Genetics

Review

The role of immunity and neuroinflammation in genetic predisposition and pathogenesis of Alzheimer's disease

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Abstract: Alzheimer's disease is an important public concern with rising prevalence across the globe. While many therapeutic approaches for Alzheimer's disease have been developed, there are currently no validated disease-modifying treatments. Thus, in order to develop novel treatment strategies, there is a significant need to progress our understanding of the pathogenesis of Alzheimer's disease. Several large genome-wide association studies and whole genome and exome sequencing studies have identified novel genes associated with late-onset Alzheimer's disease. Interestingly, many of the genes are associated with inflammation and the immune system, including complement receptor 1, clusterin, CD33, EPH receptor A1, membrane-spanning 4-domains subfamily A, ATP-binding cassette sub-family A member 7, major histocompatibility complex class II, inositol polyphosphate-5-phosphatase, myocyte enhancer factor 2C, and triggering receptor expressed on myeloid cells 2. The pathogenetic contributions of immune reaction and neuroinflammation in Alzheimer's disease have been regarded largely as part of amyloid cascade hypothesis. The neurotoxic amyloid-β (Aβ) induces activation of immune cells, such as microglia, astrocytes, perivascular macrophages and lymphocytes and decreased capability of clearing Aβ by immune system and chronic inflammation caused by activated immune cells aggravate neuronal damage and eventually Alzheimer's disease. But the precise mechanism and hereditary impact on such process is largely unknown. The current findings in genetic studies suggest that the immunological mechanisms of Alzheimer's disease may extend beyond passive reaction of Aβ, including the development of Alzheimer's disease such as time of onset and rate of progression. In this article, we aimed to review the mechanisms of immune reaction and neuroinflammation in Alzheimer's disease, with an emphasis on the function of genes known to be associated with a risk of Alzheimer's disease in terms of neuroinflammation and immune function.

Keywords: Alzheimer's disease; neuroinflammation; immune; microglia; genetics

1. Introduction

Alzheimer's disease is the major form of dementia, and is considered as important global concern. Alzheimer's disease together with other forms of dementia is the 4th leading cause of years lost due to disability (YLD) in high income countries at 3.7 million YLD, representing 5.4% of the total YLD, which is higher than that of chronic obstructive pulmonary disease, diabetes mellitus, and cancer [1]. As both life expectancy and the size of the elderly population continue to increase, the prevalence of dementia is also increasing. In 2001, 24 million people were estimated to have dementia worldwide, and the prevalence of dementia is expected to double every 20 years to 42 million by 2020 and 81 million by 2040 [2]. There were 35.6 million people with dementia as of 2010 [3], approximately 43% of which require a high level of care such as nursing home care [4]. Alzheimer's disease has a significant effect on society as well as patients and their caregivers; however, there is currently no validated disease modifying pharmacotherapy. Thus, there continues to be significant investigations aimed at understanding the pathogenesis of Alzheimer's disease and treating the condition.

The exact pathogenesis of Alzheimer's disease is largely obscure. The most representative pathological hallmarks of Alzheimer's disease are senile plaques and neurofibrillary tangles. Other pathological findings such as granulovacuolar degeneration and amyloid vascular degeneration are also present. Senile plaques, which are also known as amyloid plaques, consist of extracellular deposition of amyloid-β (Aβ) peptides, Aβ40, and Aβ42. Likewise, neurofibrillary tangles are intracellular lesions consisting of structures of paired helical filaments composed of deposits of hyperphosphorylated tau protein. The amyloid cascade hypothesis suggests that Aβ protein is neurotoxic and causes neurofibrillary tangles, cell loss, vascular damage and dementia directly [5]. Indeed, the amyloid cascade hypothesis has been considered the leading pathogenesis of Alzheimer's dementia for decades. The amyloid cascade hypothesis also suggests that, in addition to direct neurotoxic effects of Aβ on synapses and neurites, microglial and astrocytic activation induced by senile plaques and neurofibrillary tangles causes neuroinflammation, altered neuronal ionic homeostasis, and oxidative injury, eventually followed by aggravation of neuronal dysfunction and cell death. Decreased effective clearance of Aβ fibrils by microglia is also a key factor in pathogenesis of Alzheimer's disease, as well as increased generation of Aβ fibrils [6]. Thus, immunological mechanism and neuroinflammation play an important role in the progression of Alzheimer's disease. However, the process of immune reaction and neuroinflammation in Alzheimer's disease and its molecular participants remain largely unknown.

Epidemiological studies have revealed risk factors of Alzheimer's disease, largely distinguished into vascular (such as, smoking, obesity, dyslipidemia, diabetes, hypertension and asymptomatic cerebral infarction), psychosocial (such as, lower education, poor social engagement and physical activities) and genetic factors [7]. Genetic factors play important role in Alzheimer's disease and positive family history is a strong risk factor. Indeed, the Multi-Institutional Research in Alzheimer Genetic Epidemiology (MIRAGE) project reported that the lifetime risk of Alzheimer's disease in first degree relatives is more than doubled compared to the general population [8]. Likewise, several large studies on genetically identical twins have confirmed the high heritability for Alzheimer's disease, ranging from 58% to 80% [9,10]. Alzheimer's disease can be divided into two groups based on genetic aspects, namely, "early-

onset familial Alzheimer's disease" (familial EOAD) and "late-onset Alzheimer's disease" (LOAD). Familial EOAD seems to be inherited following Mendelian genetic distribution in an autosomal dominant pattern, with an average age of onset mostly before 60. The loci of causative genetic mutations are the amyloid precursor protein gene (*APP* gene in chromosome 21) and two presenilin genes (*PSEN1* and *PSEN2* located on chromosomes 14 and 1, respectively), all of which have complete penetrance. Conversely, LOAD accounts for more than 90% of Alzheimer's disease patients, and is considered sporadic without a well-defined mode of transmission. On the other hand, LOAD is associated with substantial genetic effects, having heredity as the important causal factor [11,12]. In this way, LOAD appears to be inherited following "complex" or "non-Mendelian" genetic distribution, rather than sporadic. The *APOE* (apolipoprotein E) gene, which encoding ApoE has been confirmed as a risk factor for LOAD [13,14]. Specifically, ApoE plays a role in mediating cell signaling, synaptic plasticity, and neuroinflammation as well as in cholesterol and lipid transport [15]. *APOE*-ε4 alleles significantly increase the risk of Alzheimer's disease in a dose dependent manner in both Caucasians and Asians. Furthermore, individuals with two *APOE*-ε4 alleles have from 15 to 17 times higher risk of Alzheimer's disease than those without the *APOE*-ε4 allele [16,17]. However, the *APOE* genotype explains only about 20% of Alzheimer's disease risk, and thus does not sufficiently explain the genetic variance of LOAD [18]. In addition to *APOE*, more than 500 candidate genes were suggested to be involved in LOAD between 1996 and 2005. Subsequently, the Alz Gene database (www.alzgene.org) was created in 2007 and includes updated information on 1395 studies, 320 meta-analyses, 695 genes, with 2973 polymorphisms [19]. Among the genes in the Alz Gene database, sortilin-related receptor 1 (*SORL1*) is associated with Alzheimer's disease according to multiple meta-analysis [20,21].

Genome-wide association studies (GWAS) performed since 2007 have identified many genes not previously suspected of being associated with complex diseases such as Alzheimer's disease [22]. Indeed, more than 20 genes associated with LOAD have been successfully identified with relative risks ranging from 1.1–1.3. Specifically, bridging integrator 1 (*BIN1*), complement receptor 1 (*CR1*), clusterin (*CLU*), phosphatidylinositol-binding clathrin assembly protein (*PICALM*), *CD33*, EPH receptor A1 (*EPHA1*), membrane-spanning 4-domains subfamily A (*MS4A/MS4A6*), ATP-binding cassette, sub-family A member 7 (*ABCA7*), CD2-associated protein (*CD2AP*), *SORL1*, major histocompatibility complex, class II (*HLA-DRB5/DRB1*), protein tyrosine kinase 2 beta (*PTK2B*), solute carrier family 24 member 4 (*SLC24A4-RIN3*), inositol polyphosphate-5-phosphatase (*INPP5D*), myocyte enhancer factor 2C (*MEF2C*), NME/NM23 family member 8 (*NME8*), zinc finger, CW type with PWWP domain 1 (*ZCWPW1*), CUGBP Elav-like family member 1 (*CELF1*), fermitin family member 2 (*FERMT2*), Cas scaffolding protein family member 4 (*CASS4*), and thyroid hormone receptor interactor 4 (*TRIP4*) have been identified as near-novel AD genes [23].

Whole genome sequencing methods have also been employed to identify new rare genetic variants. One such study showed that the triggering receptor expressed on myeloid cells 2 (*TREM2*) gene has an effect on the risk of developing LOAD [24]. Likewise, another whole exome sequencing (WES) study identified a rare variant of phospholipase D3 (PLD3) that doubles the risk of Alzheimer's disease [25]. The underlying biological mechanisms of these newly identified genes in the development of Alzheimer's disease are not yet fully understood, although a thorough understanding of the functional implications of these genes may suggest significant and novel pathogeneses of Alzheimer's disease, providing new biomarkers and treatment strategies. Among possible underlying causes of Alzheimer's disease, neuroinflammation remains a promising pathogenic mechanism. In this article, we aimed to review the role of neuroinflammation in

development of Alzheimer's disease and study the influence of known and novel genes.

2.   Neuroinflammation and immune reaction in Alzheimer's disease

2.1. Innate immunity

2.1.1.   Microglia

Microglia are resident macrophages in the central nervous system (CNS) and serve as key cellular elements of the innate immune system of the CNS. Microglia survey their local microenvironment and defend against invading pathogens via phagocytosis, production of cytokines, oxyradicals, and activation of the complement cascade [26]. Microglia also play roles in neurodevelopment such as synaptic remodeling and inducing apoptosis in supernumerary Purkinje cells, and may also produce neural growth factor and influence vascular development. In this way, microglia help to maintaining homeostasis of other cells such as astrocytes and neurons in the CNS [27,28].

Clustered microglia and fibrous astrocytes around extracellular deposits of Aβ have been observed in multiple publications [28,29]. Increased Aβ proteins, especially aggregation-prone Aβ42 species, lead to formation of amyloid fibrils and Aβ oligomers in Alzheimer's disease. These misfolded Aβ peptides produce chemokines and also act directly as microglial attractants. Chemokine receptors such as CCR2, CCR3, CCR5, and CX3CR1 are present both in the microglia and brain of Alzheimer's disease patients, and CXCR2 and CXCR33 are expressed in the vicinity of neuritic plaques [30]. Microglia, which are attracted to amyloid plaques, recognize and bind to Aβ oligomers and Aβ fibrils via cell surface receptors, such as CD36 [31,32], integrin-associated protein/CD47, α6β1 integrin [32], scavenger receptor A-1 (SCARA1) [33], Toll-like receptors (TLRs) [34-36], and associated receptors (e.g., CD14) [37]. After receptor ligation with Aβ fibrils, microglia engulf Aβ fibrils by phagocytosis in vitro and degrade soluble Aβ species via extracellular proteases, neprilysin, and insulin-degrading enzyme (IDE) [38,39]. In turn, ligation of CD36 and TLRs 4 and 6 with Aβ activate microglia to stimulate production of proinflammatory cytokines and chemokines [40].

Microglia activation is classified into several phenotypes, namely, the proinflammatory classical (M1) phenotype, non-inflammatory alternative (M2) phenotype, and acquired deactivation [41,42]. M1 is induced by T-helper-type 1 (Th1) cell-derived cytokines and causes a release of high levels of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), STAT3, interleukin-1β (IL-1β), IL-6, IL-12, IL-18 IL-23, reactive oxygen, and nitrogen species and corresponding toxic intermediates followed by impaired phagocytic capacity [43]. Inflammatory cytokines, reactive oxygen and nitrogen species can cause cell damage. M1 can also cause deterioration of the extracellular matrix by releasing proteolytic enzymes and degrade cellular integrity that lead to cellular destruction [42]. M2 is induced by Th2 cell-derived cytokines and causes increased release of anti-inflammatory cytokines such as IL-4, IL-10, IL-13 and transforming growth factor-β (TGF-β). This activation is also characterized by increased phagocytic capacity without toxic nitric oxide (NO) production. M2 macrophages also play a role in immunomodulation, homeostasis, neurogenesis, repair, and tissue remodeling as well as inflammation resolution [42,44]. The heterogeneity of such microglial activation states likely impacts development and progression of neurodegenerative diseases. Indeed, the characteristic switch from M2 to M1 seems to align with disease progression and age [45,46]. However, recent articles suggest that simple categorization according to the M1/M2 paradigm is insufficient, and that mononuclear phagocytes interact with various

environmental challenges, are more complex, and exhibit mixed phenotypes [47].

Activation of microglia both beneficially and adversely affects the pathogenesis of Alzheimer's disease. As the primary role of microglia is to defend against recognized pathologic changes while minimizing tissue damage, microglia have a central role in clearing toxic senile plaques via phagocytosis [48]. Microglia also degrade soluble Aβ through internalization with a fluid phase macropinocytic mechanism and subsequent proteolytic degradation in endolysosomal compartments [49]. Sphingolipid-modulated exosomes seem to promote microglia-mediated clearance of Aβ [50]. The complement system, a major constituent of the innate immune system, includes anaphylatoxins and opsonins and is associated with Aβ clearance and genetic ablation of complement factor C3 in mice, resulting in augmentation of Aβ deposition and neurodegeneration [51]. Likewise, microglia and astrocytes are responsible for production of proteins of the complement system [52]. Decreased clearance of Aβ by microglia is considered to contribute to the pathogenesis of Alzheimer's disease, and senescence of microglia and failure to appropriately convert the microglial phenotype may influence the effectiveness of microglial amyloid clearance [45,53].

Chronic microglial activation is problematic in the pathogenesis of Alzheimer's disease. Specifically, a positive feedback loop between inflammation and APP processing results in chronic, non-resolving inflammation [54-59]. As M2 promotes cessation of inflammation, conversion of microglia is important. Such conversion is mediated by modification of proinflammatory signaling pathways (e.g. NLPR3 inflammasome) and heterodimeric type II nuclear receptors (e.g., PPARγ/RXR, PPARδ/RXR and LXR/RXR) [60]. Chronic inflammation causes enormous and sustained exposure to proinflammatory cytokines, chemokines, and other inflammatory mediators resulting in neuronal damage. Studies on the progression of mild cognitive impairment to Alzheimer's dementia have shown that increased levels of proinflammatory cytokines such as TNF-α and decreased levels of the anti-inflammatory cytokine TGF-β are associated with the conversion to dementia [61]. Microglial activation and inflammatory responses promote activation of mitogen-activated protein kinase (MAPK), resulting in exacerbated hyperphosphorylation of tau protein in mouse models [57,62,63]. Specifically, cytokine-directed influences on tau pathologies interfere with neuronal transport by damaging neurons and aggravating the detrimental effects of tau on intraneuronal transport [64]. Inducible nitric oxide synthase (iNOS) in microglia and astroglia is stimulated in M1 and releases high concentrations of NO and other reactive oxygen species (ROS). ROS and reactive nitrogen species (RNS) are highly reactive and are capable of destabilizing proteins, lipids, carbohydrates, DNA, and RNA, leading to various types of damage including cell death [65]. Furthermore, oxidative stress may aggravate the Aβ aggregation process [66]. Lastly, iNOS is expressed in the brain of patients with Alzheimer's disease, and genetic ablation of iNOS in mice has a protective effect on the development of Alzheimer's disease [67].

The paradoxical actions of inflammation and microglial activation on progression of Alzheimer's disease, having both detrimental and beneficial effects, make the development of anti-inflammatory therapies difficult [68]. In PS1-APP mice study, an animal model of Alzheimer's disease, as mice getting older they exhibited decreased expression of some Aβ receptors such as SCARA, CD36, receptor for advanced-glycosylation end-products (RAGE) and Aβ degrading enzymes such as insulysin, neprilysin, and matrix metalloproteinase 9 (MMP9), whereas increased expression of the proinflammatory cytokines IL-1β and TNF-α. Further, TNF-α downregulates SCARA and CD36 expression. This findings suggest that early microglial activation is neuroprotective due to its Aβ clearance function, but as the disease progresses, proinflammatory cytokines downregulate genes involved in Aβ clearance, promoting Aβ accumulation [69].

2.1.2. Peripheral macrophages

Macrophages distinct from microglia may take part in the pathogenesis of Alzheimer's disease. Specifically, infiltrated blood macrophages from periphery and perivascular macrophages continue to be of interest in Alzheimer's disease [70]. Stimulation of perivascular macrophages reduces cerebral amyloid angiopathy and deposition of Aβ in cerebral blood vessels and leptomeninges in mouse model of Alzheimer's disease [71]. In addition, CCR2 is needed for Aβ clearance by perivascular macrophages [72]. Several mouse studies have reported higher infiltration rates of peripheral macrophages in the CNS of Alzheimer's disease model mice compared to control mice [73,74]. The function of infiltrating macrophages in the clearance of Aβ is somewhat inconclusive, appearing transient with non-phagocytic activity [73], but exhibiting co-localization with lysosomes indicative of phagocytosis [75]. The possibility of access and activity in physiological milieu of macrophage in CNS is also questionable due to the use of radiation in previous studies, which may affect the integrity of the blood brain barrier and inflammatory milieu [76,77].

2.2. Adaptive immunity

The main kind of neuroinflammation evoked in pathogenesis of traditional neuroinflammatory diseases, such as multiple sclerosis and encephalitis, and that of neurodegenerative diseases, such as Alzheimer's disease, have been known to be different. The former is driven primarily by adaptive immune system (blood derived T and B lymphocytes) but the latter by CNS-resident immune cells (microglia, perivascular macrophages and astrocytes) [78]. On the other hand, multiple studies suggested Alzheimer's disease is related with increased CNS T lymphocyte infiltration [79,80]. The exact mechanism of entering T lymphocytes into brain through blood-brain barrier and their roles in pathogenesis of Alzheimer's disease is largely unknown. But several studies provide possible explanations [81]. Aβ fibrils may served as modified self-antigen and induce Aβ specific T lymphocytes. Blood vessels adjacent to Aβ fibrils express increased level of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and lead extravasation of activated Aβ specific T lymphocytes [82]. In Alzheimer's disease model mice, infiltration of IL-17 producing Th17 cells into brain parenchyma was found, which promotes microglial activation, releases proinflammatory cytokines and increases neuroinflammation [83-85]. Some other studies reported beneficial effects of T lymphocytes in Alzheimer's disease. Injection of Aβ specific Th1 in mouse model of Alzheimer's disease showed increased Aβ uptake and facilitated neurogenesis [86].

Change in lymphocyte population was also detected in peripheral immune system. Decreased peripheral T and B lymphocyte numbers were observed in Alzheimer's disease patients [87]. And especially in severe stage, decrement in naïve CD4+ T lymphocyte subpopulation levels, CD19+ B lymphocytes, naïve B cells (IgD+CD27−) with increased double negative (IgD−CD27−) memory B cells were detected [88,89]. Decrease in naïve lymphocytes may be result of exhaustion due to chronic inflammation in Alzheimer's disease. The expression of chemokine receptors in lymphocytes also altered in Alzheimer's disease. Expression of pro-inflammatory chemokine receptors, CCR6 and CCR7, on B lymphocytes was increased, leading to chronic inflammatory condition [89]. Number of CD8+CD28− suppressor T lymphocytes and IL-10 production were decreased in Alzheimer's disease, suggesting diminished immunosuppressive capabilities [90].

2.3. Other cells (astrocytes)

Astrocytes are the most abundant glial cells in the CNS where they play important roles such as maintenance of brain homeostasis, neurotransmitter regulation, signal transmission, and blood flow regulation. There is evidence that impaired and modified astroglial function contributes to the pathogenesis of Alzheimer's disease [91-94]. Astrocytes may also contribute to progression of Alzheimer's disease by directly and indirectly contributing to neuroinflammation. Activated microglia produce cytokines, chemokines, ROS and recruit astrocytes around plaques promoting their proliferation [95]. In addition, Aβ itself could directly activate astrocytes [96]. Activated astrocytes take part in Aβ clearance via internalization and degradation of Aβ, and ApoE seems to facilitate this function [97,98]. Activated astrocytes release proinflammatory cytokines, chemokines and NO and ROS, and thus may participate in chronic inflammation resulting in neuronal damage. The overexpression of β-site amyloid precursor protein cleaving enzyme (BACE) and α1-antichymotrypsin in activated astrocytes may also induce further amyloidogenesis and tau hyperphosphorylation resulting in inhibition of Aβ breakdown, respectively [99].

3. Genes related to neuroinflammation and immune reaction in Alzheimer's disease

3.1. Formation of Aβ (APP, PSEN1&2 in EOAD, newly suggested genes in LOAD)

The amyloid cascade hypothesis is regarded as a major pathogenetic hypothesis of Alzheimer's disease. As mentioned above, amyloid fibrils and Aβ oligomers both play a major role in neuroinflammation by activating microglia and astrocytes, which in turn release proinflammatory cytokines, proteolytic enzymes, and NO, and subsequently activate the complement system and induce oxidative stress. Processing of APP can be divided into amyloidogenic and non-amyloidogenic pathways. In the non-amyloidogenic pathway, APP is cleaved by α-secretase, which is followed by further cleavage by γ-secretase. Non-toxic P3 peptide, α-APP, and the intracellular domain of APP (AICD) are secreted by this pathway. Conversely, β-secretase and γ-secretase producing β-APP, AICD, and Aβ peptides, which are the major pathological findings in Alzheimer's disease, are secreted via the amyloidogenic pathway. The exact mechanism is largely unknown, but mutations in the *APP* genes (Chromosome 21) and genetic mutation of presenilin, the catalytic core of the γ-secretase (*PSEN1* and *PSEN2* on Chromosomes 14 and 1, respectively) results in relatively increased generation of aggregation prone Aβ42 species, which are largely responsible for familial EOAD [100].

Recent studies have suggested that some novel susceptibility genes may also influence Aβ production in LOAD. Specifically, the A disintegrin and metalloproteinase domain (ADAM) protease family is the executer of APP cleavage by α-secretase, and two rare mutations in *ADAM10* have been suggested to predispose individuals to LOAD [101]. β-secretase cleavage requires recycling of APP from the cell surface, which in turn requires clathrin-mediated endocytosis of APP and its targeting to specific subcellular compartments. PICALM and SORL1 are involved in this process, and variants of these genes are associated with LOAD [102-105]. Formation of oligomers seems to exert increased Aβ neurotoxicity. In addition, clusterin influences aggregation and disaggregation of Aβ, and variants of *CLU* are associated with LOAD [106,107].

3.2. ApoE modulates neuroinflammation in Alzheimer's disease

ApoE is a high density lipoprotein that plays important roles in the CNS, including regulation of phospholipid and cholesterol distribution, synaptogenesis, and synaptoplasticity. There are three common alleles of *APOE* (ε2, ε3, and ε4), among which *APOE*-ε4 significantly increases the risk of Alzheimer's disease in a dose dependent manner while *APOE*-ε2 has a protective effect [16,17]. ApoE also seems to regulate innate immunity in the brain, influencing neuroinflammation and pathogenesis of Alzheimer's disease. In addition, ApoE promotes co-localization, degradation and clearance of Aβ by astrocytes, and astrocyte-dependent lipidation of ApoE facilitates Aβ clearance by microglia [98,108]. ApoE also enhances endolytic degradation of Aβ by microglial neprilysin and related enzymes [109]. Interestingly, different isoforms of ApoE seem to influence inflammatory receptor signaling differently. In animal studies, binding of lipopolysaccharide (LPS) to CD14/TLR4 co-receptor (which bind to Aβ in Alzheimer's disease) leads to dual pathway activation, namely, NF-κB mediating signaling, which is associated with immune modulation, and p38/MAPK-dependant signaling, which is related to increased paracrine damage by neurons. Targeted replacement (TR) of ε4 in mice is also associated with microglial p38/MAPK-dependent cytokine secretion and neuronal damage. Indeed, TR of ε2 revealed an association with the NF-κB mediated pathway [110,111]. Macrophages expressing *APO*-ε2 also exhibit greater Aβ degradation via MMP9 compared with *APO*-ε3 or ε4 [112].

3.3. Novel risk genes for Alzheimer's disease associated with inflammation and immune reaction (CD33, TREM2, CR1, CLU, ABCA7, MS4A, EPHA, INPP5D, MEF2C and HLA- DRB5/DRB1)

GWAS and meta-analysis of LOAD consortium data have suggested several novel loci associated with LOAD risk: *BIN1, CR1, CLU, PICALM, CD33, EPHA1, MS4A/MS4A6, ABCA7, CD2AP, SORL1, HLA-DRB5/DRb1, PTK2B, SLC24A4-RIN3, INPP5D, MEF2C, NME8, ZCWPW1, CELF1, FERMT2, CASS4,* and *TRIP4* [22,113-115]. In addition, whole genome sequencing methods such as WES identified rare genetic variants of *TREM2* and *PLD3* that are associated with increased risk of LOAD [24,25]. These loci may help to identify novel mechanisms of the pathogenesis in Alzheimer's disease, deepening our understanding of the disease, and raising the possibility of finding new therapeutic options based on its pathogenetic mechanisms. Importantly, these novel genes seem to be associated with multiple mechanisms such as cell migration, lipid transport and endocytosis, amyloidogenesis, tauopathy, synaptic, and cytoskeletal function, and may have a role in immune processes and neuroinflammation [116].

CD33, TREM2, and *CR1* are expressed on and modulate microglia, which play a major role in Aβ clearance and neuroinflammation as described above. Genetic variations of *CD33* are associated with Alzheimer's disease [114,115,117,118]. *CD33* is located on 19q13.3 and encodes a member of the sialic acid-binding Ig-like lectin family, which is expressed on myeloid/microglial cells. CD33 is an inhibitory lectin, and mediates cell-cell interactions and inhibit immune cell function. In the CNS, CD33 impairs microglia-mediated Aβ clearance, resulting in facilitation of Aβ pathology. The C allele of rs3865444 is associated with greater cell surface expression of *CD33* and increased Alzheimer's disease risk [119,120]. Consistently, microglia with higher expression of *CD33* exhibit diminished Aβ internalization resulting in accumulation of Aβ pathology on brain imaging with increased numbers of microglial activation [120]. CD33 may also play a role in other neuroinflammatory pathways mediated by microglia and should be studied further [119].

TREM2 is located on chromosome 6q21.1, and its expression on microglia facilitates phagocytosis and down-regulation of cytokine production and inflammation [121]. Mutation of *TREM2* is associated with some forms of dementia such as Nasu-Hakola disease, a rare autosomal recessive form of dementia that presents with bone cysts, and familial frontotemporal dementia-like syndrome [122,123]. Rare missense mutations and rare multiple coding variants in *TREM2* increase the risk of LOAD. According to recent meta-analyses, the most common variant of *TREM2*, R47H (rs75932628), is associated with from 2.70 to 4.11 increased LOAD risk relative to non-carriers [124,125]. Other *TREM2* variants rs104894002 and rs143332484 are also associated with higher risk of Alzheimer's disease with ORs of 7.21 and 1.65 respectively, although these risks were established with a small sample size and limited ethnicity data [124]. Consistently, Alzheimer's disease patients that carry a *TREM2* mutation have more extensive brain atrophy than non-carriers [126].

CR1 is located on chromosome 1q32 and encodes CR1 protein, which functions in the complement response as a receptor for C3b and C4b. Activation of complement cascade leads to opsonization and lysis of pathogens. Aβ can activate the complement system in vitro [127]. Single nucleotide polymorphisms (SNPs) rs6656401 and rs3818361 of *CR1* are associated with increased LOAD risk, especially the latter in the presence of the *APOE*-ε4 allele [107]. Measurement of gene expression level on brain tissue revealed that higher *CR1* expression is associated with increased cognitive decline and Alzheimer's disease [128]. Among the isoforms of CR1, CR1-B/S increases complement activity via extra binding site for C3b/C4b, which is associated with increased Alzheimer's disease risk [129]. CR1 and C3b moderate the complement activity, and presence of CR1 on phagocytic cells make them ingest particles that activate the complement response. A previous study demonstrated that clearance of Aβ in the circulation is dependent upon binding of C3b to erythrocyte–associated CR1, and also suggested that the purpose of CR1 expression in the brain is to act as a receptor for Aβ [130]. Together, these findings suggest that genetic variance in *CR1* can alter the rate of Aβ clearance by the immune response. The complement system may also exert detrimental inflammation. Presence of complement system element and membrane attack complex C5b-9 is correlated with a high level of synaptic loss and Alzheimer's disease [131]. Insufficient clearance of Aβ and increased chronic inflammation by *CR1* genetic variation seems to affect pathogenesis of Alzheimer's disease, with a recent study suggesting that forms of CR1 with impaired function are associated with increased risk of Alzheimer's disease through decreased clearance of Aβ [132].

Other LOAD related genes such as *CLU* and *ABCA7* are also associated with the complement system. *CLU* is located on chromosome 8p21.1 and encodes the stress activated chaperone protein clusterin. SNPs including rs11136000, rs9331888, rs2279590, rs7982, and rs7012010 have a protective effect on LOAD risk, while rs9331896 is associated with increased risk of LOAD [114,115,118,133]. Increased plasma concentrations of clusterin are associated with entorhinal cortex atrophy and increased Aβ burden in the medial temporal lobe [134]. Clusterin was also described above as being involved in Aβ aggregation, with functions in lipid transport, membrane protection, cell-cell interaction, apoptosis, and complement regulation. With respect to the complement system, clusterin modulates the membrane attack complex and inhibits complement activation associated inflammatory response [135]. Furthermore, a mouse study showed that binding of Aβ to clusterin increases the rate of Aβ clearance and efflux through the blood-brain barrier [136]. *ABCA7* is located on chromosome 19p13.3 and several SNPs, namely rs3764650, rs4147929, are associated with LOAD [115,118,133]. ABCA7, a member of the ABC transporter superfamily, transports substrates across cell membranes. ABCA7 modulates the C1q complement pathway and affects microglial phagocytosis of apoptotic cells and Aβ [137-139]. ABCA7 may also affect

cholesterol transfer to ApoE and clearing Aβ aggregates [140,141]. Despite these findings and ongoing research, the precise mechanism of how the complement system influences the pathogenesis of Alzheimer's disease remains largely unknown.

Several other LOAD associated genes such as *MS4A, EPHA, INPP5D, MEF2C,* and *HLA- DRB5-DRB1* are related with immunity and inflammation, although the functional implication of these genes in the pathogenesis of Alzheimer's disease is largely unknown. The *MS4A* gene cluster, composed of 12 related genes, is located on chromosome 11q12 [142], of which the SNPs rs983392 and rs670139 are associated with LOAD [114,115,118]. Overall the *MS4A* gene cluster is poorly characterized; however, *MS4A1* (CD20), *MS4A2* (FcεRIβ), and *MS4A3* (HTm4) are best characterized of the cluster. It is known that the MS4A family functions to regulate calcium homeostasis, and that increased intracellular calcium may be associated with Aβ accumulation, tau hyperphosphorylation, and neuronal death. The MS4A family also affects progression of Alzheimer's disease by modulating neuroinflammation. Specifically, *MS4A1* (CD20) is expressed mainly by B lymphocytes, regulating calcium influx after activating B lymphocyte antigen receptor and related with immunological diseases [143,144]. In mouse study, neuroinflammation with B lymphocytes infiltration after stroke was related with development of dementia and anti-CD20 antibody prevented cognitive deficits [145]. Likewise, overexpression of *MS4A* genes increases T lymphocyte activation, facilitating the trafficking of T lymphocyte through the blood-brain barrier and decreasing T lymphocyte apoptosis. Activated T lymphocytes may also interact with microglia to promote the release of proinflammatory cytokines leading to immune system dysfunction and ensuing neuronal damage [142,146].

EPHA1 is located on chromosome 7q34 and encodes the ephrin type-A receptor, a member of the ephrin receptor subfamily of tyrosine kinase family. The SNPs rs11767557 and rs11771145 are associated with decreased LOAD risk [114,115,118]. In addition, a recent study found that EPHA1 (rs11771145) prevents hippocampal atrophy in mild cognitive impairment over a 2-year follow up period and is associated with decreased atrophy and greater cerebral glucose metabolic rate in the lateral occipitotemporal and inferior temporal gyrus in Alzheimer's disease, without association with Aβ and tauopathy [147]. Thus, EPHA1 seems to contribute to axonal guidance and synaptic plasticity, modulation of the MAPK pathway, and response at glutamatergic synapses [148-150]. EPHA1 also seems to modulate immune function and chronic inflammation. The relationship between Eph/Ephrin signaling and inflammatory disease such as atherosclerosis, rheumatoid arthritis, and inflammatory bowel disease has also been reported [151,152]. Furthermore, Eph receptors reduce monocyte chemotaxis via expression in CD4-positive T lymphocytes and monocytes [153]. Ultimately, the pathogenetic contribution of Eph receptors to Alzheimer's disease remains unclear.

INPP5D, a member of the *INPP5* family, is located on chromosome 2q37.1 and encodes Src homology 2 domain-containing inositol-5-phosphatase (SHIP1), of which the SNP rs35349669 is associated with increased LOAD risk [115]. SHIP plays a central role in multiple signaling pathways, such as phosphatidylinositol 3-kinase pathway. SHIP1 attenuates immunoreceptor signaling and hematopoietic progenitor cell proliferation [154]. SHIP1 knockout mice also exhibit immunologic dysfunction with increased myeloid-derived suppressor cells and regulatory T cells as well as altered natural killer cell development [155]. *MEF2C* is located on 5q14.3, of which the SNP rs190982 is associated with increased LOAD risk [115]. MEF2C participates in the development and maintenance of multiple organ systems, and is also an effector of neurogenesis. Indeed, mutations and deletions in *MEF2C* are associated with mental retardation, seizure, cerebral malformation, stereotypic movements, and atony [156]. MEF2C also

appears to downregulate inflammation. Specifically, *MEF2C* knockout mice have increased expression of proinflammatory molecules and NF-κB, a proinflammatory transcription factor. Consistently, overexpression of *MEF2C* suppresses both TNF-α levels and induction of proinflammatory molecules such as those involved in leukocyte adhesion [157]. *HLA-DRB5/DRB1* is located on chromosome 6 and encodes HLA class II, DRβ5, and DRβ1, and is also associated with Alzheimer's disease [115]. These HLA class II beta chain paralogues participate in the immune system by presenting peptides derived from extracellular protein. HLA-DRs seem to affect glial activity differently according to subtype. One study reported decreased glial fibrillary acidic protein in the hippocampus of Alzheimer's disease patients who are *HLA-DR4* allele carriers compared with *HLA-DR4* negative subjects [158]. HLA-DR also influences T lymphocyte responses to Aβ differently according to subtype, and this difference in Aβ immunogenicity is considered critical for evaluating therapeutic and/or pathogenic potential of Aβ vaccines [159]. Currently, however, the autoimmune response in Alzheimer's disease and how HLA subtypes affect the pathogenesis of Alzheimer's disease is not well understood [160].

4. Conclusions

Neuroinflammation and immune reactions affect the pathogenesis of psychiatric disorders such as dementia, depression, and schizophrenia [161-163]. The pathogenesis of Alzheimer's disease is also affected by immunological mechanisms. Aβ induced activation of immune system, such as microglia, astrocytes, other macrophages and lymphocytes, causes chronic inflammation with increased levels of proinflammatory cytokines, ROS, and complement system giving detrimental effects on CNS. To date, the significance of immunological mechanisms has likely been underestimated as part of the amyloid cascade hypothesis and much of the hereditary aspects of Alzheimer's disease have pointed to APP processing and generation of Aβ. Conversely, GWAS studies and relatively new whole exome and genome sequencing techniques have revealed multiple novel genes associated with LOAD risk, many of which are related to the immune system. These findings suggest that immunological mechanisms are more than just a passive reaction to Aβ, and that alterations and the complex heterogeneity of the immune system in different individuals may modify development of Alzheimer's disease, including time of onset and progression.

Many studies have attempted to target immune reactions and neuroinflammation as therapeutic targets in Alzheimer's disease, but the results of these efforts have not been successful to date. Indeed, the heterogeneity of immunity in different individuals, different stage specific roles of genes, and different microglia phenotypes that may be modulated by multiple factors make such approaches difficult to pursue. Nevertheless, the detailed mechanisms of neuroinflammation and immune reactions associated with Alzheimer's disease, including the identity of involved molecules, should be investigated more deeply. Newly identified LOAD risk genes indicate the possibility of novel, more complicated, and multifaceted mechanisms of neuroinflammation. Thus, future studies should be performed to determine the exact and precise mechanisms of these novel genes on Alzheimer's disease, and integrate these findings together with those of previous studies. Together, such efforts will help to suggest new biomarkers beneficial for diagnosing Alzheimer's disease as well as developing novel, individualized treatment strategies with superior disease-modifying outcomes.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this paper.

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