in APOE binding compared to normal TREM2. This further provides evidence that the TREM-APOE axis regulates functions of microglia and so plays a role in AD.

Interestingly, Gratuze et al. [ea23a], which investigated P301S models of mice expressing human APOE4 or no APOE at all in a background of normal and no TREM2, found that TREM2 ablation did not affect synaptic loss and tau accumulation if APOE4 was present. Homeostatic microglia were also found to be converted to their active states in conditions expressing human APOE4, regardless of TREM2 presence, and expression of interferon-responsive genes, which cause inflammation, was elevated. TREM2 independent microgliosis specifically was found to be increased in conditions of TREM2 KO alongside elevated APOE4 levels. The study thus suggests that in the ablation or knockout of TREM2 during tau pathology, APOE4 makes up for the loss of TREM2. This should be further studied so that the extent to which the APOE-TREM2 axis, and not each factor alone, affects the tau stages of AD can be understood.

7 Conclusion:

Substantial evidence thus indicates that APOE and TREM2 play a significant role in the tau stages of AD.

Literature supports that the effect of TREM2 on tau is influenced by the presence of A, with TREM2 facilitating microglial activation and phagocytosis of A plaques to prevent seeding and spreading. On the other hand, in the absence of A pathology, TREM2-induced activation of microglia leads to the phagocytosis of synapses and neurons. Experiments investigating the ablation of TREM2 along with the effects of LOF TREM2 variants highlight the potential of TREM2 as a therapy target. However, the complexity of its context dependent mechanism reduces this potential. This context dependence must be thus further understood for effective TREM2 therapies to be developed.

APOE, on the other hand, does not act on tau pathology in a A-dependent manner. Rather, studies show that throughout all stages of AD the APOE4

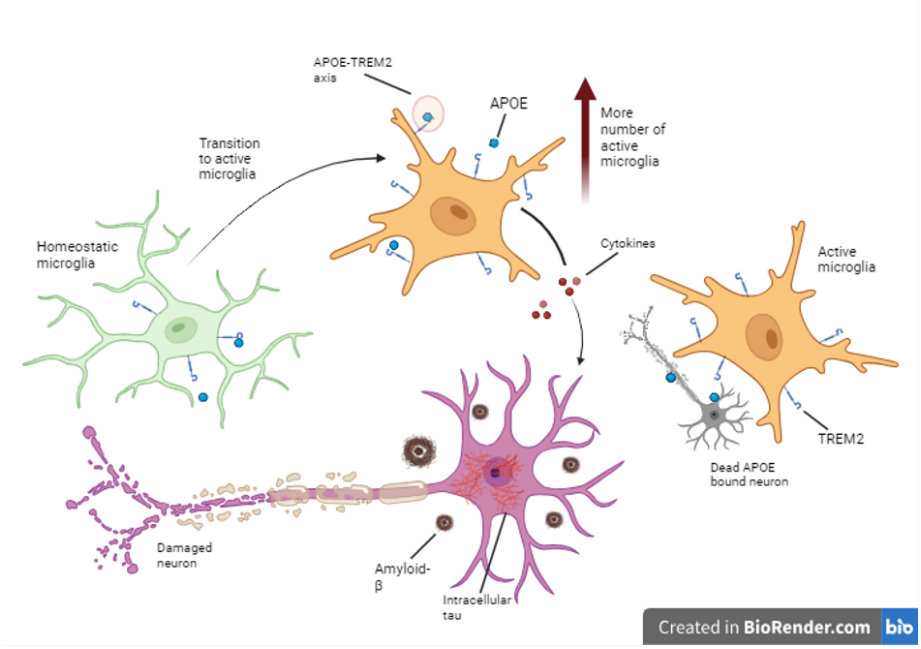

Figure 1: The APOE-TREM2 axis triggers the transition of homoestatic microglia to active microglia. Active microglia also phagocytose APOE-bound neurons, increasing brain loss

isoform is associated with the highest risk of AD, followed by APOE3 and then APOE2, which actually reduces AD risk. This effect of APOE4 is explained by its effect on tau structure and phosphorylation as well as its mediating effect on microglial activation, as explored by the TREM2-APOE axis. This highlights the potential of APOE4 as a therapy target, seen in papers such as the one by Gratuze et al. [ea22d], which uses a therapy to target APOE4 causing reduced A plaques and A driven tau pathology, and the one by Koutsodendris et al.1 [ea23e], which showcased how the selective genetic removal of APOE4 from neurons leads to a significant reduction in tau pathology, gliosis, neurodegeneration and myelin deficits. Thus, the targeting of APOE4 can mitigate the effects of tau pathology and is a promising future treatment.

The APOE-TREM2 axis was also explored in this review and showcases how both factors play a powerful role in regulating microglial signatures and microglial functions and so may affect AD tau pathology by driving phagocytosis, inflammation and other glial-related function (Fig. 1). The extent of its effect, however, must fully be understood, but targeting this axis or blocking this pathway is another promising future therapy.

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