[Frontiers in Bioscience, Landmark, 22, 180-192, January 1, 2017]

Molecular mechanisms of the genetic risk factors in pathogenesis of Alzheimer disease

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1. ABSTRACT

Alzheimer disease (AD) is a neurodegenerative disease characterized by the extensive deposition of senile plaques and neurofibrillary tangles. Until recently, only the *APOE* gene had been known as a genetic risk factor for late-onset AD (LOAD), which accounts for more than 95% of all AD cases. However, in addition to this well-established genetic risk factor, genome-wide association studies have identified several single nucleotide polymorphisms as genetic risk factors of LOAD, such as *PICALM* and *BIN1*. In addition, whole genome sequencing and exome sequencing have identified rare variants associated with LOAD, including *TREM2*. We review the recent findings related to the molecular mechanisms by which these genetic risk factors contribute to AD, and our perspectives regarding the etiology of AD for the development of therapeutic agents.

2. INTRODUCTION

Alzheimer disease (AD) is defined pathologically by extensive neuronal loss and the deposition of senile plaques and neurofibrillary tangles, which are composed mainly of amyloid-β peptide (Aβ) and tau, respectively. Genetic, biochemical, and cell biological studies suggest that Aβ plays a central role in initiating the pathogenic process and tau contributes to the clinical progression of AD, including neuronal cell death (1, 2). Aβ is produced by the sequential proteolytic cleavage of amyloid-β precursor protein (APP) by β-site APP-cleaving enzyme 1 (BACE1) and γ-secretase, and secreted into the brain parenchyma (3). γ-Secretase-mediated cleavage generates Aβ fragments with various the C-terminal lengths (4). Among the various Aβ species generated, Aβ40 is a major secreted peptide (80~90% of total Aβ) in the physiological condition. However, Aβ42, which consists with ~10% of total Aβ, is aggregation-prone and is the predominant species deposited in AD brains (5). Aβ is proteolytically degraded by several proteases including Neprilysin. Some of the Aβ is transported to blood through the blood-brain barrier and is degraded in peripheral organs. APP is also processed by a non-amyloidogenic pathway, in which ADAM10 and γ-secretase are involved. The rate of production and clearance of Aβ affect the brain's "Aβ economy" (2). An unbalanced Aβ economy leads to the aggregation and deposition amyloid, thereby causing AD.

3. GENETICS OF ALZHEIMER DISEASE

Mutations linked to early-onset familial AD (FAD) have been identified in genes encoding *APP*, presenilin (PS) 1 (*PSEN1*), and presenilin 2 (*PSEN2*) (6). *APP* mutations cause an increased production of or aggregation propensity of Aβ. In addition, *APP* duplications have been identified in autosomal dominant early-onset families. Furthermore, almost all FAD mutations in *PSEN1* and *PSEN2* affect γ-secretase-mediated cleavage to increase the production ratio of Aβ42 (4). Chemical biology approaches revealed that PS is a catalytic subunit of the γ-secretase complex, which is composed of four membrane proteins (7, 8). Recently, the cosegregation of rare coding variants in *ADAM10*, an APP-cleaving enzyme responsible for the non-amyloidogenic pathway, with LOAD have been reported (9). These variants cause a decrease in α-secretase activity, leading to an increase

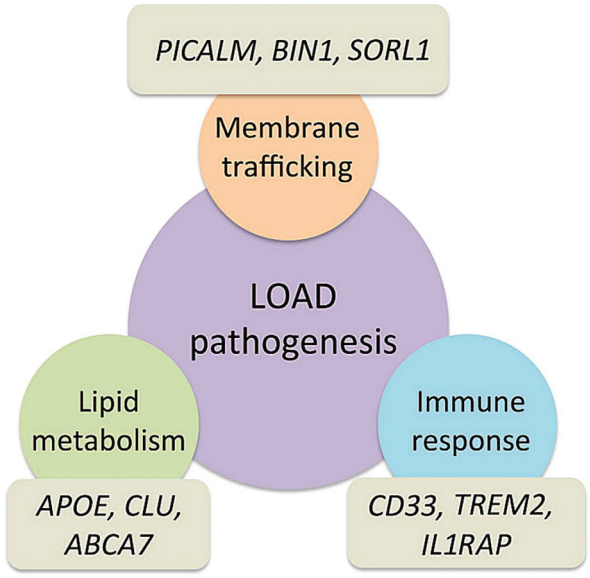

Figure 1. Classification of genetic risk factors for AD. Molecular functions of gene products encoded by the LOAD risk factors are classified into three main groups; membrane trafficking, lipid metabolism, and immune response. Genes described in this review are shown.

in amyloidogenic processing by BACE1. Moreover, a rare protective variant of *APP* (A673T) has been identified in the Icelandic population (10). This variant was located near the β-cleavage site to reduce Aβ secretion and AD risk. These genetic and biochemical analyses strongly support the notion that an impairment of brain Aβ economy affects the onset of AD.

On the other hand, the vast majority of AD cases are sporadic LOAD. LOAD has been considered as a multifactorial disease, and multiple genetic and environmental factors contribute to its pathogenesis. However, in 1993, association of the *Apolipoprotein E (APOE)* allele ε4 with both sporadic and familial LOAD was reported (11, 12). Moreover, the recent remarkable advancement in genome sequencing techniques has enabled genome-wide association studies (GWAS) to be performed for LOAD patients (6). These GWAS have identified several single nucleotide polymorphisms (SNPs) as genetic risk factors of LOAD, including *ABCA7*, *BIN1*, *CD33*, *CLU*, *CR1*, *CD2AP*, *EPHA1*, *MS4A6A/MS4A4E*, and *PICALM* (13-17). In addition, meta-analyses of the GWAS studies have identified new genetic risk factors, namely, *CASS4*, *CELF1*, *DSG2*, *FERMT2*, *HLA-DRB5-HLA-DRB1*, *INPP5D*, *MEF2C*, *NME8*, *PTK2B*, *SLC24A4-RIN3*, *SORL1*, and *ZCWPW1* (18). In parallel with GWAS, the search for rare variants that occur at very low incidence but have large effects on the etiology of AD has been performed by whole genome sequencing or exome sequencing, and coding variants in *TREM2*, *PLD3*, *UNC5C*, *and AKAP9* were identified (19-22). Interestingly, GWAS for cerebrospinal fluid (CSF) tau levels, which correlates with neurodegeneration, identified SNPs within *APOE*, between *GEMC1* and *OSTN*, within *GLIS3*, and within the *TREM* gene cluster (23). Differences in the identified SNPs among the studies are thought to reflect the pathomechanisms of the risk factors on the endophenotypes utilized in each GWAS (i.e. onset of LOAD vs CSF tau levels).

4. MOLECULAR MECHANISMS OF RISK FACTORS

The LOAD risk factors identified by these genetic studies are classified into three main groups; proteins involved in membrane trafficking, lipid metabolism, and immune responses (Figure 1) (6). We have summarized the molecular mechanisms by which these risk factors are involved in LOAD, in the following sections.

4.1. Membrane trafficking

Several lines of evidence suggest that membrane trafficking plays a crucial role in the amyloidogenic cleavage of APP. Importantly, the internalization of APP is required for Aβ generation, suggesting that an endocytic mechanism is critical for BACE1- and γ-secretase-mediated cleavage (3, 24). In fact, the optimal pH for BACE1 is acidic, corresponding to that of early endosomes. In contrast, the association between the subcellular localization and proteolytic activity of γ-secretase has been enigmatic. Several genes involved in membrane trafficking were identified from GWAS, and molecular functions of these gene products were investigated; *PICALM*, *BIN1* and *SORL1* (Figure 2).

The *PICALM* gene encodes a protein called clathrin assembly lymphoid myeloid leukemia (CALM), which functions in the initial step of clathrin-mediated endocytosis by facilitating the proper formation of clathrin-coated pits (25, 26). CALM also recognizes endocytic cargo proteins, such as VAMP8 (27). Recently, it was suggested that CALM affects the endocytosis of APP/secreted Aβ, as well as clearance of the C-terminal fragment of APP by autophagy (28, 29). However, its mechanistic details and the effects of the SNPs reported by GWAS were largely unknown. We found that CALM regulates the endocytosis and subcellular localization of γ-secretase and affects the production ratio of Aβ42 (30). Our results raise the possibility that CALM affects the onset of AD by altering the Aβ42 production ratio in an opposite manner to that by FAD-linked mutations in *APP* and *PSEN* genes.

Bridging integrator 1 (BIN1) encoded by *BIN1* was initially identified as a tumor suppressor (31). BIN1 is a cytosolic adaptor protein with an N-terminal BIN1-Amphiphysin-RVS167 (BAR) domain and a C-terminal SH3 domain, suggesting that BIN1 functions in clathrin-mediated endocytosis and endosomal transport (32). However, studies on genetically modified animal models suggest that BIN1 is involved in various events, such

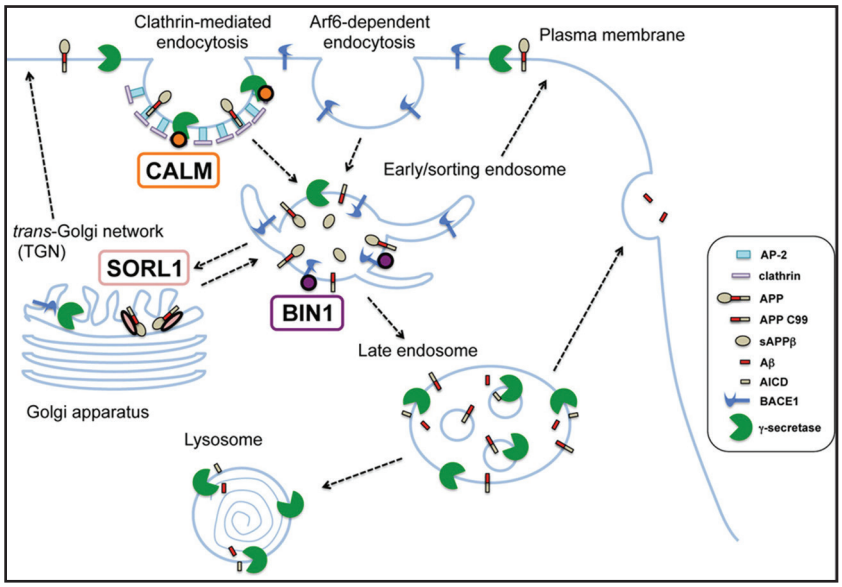

Figure 2. Molecular mechanisms of membrane trafficking-related risk factors in Aβ production pathway. CALM binds to γ-secretase and regulates its clathrin-mediated endocytosis. BIN1 associates with BACE1 at early endosomes and controls its transport to late endosomes/lysosomes. SORL1 interacts with APP to guide its trafficking between TGN and early endosomes.

as inflammation, calcium homeostasis, apoptosis, and cell growth. In an analysis of the pathobiology of AD, a genetic and physical interaction between BIN1 and MAPT was identified (33). In addition, we found that BIN1 regulates the intracellular trafficking of BACE1. BACE1 is endocytosed by Arf6 dependent pathway from the plasma membrane (34) and targeted to late endosome/lysosome via BIN1 dependent fashion. Genetic depletion of BIN1 causes the accumulation of BACE1 within early endosomes, leading to an increase in Aβ generation (Miyagawa and Tomita, unpublished observations). Thus one of the pathological functions of BIN1 in AD would be a regulator for Aβ production.

Sortilin-related receptor (SORL1, also known as SORLA or LR11) belongs to the low-density lipoprotein receptor (LDLR) family as well as the vacuolar protein sorting 10 domain receptor family (35). *SORL1* was originally identified as a candidate gene associated with AD by GWAS (36). To date, in addition to the several coding mutations and genetic variants associated with LOAD (18), early-onset FAD-linked nonsense and missense mutations in *SORL* have been identified (37), indicating the pathological significance of SORL1 function in the development of AD. Consistent with the results that AD patients have a reduced expression of SORL1 (38), SORL1 levels were found to correlate with brain Aβ levels in mouse AD models (39, 40). At present, the regulation of APP/Aβ trafficking and processing is the most promising mechanism via which SORL1 is involved in LOAD. SORL1 interacts with APP mainly in the *trans*-Golgi network (TGN) to regulate its transport between the TGN and early endosomes, which is the main Aβ-generating compartment (41). Moreover, SORL1 binds to Aβ and is involved in the lysosomal sorting of nascent Aβ (39, 42).

4.2. Lipid metabolism

APOE ε4 is the strongest known risk factor for AD. *APOE* encodes apolipoprotein E (ApoE), which functions as a ligand in the receptor-mediated endocytosis of lipoprotein particles and cholesterol at the periphery, suggesting that lipid metabolism plays a crucial role in the pathogenesis of AD (43). The most common three SNPs of *APOE* are located in the coding region. Among the three *APOE* alleles (ε2, ε3, and ε4), the *APOE* ε4 allele increases the risk for AD; 2-3-fold by one allele, and 12-fold by two alleles, whereas the *APOE* ε2 allele has a protective effect (11, 12). In the AD field, ApoE was originally identified as one of the main components of senile plaques and as an Aβ-binding protein (44). The interaction of ApoE with Aβ affects Aβ aggregation (45) and clearance (46), the latter being mediated by the LDLR family, such as LRP1 and LDLR. In fact, *Apoe* knockout mice as well as human *APOE* transgenic mice showed altered amyloid deposition (47, 48). In addition, other pathological mechanisms of ApoE, including its effects on synaptic function, tau phosphorylation, and the inflammatory response may also contribute to LOAD pathogenesis (43); however, the mechanistic details remain to be clarified. Recently, ApoE was identified as a major ligand of TREM2 (49, 50), which is known as a risk factor for AD and a receptor for zwitterionic lipids found on damaged neurons (see below). Thus, ApoE might be involved in the inflammatory response that is induced by lipids.

Additional genes involved in lipid metabolism were identified from GWAS and genome sequencing studies, such as *CLU*, *ABCA7*, and *PLD3 (*6). Clusterin (also known as apolipoprotein J) encoded by the *CLU* gene is a major apolipoprotein in the brain and is found in amyloid plaques (51). To date, various roles of clusterin in LOAD pathogenesis have been proposed, including Aβ aggregation, Aβ clearance, lipid metabolism/cholesterol transport and inflammatory responses (46, 52). *CLU* SNPs found by GWAS were found to be associated with its alternative splicing or expression levels (53).

ATP-binding cassette transporter A7 (ABCA7) is a member of the ABC transporters, and transports lipids and cholesterols across membranes to apolipoproteins (54). In addition to from GWAS, loss-of-function variants in *ABCA7* associated with an increased risk of AD were identified (55-57). ABCA7 is highly expressed in microglia and its deletion reduces the cellular uptake of Aβ. In addition, the deletion of *Abca7* increased amyloid deposition levels in a mouse model of AD (58, 59). However, the precise mechanisms by which ABCA7 affects AD risk via APOE/lipid metabolism, the immune response, and Aβ clearance still remain unclear.

4.3. Immune response

GWAS identified several genes involved in the inflammatory response to be associated with AD, namely, *CD33*, *CR1*, *MS4A6A/MS4A4E*, and *IL1RAP*. In addition, rare variants of *TREM2* were found to be associated with AD by genome sequencing studies, highlighting the pathological function of inflammatory genes in AD (6).

CD33 is a member of the sialic acid-binding immunoglobulin-like lectins, and is expressed in immune and hematopoietic cells. In AD brains, the level of CD33 and the number of CD33-immunoreactive microglia are increased (60). Consistent with these results, the protective *CD33* SNP rs3865444 is associated with a reduction in CD33 expression, insoluble Aβ42 levels, and Aβ plaque deposition. Moreover, CD33 inhibits the microglial uptake of Aβ42, suggesting that CD33 plays a role in AD risk by affecting Aβ clearance by microglia (61).

TREM2 (Triggering Receptor Expressed on Myeloid cells 2) is an innate immune receptor expressed in microglia, which plays a role in phagocytosis and the inflammatory response (62). TREM2 is known to bind various polyanionic molecules, including bacterial antigens, nucleic acids, and phospholipids. Recently, ApoE was identified as an endogenous ligand for TREM2 (49, 50). A genetic link between *TREM2* and a neurological disorder was first identified for Nasu-Hakola disease (also known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy) (63). In addition, mutations in *TREM2* were identified in frontotemporal dementia patients and AD patients (64, 65). The observed effect of the *TREM2* p.R47H mutation was of a comparable extent to that of the *APOE* ε4 allele (20, 66). TREM2 associates with the transmembrane adaptor and signaling molecule TYROBP (DAP12). Loss-of-function mutations in *TREM2* or *TYROBP* causes impaired tissue debris clearance by the phagocytic function of microglia, thereby causing chronic neurodegeneration. In contrast, the pathological role of the TREM2 p.R47H mutation in AD still remains unclear. In fact, completely opposite effects of *Trem2* deficiency on Aβ deposition have been observed in AD mouse models. Wang *et al*. found that *Trem2* deficiency exacerbated Aβ deposition by the incomplete sensing of anionic lipids associated with Aβ plaques (67). On the other hand, Jay *et al*. found that *Trem2* knockout ameliorated Aβ deposition, tau phosphorylation, and brain inflammation due to the reduction in Aβ-associated $CD45^{hi}Ly6C^{+}$ macrophages (68). Thus, neuropathological analyses of individuals who carry the rare *TREM2* variant will provide important clues regarding the pathological function of TREM2. Intriguingly, cell surface levels of TREM2 are regulated proteolytically (69). The *TREM2* p.R47H mutation is associated with reduced shedding of TREM2 and reduced secretion of TREM2 in the CSF of human AD patients, supporting the notion that the loss-of-function of TREM2 affects the onset of AD. TREM and TREM-like receptors are a protein family composed of structurally similar proteins encoded by genes clustered on chromosome 6 (6). Interestingly, variants in *TREML2* and *TREM1* have been identified as genetic modifiers of AD (70, 71). In addition, a SNP located in the *TREM* gene cluster was also found to be associated with CSF tau levels (23). These data suggest that the molecular functions of the TREM protein family are critical to AD pathogenesis.

Positron emission tomography for amyloid (amyloid PET) enables us to measure amyloid plaque burden and longitudinal changes in individuals. Recently, a SNP in *IL1RAP* was found to be associated with higher rates of amyloid accumulation (72). IL1RAP encodes a pro-inflammatory IL-1 signaling component expressed in microglia. The IL-1 pathway has been implicated in the pathology of AD in patients and mouse models (73, 74). Importantly, this *IL1RAP* SNP was associated with cortical microglial activation, temporal cortex atrophy, and disease progression including cognitive decline. Nevertheless, further biological analyses are required to understand the molecular basis of these immune factors in the pathogenesis of LOAD.

5. RELEVANCE OF GENETIC FACTORS TO THE PATHOGENESIS OF ALZHEIMER DISEASE

Identification of the genetic risk factors of LOAD and subsequent analyses of the molecular mechanisms by which they are involved in AD have highlighted the

importance of Aβ metabolism, including its production, aggregation, and clearance, in LOAD pathogenesis. In addition, these studies have provided several new insights on the molecular mechanisms underlying the etiology of AD, namely, membrane trafficking, lipid metabolism, and the innate inflammatory response in the brain. Intriguingly, different loci associated with CSF tau levels were identified (23). The clinical evidence that CSF tau levels are associated with the cognitive decline of AD patients suggests that "AD risk genes" affect the pathological process of AD, such as disease duration or age at onset. Intriguingly, a rare coding mutation in *UNC5C* was identified in autosomal-dominant LOAD patients (22). *UNC5C* loci were associated with the rate of brain atrophy. Neurons expressing mutant UNC5C are more susceptible to cell death by Aβ. In addition, genetic analyses based on quantitative imaging phenotypes identified brain atrophy-associated missense mutations in *CARD10* and *PARP1 (*75). Functional analyses of these genes should provide a mechanistic explanation to the susceptibility to neuronal cell death, thereby predisposing patients to AD. Nevertheless, genetic studies using other biomarkers may identify new aspects in the pathological process of AD.

6. CONCLUSIONS

Recent advances in genetics and clinically relevant sequencing applications have provided important clues regarding the pathomechanism of AD (6). However, it is known that generally, common variants/SNPs are involved in the etiology of AD in a large number of patients but with a small contribution, whereas rare variants are involved in that of a small number of patients but with a large contribution. In addition, the identification of ApoE as an endogenous TREM2 ligand suggests the pathobiological connections/cross-talk between risk factors. In fact, it was reported that the *NME8* locus affects *PTK2B* expression, and that the *CD33* locus affects *TREM2* expression (76). Therefore, the precise mapping of the pathological functions of risk factors is important for understanding the overall picture of AD at the molecular level. These findings may provide new promising therapeutic strategies and diagnostic targets, leading to the development of personalized treatments for AD patients.

7. ACKNOWLEDGEMENTS

We thank Mr. Ihori Ebinuma for critical reading. This work was supported in part by a Grant-in-Aid for Scientific Research (A) from the Japan Society for the Promotion of Science (JSPS), by grants from Takeda Science Foundation, the Cell Science Research Foundation, the Tokyo Biochemical Research Foundation, the Daiichi Sankyo Foundation of Life Science, NAGASE Science Technology Foundation and Ono Medical Research Foundation. K.K. is a research fellow of JSPS.

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Key Words: Alzheimer disease, Genetic Risk Factor, Trafficking, Lipid, Immune System, Review

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