

# Genes associated with Alzheimer's disease: an overview and current status

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**Abstract:** Alzheimer's disease (AD) is a progressive, neurodegenerative disease and the most common form of dementia in elderly people. It is an emerging public health problem that poses a huge societal burden. Linkage analysis was the first milestone in unraveling the mutations in APP, PSEN1, and PSEN2 that cause early-onset AD, followed by the discovery of apolipoprotein E-ε4 allele as the only one genetic risk factor for late-onset AD. Genome-wide association studies have revolutionized genetic research and have identified over 20 genetic loci associated with late-onset AD. Recently, next-generation sequencing technologies have enabled the identification of rare disease variants, including unmasking small mutations with intermediate risk of AD in PLD3, TREM2, UNC5C, AKAP9, and ADAM10. This review provides an overview of the genetic basis of AD and the relationship between these risk genes and the neuropathologic features of AD. An understanding of genetic mechanisms underlying AD pathogenesis and the potentially implicated pathways will lead to the development of novel treatment for this devastating disease.

**Keywords:** Alzheimer's disease, amyloid precursor protein, genome-wide association studies, biological pathways, presenilin 1, presenilin 2, neuropathology

## Introduction

Currently, there are ~46.8 million dementia cases worldwide, with that number projected to reach 74.7 million in 2030 and over 131.5 million by 2050.<sup>1</sup> Alzheimer's disease (AD) is a major public health problem in the world. Health care costs of dementia in 2015 surpassed \$818 billion (USD), and this figure is estimated to be as high as \$2 trillion by 2030.<sup>1</sup> AD is the most common form of dementia and one of the most common diseases in the developed countries. AD is defined clinically by gradual decline in memory and progressive loss of cognitive functions. Neuropathologically, AD is characterized by gross cortical atrophy and ventricular dilatation and pathological hallmarks of accumulation of Aβ protein in the form of senile plaques and intraneuronal tangles of hyperphosphorylated tau (t) protein. AD is a multifactorial and complex disease and leading cause of dementia among elderly people. Although advanced age is the best known risk factor for AD, some individuals may develop AD at younger age. Hence, based on the time of onset, AD is classified into two types.<sup>2</sup> Early-onset AD (EOAD), which typically develops before the age 65 years, and late-onset AD (LOAD) in those older than 65 years. EOAD is caused by rare and dominantly inherited mutations in APP, PSEN1, and PSEN2. LOAD has a strong genetic component, and it is also called sporadic AD. Up to 60%–80% of LOAD is inheritable, but genetic and environmental factors play a key role in onset, progression, and severity of disease.<sup>3</sup>



The  $\epsilon 4$  allele of the *APOE* gene is the major risk factor for pathogenesis of LOAD.<sup>4,5</sup> Using revolutionizing technologies such as genome-wide association study (GWAS), researchers have identified a number of regions of interest in the genome that may increase a person's risk for LOAD to varying degrees.<sup>6,7</sup> Furthermore, next-generation sequencing (NGS) techniques have identified rare susceptibility modifying alleles in different AD genes.<sup>8</sup> However, other causative and risk genes are involved in AD and need to be identified to fully elucidate the etiology of AD. In this review, we provide a summary of the genetic basis of both EOAD and LOAD, the relationship between these risk genes and the pathogenesis of AD.

## Types of studies in AD genetics

Mainly four strategies have governed the field of AD genetics: genetic linkage analysis, study of candidate genes, GWASs, and NGS technology. Genetic linkage family studies have led to the identification of dominantly inherited, rare mutations in genes of EOAD (APP, PSEN1, and PSEN2). The development of whole-genome genotyping by several independent GWASs has allowed for the study of the involvement of common variants with low risk in disease.

## Genetic linkage analysis

Linkage analysis was the first milestone in unraveling the genetic basis of AD studies of families displaying autosomal dominant inheritance. Genetic linkage studies aim to identify chromosomal regions associated with disease but do not identify one gene or one mutation associated with a disease. Genetic linkage studies in families led to the identification of dominantly inherited mutations in APP on chromosome 21q, PSEN1 on 14q, and PSEN2 on 1q that are associated with EOAD.<sup>9</sup> The  $\epsilon 4$  allele of the *APOE*, which is considered as only one genetic risk factor for LOAD, was also identified with the help of genetic linkage studies.<sup>10</sup> Nonetheless, linkage mapping has been very useful in identifying monogenic traits in EOAD, but it has largely failed to identify risk factors in LOAD, probably due to the complex trait with unidentified variants.

## Study of candidate genes

In candidate gene studies, genetic variants of people with a particular disease are genotyped and compared with a similar group of healthy individuals. Susceptibility genes are revealed when case and control frequencies differ significantly. Candidate gene approach elucidated that *APOE* risk alleles are firmly implicated in LOAD. Over 1,000

candidate genes were tested for AD susceptibility, with mostly inconsistent results. The success of this approach relies on the existing knowledge on disease pathogenesis. Because of small sample size, inadequately evaluated population groups, and insufficient *P*-value threshold, most of the candidate studies could not be replicated.<sup>11</sup>

## Genome-wide association studies

The development of microarray technology revolutionized genetics research, allowing for the simultaneous evaluation of millions of single-nucleotide polymorphisms (SNPs) in thousands of samples. Such cheap and comprehensive GWAS allows one to perform genome-wide association testing in a hypothesis-free manner. Besides *APOE*, GWAS has allowed identification of >20 genetic loci associated with increased susceptibility for LOAD.<sup>12</sup> GWAS has successfully identified numerous susceptibility genes for AD, but, rare variants will not be detectable by GWAS. Thus, the success of GWAS depends on sample size, frequency of risk alleles, and individual effect sizes.

## NGS technologies

NGS technologies provide fast and cost-effective sequencing strategies that allow an entire genome to be sequenced in <1 day. NGS technology has important implications in understanding the basis of many Mendelian neurological conditions and complex neurological diseases that have enabled the identification of rare disease variants, including unmasking small mutations. Recently, NGS has been applied to identify genetic factors in small families with unexplained EOAD. The primary success of NGS in AD has been the identification of rare susceptibility modifying alleles in APP, TREM2, and PLD3.<sup>8</sup>

## EOAD susceptibility genes

Studies of families with autosomal dominant patterns of inheritance were a cornerstone in understanding the genetics of AD. Three genes are considered the main risk factors for EOAD: APP on chromosome 21q, PSEN1 on 14q, and PSEN2 on 1q that are associated with EOAD (Table 1). To date, >270 highly penetrant mutations have been described in these genes that cause familial AD and many more are discovered each year<sup>13</sup> (<http://www.molgen.ua.ac.be/admutations/>).

## APP

The gene encoding APP is located on chromosome 21q21.3. The three isoforms, APP751, APP770, and APP695, are

**Table 1** Genes implicated in risk of early-onset Alzheimer's disease

Gene symbol	Location	Function	Pathway	References
<i>APP</i>	21q21.3	Neuronal development, Synaptic formation and repair, $\beta$ -Amyloid production	APP processing	13–15,18
<i>PSEN1</i>	14q24.3	$\gamma$ -Secretase activity, Intracellular signaling, $\beta$ -Amyloid production	APP processing	9,26,27
<i>PSEN2</i>	1q42.13	$\gamma$ -Secretase activity, $\beta$ -Amyloid production, Synaptic plasticity	APP processing	13,27,36

produced by splicing of APP. The major isoform found in brain is APP695.<sup>14</sup> APP is proteolytically cleaved following nonamyloidogenic or amyloidogenic pathway by three enzymes, known as  $\alpha$ -,  $\beta$ -, and  $\gamma$ -secretases. Proteolysis of APP by  $\alpha$ - and  $\gamma$ -secretases results in nonpathogenic fragments (sAPP $\alpha$  and  $\alpha$ -C-terminal fragment) in non-amyloidogenic pathway. In the amyloidogenic pathway, proteolysis of APP by  $\beta$ -secretase and  $\gamma$ -secretase results in two major A $\beta$  species that include sAPP $\beta$  and  $\beta$ -CTF.<sup>15</sup> The action of  $\gamma$ -secretases on  $\beta$ -CTF generates A $\beta$ , which forms a key component of amyloid plaques known as extracellular fibrils in AD brain.<sup>16</sup> To date, 49 APP mutations in 119 families are known to cause AD.<sup>13</sup> Most of the APP mutations are dominantly inherited but two recessive mutations, A673V and E693 $\Delta$  have been reported to cause EOAD.<sup>17</sup> The clustering of most pathogenic APP mutations either adjacent to or within the cleavage sites of  $\beta$ - and  $\gamma$ -secretase (exons 16 and 17 of APP) shows that they might be involved in AD pathogenesis.<sup>13,18</sup> Swedish APP Mutation (KM670/671NL) is a double point mutation located at the N-terminus of the A $\beta$  domain adjacent to the  $\beta$ -secretase site in which lysine–methionine is replaced by asparagine–leucine. In Swedish mutation, total A $\beta$  level increases by twofold to threefold, thus affecting the efficiency of  $\beta$ -secretase cleavage.<sup>19</sup> In a case with Swedish mutation, autopsy revealed cortical atrophy, enlargement of the cerebral ventricles, and sulcal widening. Studies have shown that patients with Down syndrome (trisomy 21) develop AD pathology earlier than the normal person.<sup>17,20</sup> Thus, AD pathology may be related to overexpression of APP. Along with Swedish APP Mutation, Tottori mutation (Asp678Asn) and Leuven mutations (Glu682Lys) are located at the N-terminus of the A $\beta$  domain adjacent to the  $\beta$ -secretase site. London mutation (V717I) is one of the most common APP mutations worldwide. APP mutation at or after C-terminus of the A $\beta$  domain alters the  $\gamma$ -secretase function with increased A $\beta$ 42/A $\beta$ 40 ratio by increasing A $\beta$ 42 levels and decreasing A $\beta$ 40 levels. Neuropathological findings in an English family were cortical Lewy bodies and mild amyloid angiopathy.

$\beta$ -Amyloid plaques and neurofibrillary tangle were found in the American family, with the absence of amyloid angiopathy or Lewy bodies.<sup>21</sup> Arctic mutation (E693G) occurs in the coding region of A $\beta$  domain. This mutation fails to alter total A $\beta$  level or the ratio of A $\beta$ 42/A $\beta$ 40. Arctic mutation results in increased aggregation rate of mutant peptide.<sup>22</sup> Brain imaging showed no signs of strokes or vascular lesions but neuritic plaques and neurofibrillary tangles consistent with a diagnosis of AD were seen in one mutation carrier.<sup>23</sup> Flemish mutation (A692G) falls within the region of A $\beta$  domain and increases the level of A $\beta$ 42 and A $\beta$ 40 by twofold to fourfold, affecting  $\gamma$ -secretase activity for A $\beta$  production. Flemish mutation results in A $\beta$  deposition in the blood vessels of the brain and senile plaques.<sup>24</sup> Jonsson et al<sup>25</sup> found a rare mutation (A673T) in the APP gene that showed a strong protective effect against AD.

Abovementioned examples show that altered APP processing and A $\beta$  accumulation are key in the AD pathogenesis.

## PSEN1

PSEN1 gene is located on chromosome 14q24.3, and it is a vital component of the  $\gamma$ -secretase complex, which cleaves APP into A $\beta$  fragments. PSEN1 is localized primarily to the endoplasmic reticulum and helps in protein processing. To date, 215 pathogenic mutations have been identified in PSEN1, of which >70% mutations occur in exons 5, 6, 7, and 8.<sup>13</sup> Mutations in PSEN1 account for up to 50% of EOAD, with complete penetrance and early age of onset. Mutant  $\gamma$ -secretase increases A $\beta$ 42 level while it decreases A $\beta$ 40 level, leading to an increase in the A $\beta$  42/40 ratio. Studies have shown that A $\beta$ 42 is more amyloidogenic and more prone to aggregate in brain than A $\beta$ 40.<sup>9,26,27</sup> Morphologic variants in A $\beta$  plaques due to PSEN1 mutations may result in cotton wool plaques. Cotton wool plaque is formed by large rounded A $\beta$  deposits, and it tends to be immunopositive for A $\beta$ 42 rather than for A $\beta$ 40.<sup>28–30</sup> The vast majority of PSEN1 mutations are missense mutations, but a few insertion and deletions have also been detected. Neuronal loss of 30% in the hippocampal

CA1 region, decreased synaptic plasticity, and 18% of hippocampus atrophy was demonstrated by Breyhan et al<sup>31</sup> due to the intraneuronal accumulation of A $\beta$  peptides and fibrillary accumulation species. In addition to their role in  $\gamma$ -secretase activity, PSEN1 mutations may compromise neuronal function, affecting GSK-3 $\beta$  activity and kinesin-I-based motility, thus leading to neurodegeneration.<sup>32</sup>

## PSEN2

PSEN2 gene is located on chromosome 1q31-q42, and it is very similar in structure and function to PSEN1. PSEN2 mutations are very rare, and to date 13 pathogenic PSEN2 mutations have been detected in 29 families.<sup>13</sup> PSEN2 is a main component of the  $\gamma$ -secretase complex along with PSEN1, nicastrin, Aph-1, and PEN-2.<sup>33</sup> PSEN2 mutation alters the  $\gamma$ -secretase activity and results in increase in the A $\beta$  42/40 ratio in similar manner to the PSEN1 mutation.<sup>9,26,27</sup> PSEN2 mutations carriers have older age of onset with a wide range from 39 years to 75 years. Neuritic plaque formation and neurofibrillary tangle accumulation may be seen as neuropathological changes in some people with PSEN2 mutations.<sup>34</sup> Park et al<sup>35</sup> demonstrated that  $\beta$ -secretase activity is enhanced by PSEN2 mutation, through reactive oxygen species-dependent activation of extracellular signal-regulated kinase. Although, PSEN2 shows close homology to PSEN1, but less amyloid peptide is produced by PSEN2.<sup>36</sup>

## LOAD susceptibility genes

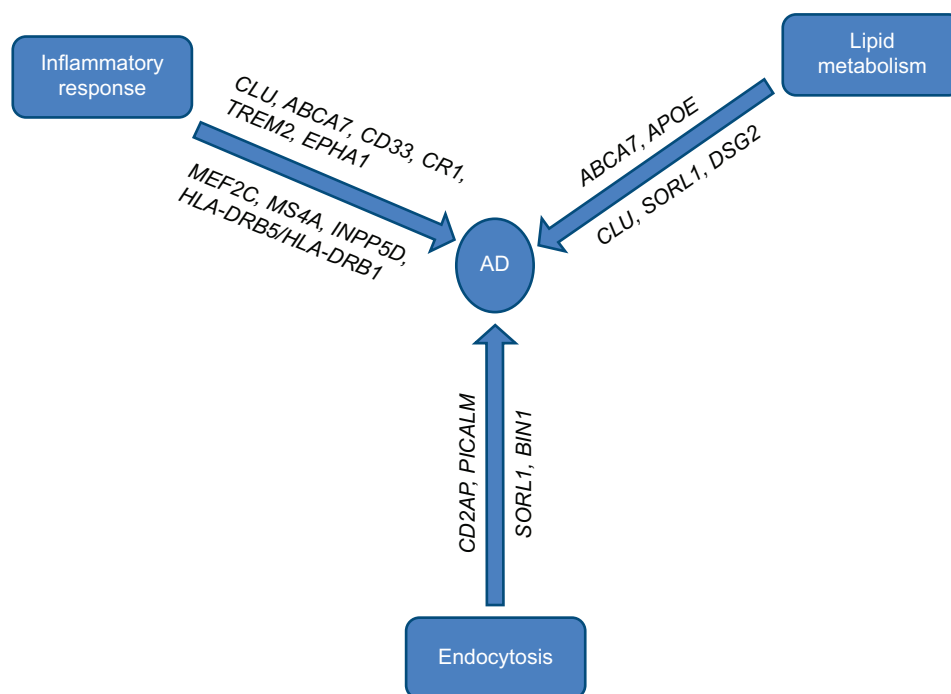
LOAD is genetically far more complex than EOAD with the possible involvement of multiple genes and environmental factors. Most LOAD cases are sporadic with no family history of the disease. Before the era of large-scale GWAS,  $\epsilon$ 4 allele of the APOE gene was the only well-established risk factor for the pathogenesis of LOAD, but with technological advances, researchers have identified a number of regions of interest in the genome that may increase a person's risk for LOAD to varying degrees. It was striking to note that most of the genes identified by GWAS that could be linked with the A $\beta$  cascade or tau pathology roughly cluster within three pathways (Figure 1). We will briefly describe the known genes of LOAD, biological pathways, and mechanisms that might be relevant to AD biology.

## Genes involved in cholesterol metabolism

The genes implicated in cholesterol metabolism are APOE, SORL1, ABCA7, and CLU<sup>6,37–39</sup> (Table 2). Solomon et al<sup>40</sup> demonstrated that having high cholesterol levels in midlife increased the risk of developing AD in late life.

## APOE gene

The APOE gene, located on chromosome 19q13.2, is the strongest genetic risk factor for LOAD. APOE protein is the major cholesterol carrier in the brain. There are three



**Figure 1** Major pathways involved in AD and affected genes.  
**Abbreviation:** AD, Alzheimer's disease.

**Table 2** Common and rare variants associated with Alzheimer's disease

Gene	Chromosome	SNP	Function	Pathway	References
<i>APOE</i>	19q13.2		Lipid transport Synaptic vesicle endocytosis Cytoskeletal dynamics	Lipid metabolism	41–44
<i>CLU</i>	8p21-p12	rs11136000	Synapse turnover Complement regulation Chaperone protein	Cholesterol metabolism and immune response	37,66,67
<i>ABCA7</i>	9p13.3	rs3764650	Phagocytosis	Lipid metabolism	72,74,78
<i>SORL1</i>	11q23.2-q24.2	rs3752246 rs12285364	Lipid homeostasis Endocytosis Receptor for APOE Processing of APP	Immune response Endocytosis Lipid metabolism	79,81,88
<i>CRI</i>	1q32	rs3818361 rs6656401	Amyloid $\beta$ clearance Complement activation	Immune response	91,92,94
<i>CD33</i>	19q13.3	rs3865444 rs3826656	Clathrin-mediated endocytosis Cell signaling	Immune response	97,101
<i>MS4A</i>	11q12.2	rs610932 rs670139 rs4938933	Signal transduction Immune function	Immune response	102,103
<i>TREM2</i>	6p21.1	rs75932628	Inflammatory response	Immune response	104,107,110
<i>BIN1</i>	2q14.3	rs744373 rs7561528	Synaptic vesicle endocytosis APP trafficking Cytoskeletal dynamics	Synaptic vesicle endocytosis	114,118
<i>CD2AP</i>	6p12	rs9296559 rs9349407	Receptor-mediated endocytosis Cytokinesis	Endocytosis Synapse function	119,120,122
<i>PICALM</i>	11q14	rs3851179 rs541458	Cytoskeletal dynamics Clathrin-mediated endocytosis	Endocytosis Synapse function	114,123,125
<i>EPHA1</i>	7q34	rs11771145 rs11767557	Synaptic development Immune function Neural development	Immune response	38,132,133
<i>HLA-DRB5/HLA-DRB1</i>	6p21.3	rs9271192	Immune function Histocompatibility	Immune response	38,135,137
<i>INPP5D</i>	2q37.1	rs35349669	Cytokine signaling Immune function	Immune response	38,138
<i>MEF2C</i>	5q14.3	rs190982	Myogenesis	Immune response	140,141
<i>CASS4</i>	20q13.31	rs7274581 rs6024870 rs16979934	Synapse formation Cell migration Cell adhesion	Hippocampal synaptic function Cytoskeletal function and axonal transport	134,142,144
<i>PTK2B</i>	8p21.1	rs28834970	Calcium homeostasis MAP kinase signaling	Hippocampal synaptic function	146,147
<i>NME8</i>	7p14.1	rs2718058	Ciliary function Neuronal cell proliferation	Axonal transport and cytoskeletal function	149,150
<i>ZCWPW1</i>	7q22.1	rs1476679	Epigenetic regulation Neural development	Epigenetic regulation	153,154
<i>CELF1</i>	11p11	rs10838725	mRNA editing Pre-mRNA splicing	Cytoskeletal function and axonal transport	144,155
<i>FERMT2</i>	14q22.1	rs17125944	Cell–cell adhesion Angiogenesis	Tau pathology Angiogenesis	134,160
<i>SLC24A4/RIN3</i>	14q32.12	rs10498633	Cell signaling Neural development	Synapse function endocytosis	166,169
<i>DSG2</i>	18q12.1	rs8093731	Cell–cell adhesion		38,172,173
<i>PLD3</i>	19q13.2	rs145999145	Signal transduction Epigenetic modification	Lipid metabolism	174,175
<i>UNC5C</i>	4q22.3	rs137875858	Neural development		177
<i>AKAP9</i>	7q21-q22	rs144662445 rs149979685	Signal transduction		183,184
<i>ADAM10</i>	15q22	rs2305421	Hippocampal neurogenesis Cell adhesion		186,187

**Abbreviation:** SNP, single-nucleotide polymorphism.



common alleles of APOE called  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$  alleles. APOE  $\epsilon 4$  increases LOAD risk, whereas APOE  $\epsilon 2$  is associated with decreased LOAD risk.<sup>41–44</sup> There is a threefold increased risk of AD by one copy of APOE  $\epsilon 4$  and 12-fold by two APOE  $\epsilon 4$  alleles. APOE is involved in the control of inflammation, cholesterol metabolism, lipid transport, synaptic function, neurogenesis, or the generation and trafficking of APP and A $\beta$ .<sup>45–47</sup> In the study of large Colombian kindred, APOE  $\epsilon 4$  was associated with earlier onset of dementia in individuals with PSEN1 mutation.<sup>48</sup> More recently, Vélez et al<sup>49</sup> reported ApoE2 allele delays age of onset for carriers of the Paisa mutation (PSEN1 E280A). The mechanism that underlies the link between APOE4 genotype and AD is complex. Studies in humans and transgenic mice showed that APOE influences A $\beta$  clearance, aggregation, and deposition in an isoform-dependent manner.<sup>50–52</sup> Studies suggest that ApoE4 inhibits A $\beta$  clearance and is less efficient in mediating A $\beta$  clearance compared with ApoE3 and APOE2. ApoE4 also contributes to AD pathogenesis by A $\beta$ -independent mechanisms that involve synaptic plasticity, cholesterol homeostasis, neurovascular functions, and neuroinflammation. In APOE4 carriers, there is accelerated A $\beta$  deposition as senile plaques compared with noncarriers.<sup>53,54</sup> Recently, Dorey et al<sup>55</sup> showed that APOE isoforms differentially regulate and modify the A $\beta$ -induced inflammatory response in neural cells in AD, with ApoE2 suppressing and ApoE4 promoting the response. Levels of total tau (t-tau) and phosphorylated tau (p-tau) 181 were increased in AD patients who were APOE4 homozygotes.<sup>56</sup> Conversely, lower cerebrospinal fluid (CSF) p-tau and t-tau concentrations and decreased rate of hippocampal atrophy were seen in APOE2 carriers. This shows that APOE  $\epsilon 2$  is associated with decreased AD pathology.<sup>57</sup> APOE2 has higher affinity for A $\beta$  than does APOE4.<sup>58</sup> Therefore, APOE2 may be more efficient than APOE4 at clearing A $\beta$  fragments from the extracellular space.<sup>59</sup> Neuroimaging study of APOE  $\epsilon 4$  carriers showed gray matter volume decline with age, elevated hippocampal atrophy, increased amyloid load, impaired glucose metabolism, and cerebral amyloid angiopathy with increased microbleeds.<sup>60–63</sup>

## CLU

The clusterin (CLU) gene has been identified as an important risk locus for AD in more than two GWASs.<sup>37,64</sup> In these GWASs, three SNPs within CLU (rs11136000, rs2279590, and rs9331888) showed statistically significant association with AD. CLU, also known as apolipoprotein J, is an apolipoprotein that is widely expressed in both the

periphery and the brain.<sup>65</sup> CLU is situated on chromosome 8p21-p12. CLU is hypothesized to act as an extracellular chaperone that plays an important role in lipid transport, complement regulation, apoptosis, sperm maturation, endocrine secretion, and membrane protection.<sup>66,67</sup> AD patients have elevated levels of CLU in the frontal cortex and hippocampus.<sup>68</sup> CLU concentration is increased in the CSF of patients with AD, suggesting its utility as a prognostic or diagnostic biomarkers in AD. Plasma CLU level was shown to be linked with brain atrophy, disease severity, and clinical progression in AD patients. AD patients with increased CLU mRNA expression deteriorate faster than normal.<sup>69,70</sup> Similar to APOE, CLU is present in amyloid plaques, binds to A $\beta$  peptides, and interacts with A $\beta 40$  and A $\beta 42$ . Thus CLU might play an important role in AD pathology. Recently, Deming et al<sup>71</sup> found that CLU levels were significantly associated with AD status and CSF tau/A $\beta$  ratio, thus influencing the immune system and neurogenesis in AD. CLU suppresses A $\beta$  deposition, inhibits the complement system to prevent inflammation, and decreases apoptosis and oxidative stress in AD. These findings generally support a biochemical role for CLU in the development of AD pathogenesis.

## ABCA7

ABCA7, located on chromosome 19p13.3, has recently been identified as a strong genetic locus associated with LOAD in GWASs.<sup>37,72</sup> ABCA7 is a member of the ATP-binding cassette genes, and its expression was detected in hippocampal and microglia cells in the brain.<sup>73</sup> ABCA7 mediates transport of high-density lipoprotein cholesterol, effluxes lipids from cell into lipoprotein particle, and generates phospholipids. It can also play a vital role in cholesterol homeostasis and the immune system. Meta-analyses of all data provided compelling evidence that SNPs rs3752246 and rs3764650 near ABCA7 have been associated with LOAD.<sup>38,74</sup> Recently, ABCA7 variant SNP (rs115550680) was found to nearly double the risk of LOAD in African-Americans.<sup>75</sup> ABCA7 also regulates phagocytosis by macrophage uptake of A $\beta$  and mediates processing of APP.<sup>76,77</sup> The recent study by Steinberg et al<sup>78</sup> in Icelandic population identified ABCA7 as the most significant gene for AD. Studies in adult mice showed that ABCA7 is expressed most abundantly by macrophages (ie, tenfold higher than neurons), and A $\beta$  is accumulated in brain of ABCA7-deficient mice.<sup>76</sup> Emerging data suggest that ABCA7 could be associated with AD via various pathways, possibly including A $\beta$  accumulation, lipid metabolism, and phagocytosis.

## SORL1

SORL1 (also known as SorLA and LR11) is located on chromosome 11q23.2-q24.2, and it is a member of the Vsp10p domain receptor family and is involved in intracellular transport and processing of APP, resulting in decreased production of A $\beta$  peptide.<sup>79,80</sup> Although the actual functional risk loci within the SORL1 gene still remain unknown, studies have showed that SORL1 plays a key role in AD susceptibility.<sup>81–83</sup> Disruption of SORL1 has also been shown to influence A $\beta$  pathway and tau-related cellular processes.<sup>84,85</sup> Significant associations between SORL1 genetic variants and AD have been reported in several ethnic groups.<sup>86,87</sup> SORL1 risk variants have also been associated with hippocampal atrophy, and altered CSF A $\beta$  level, suggesting its potential role in LOAD pathogenesis.<sup>88</sup> The current data suggest that underexpression of SORL1 leads to overexpression of A $\beta$ , which has been associated with a higher risk of developing AD.<sup>84,89</sup> SORL1 also mediates APP processing via endocytotic pathways and plays a key role in A $\beta$  production.<sup>84</sup> Meta-analysis of the association between variants in SORL1 and AD has identified that SNP rs12285364 was significantly associated with increased risk of AD.<sup>90</sup>

## Genes involved in immune response

Involvement of the immune system in the pathogenesis of LOAD was revealed in several GWASs. Neuroinflammation is a pathological hallmark of AD. LOAD genes implicated in immune response are CR1, CD33, MS4A, ABCA7, EPHA1, TREM2, and CLU<sup>6,37–39,72</sup> (Table 2).

## CR1

CR1 (also known as C3b/C4b receptor or CD35) is located on chromosome 1q32, and it is a member of the receptors of complement activation family. CR1 is a single-chain type I transmembrane glycoprotein comprising four main structural domains and mainly expressed on erythrocytes, leukocytes, and splenic dendritic cells.<sup>91</sup> CR1 acts as a receptor in classical pathway and alternative pathway; CR1 on phagocytes facilitates the uptake and removal of immune complexes; CR1 components have neuroprotective effect in AD. Thus, CR1 functions as a complement regulatory protein and helps in immune regulation.<sup>92,93</sup> In recent GWASs, SNPs in CR1 have been associated with LOAD risk.<sup>38,64,72</sup> It has been reported that SNPs rs3818361 and rs6656401 in CR1 are associated with increased risk of AD.<sup>64</sup> SNP rs1408077 was associated with plaque load in the brain of patients with AD.<sup>94</sup> The deposition of smaller local gray matter volume in the entorhinal cortex of carriers of the CR1 rs6656401 might lead to an increased risk

of AD later in life.<sup>95</sup> CR1 plays a key role in AD pathogenesis via reducing activation of complement cascade activity. CR1 variant is associated with increased CSF A $\beta$ 42 levels, and some variants lead to vascular amyloid deposition.<sup>96,97</sup> CR1 mRNA is involved in the formation of neurofibrillary tangles and thus results in cognitive decline.<sup>70</sup>

## CD33

CD33 is located on chromosome 19q13.3, and it is a type I transmembrane protein, which belongs to the sialic acid-binding immunoglobulin-like lectins, and it is expressed on myeloid cells and microglia. CD33 plays an important role in mediating cell–cell interaction, facilitates clathrin-independent endocytosis, inhibits immune cell functions, and regulates cell growth and survival, via induction of apoptosis.<sup>98,99</sup> Recent GWASs have identified CD33 as a strong genetic locus associated with LOAD. The two SNPs at the CD33 gene cluster, rs3865444 and rs3826656, have been associated with LOAD.<sup>39,72</sup> In AD patients, the minor allele of the CD33 SNP rs3865444 was associated with reductions in both CD33 level and amyloid plaque burdens in the brain.<sup>100</sup> There is positive correlation between CD33 expression in microglia with plaque burden and declined cognitive function. Bradshaw et al<sup>101</sup> demonstrated that the CD33 risk allele rs3865444 increases CD33 expression in monocytes and is associated with increased amyloid pathology in patients with AD. CD33 contributed to the pathogenesis of LOAD by impairing microglia-mediated clearance of A $\beta$ . Furthermore, the increased A $\beta$  clearance and reduced A $\beta$  levels in vitro and in vivo are due to deletion of CD33.

## MS4A

The MS4A gene is located on chromosome 11q12.2, and it encodes protein that contains at least four potential transmembrane domains, two extracellular loops with cytoplasmic N- and C-terminal domain, and one intracellular loop. MS4A genes are highly expressed in hematopoietic cells such as myeloid cells and monocytes. Although MS4A genes are poorly characterized, MS4A1 (CD20), MS4A2 (Fc $\epsilon$ RI $\beta$ ), and MS4A3 (HTm4) play an important role in immunity.<sup>102</sup> MS4A1 was found to interact with activated B-cell antigen receptor complex to regulate calcium influx. Dysregulation of intracellular calcium signaling plays an important role in the pathogenesis of AD. Recent GWASs have identified three members of MS4A family; MS4A4A, MS4A4E, and MS4A6E associated with LOAD.<sup>6,103</sup> To date, many SNPs have been identified in LOAD GWASs, including rs670139 in MS4A4E, rs4938933, and rs1562990 located within the

region between MS4A4E and MS4A4A, and rs610932 and rs983392 in MS4A6A.<sup>6,103</sup> Aside from rs983392 in MS4A6A, the SNP rs4938933 was also associated with reduced AD risk, while SNPs rs670139 and rs610932 were associated with increased LOAD risk. Recently, Karch et al<sup>70</sup> found that MS4A6A expression level and minor allele of rs670139 in MS4A6E were associated with increased Braak tangle and plaque in AD patients. MS4A families affect AD progression and pathogenesis including A $\beta$  generation, tau phosphorylation, and apoptosis by regulating calcium homeostasis. Elevated levels of MS4A4A and MS4A6A may cause detrimental effect on LOAD pathogenesis.

## TREM2

TREM2 gene is located on chromosome 6q21.1, which encodes a single-pass type I membrane protein made up of an extracellular immunoglobulin-like domain, a transmembrane domain, and a cytoplasmic tail, which interacts with DAP12 or TYROBP for its signaling function.<sup>104</sup> TREM2 is one of the highly expressed cell surface receptors on microglia that facilitates phagocytosis and downregulation of inflammation. TREM2 is expressed throughout the central nervous system, with highest concentrations in white matter and lowest concentrations in the cerebellum.<sup>7</sup> Jonsson et al<sup>104</sup> performed whole-genome sequencing in Icelandic population and reported that a missense mutation R47H (rs75932628) within TREM2 was strongly associated with AD with an odds ratio as strong as that for APOE  $\epsilon$ 4. Similar associations were found in cohorts from the US, Germany, the Netherlands, and Norway.<sup>104–106</sup> Missense mutation R47H in TREM2 has been reported to increase LOAD risk in a meta-analysis of three imputed data sets of GWASs (EADI, GERAD, and ANM).<sup>105</sup> Among six additional variants found by Guerreiro et al,<sup>107</sup> three variants (Q33X, Y38C, and T66M) had been related to a frontotemporal dementia-like syndrome without bone involvement in the homozygous state. Recent study by Jin et al<sup>106</sup> found 16 rare coding variants in TREM2, of which two variants R47H and R62H were associated with increased LOAD risk. A homozygous mutation in TREM2 was associated with an autosomal recessive form of early-onset dementia called Nasu–Hakola disease.<sup>108,109</sup> These patients have multifocal bone cysts predisposing to pathological fracture. Patients with heterozygous loss-of-function mutations have increased risk for LOAD.<sup>104,105</sup> TREM2 variant R47H increased risk of neurodegenerative disorders such as frontotemporal dementia, Parkinson's disease, and amyotrophic lateral sclerosis.<sup>107,110,111</sup> TREM2 R47H variant showed accelerated CSF tau and hyperphosphorylated tau

protein.<sup>112</sup> More extensive brain atrophy was seen in AD patients who carry a TREM2 mutation than noncarriers.<sup>113</sup>

## Genes involved in endocytosis/vesicle-mediated transport

Endocytosis is a critical process in synaptic transmission and response to neural damage. In recent LOAD GWASs, several genes were revealed, which are involved in regulating endocytosis, including BIN1, CD2AP, PICALM, EPHA1, SORL1, etc.<sup>6,37,38,64,72</sup> (Table 2). Most of these genes are involved in APP trafficking, which plays a key role in AD pathogenesis.

### BIN1

BIN1 is located on chromosome 2q14.3, and it has more than ten isoforms produced by alternative splicing. BIN1 is implicated in synaptic vesicle endocytosis, intracellular APP trafficking, immune response, apoptosis, and clathrin-mediated endocytosis.<sup>114,115</sup> The BIN1 gene locus was initially identified as having a possible association with AD in large GWASs.<sup>6,116</sup> Several independent candidate gene studies have replicated and confirmed that SNPs rs744373 and rs7561528 located near BIN1 coding region are associated with LOAD risk. Neuroimaging measures have shown that BIN1 risk variant (SNP rs7561528) was associated with thickening of temporal pole and entorhinal cortex.<sup>117</sup> In AD patients, upstream of SNP rs59335482 within the BIN1 was associated with increased tau loads but not with A $\beta$ , suggesting that BIN1 might modulate tau-related AD pathogenesis.<sup>118</sup> BIN1 knockdown suppresses tau-mediated neurotoxicity; therefore, BIN1 can act as a therapeutic target for AD. Little is known about the role of BIN1 in neurodegeneration. Therefore, further investigation is required to reveal the role of BIN1 in AD pathogenesis.

### CD2AP

CD2AP is an 80 kDa cytoplasmic scaffolding protein located on chromosome 6p12, and it is responsible for regulation of the cytoskeletal structure. CD2AP is also involved in receptor-mediated endocytosis, cell adhesion, intracellular trafficking, cytokinesis, and apoptosis.<sup>119</sup> Recently, CD2AP was detected as a risk factor for AD by several GWASs.<sup>6,38,72</sup> GWASs of LOAD have identified SNPs rs9296559 and rs9349407 in CD2AP as AD susceptibility loci. CD2AP is expressed in brain and in AD patients. SNP rs9349407 is associated with increased neuritic plaque pathology.<sup>120</sup> CD2AP interacts with the presynaptic transmembrane protein called neurexin and thus helps in vesicle trafficking and cell adhesion. A recent APP transgenic mouse model revealed



that suppressing CD2AP expression altered A $\beta$  levels and decreased A $\beta$ 42/A $\beta$ 40 ratio in vitro. In vivo CD2AP was associated with subtle alteration in the level of A $\beta$ .<sup>121</sup> Previous GWASs have failed to show significant association between SNP rs9349407 and CD2AP in Chinese, Japanese, African-American, Canadian, and European populations.<sup>6,72</sup> Recently, Chen et al<sup>122</sup> reported significant association between CD2AP rs9349407 polymorphism and AD in East Asian, American, Canadian, and European populations.

### PICALM

The PICALM gene is situated on chromosome 11q14, and it is ubiquitously expressed with particularly high levels in neurons. Similar to BIN1, PICALM is also implicated in clathrin-mediated endocytosis and intracellular trafficking. The SNPs rs3851179 and rs541458 were found to be associated with LOAD in GWASs for AD risk factors.<sup>37,38</sup> PICALM plays a key role in synaptic transmission by the trafficking of VAMP2. In cell culture models and APP/PS1 mice, Xiao et al<sup>123</sup> revealed that PICALM facilitates A $\beta$  production by internalizing APP but knockdown of PICALM influenced APP internalization and decreased A $\beta$  production and release. Therefore, PICALM was regarded as a key regulator of brain A $\beta$  production, amyloid plaque load, and APP internalization. SNP rs3851179 in PICALM has been associated with thickening of entorhinal cortex and hippocampal degeneration.<sup>117,124</sup> Schjeide et al<sup>125</sup> have revealed that the risk allele of PICALM SNP rs541458 was associated with decreased levels of CSF A $\beta$ 42 in a dose-dependent manner. Studies have failed to find any associations between PICALM SNPs and tangles, but the PICALM expression was increased in the frontal cortex in AD.<sup>126</sup> PICALM plays an important role in A $\beta$  blood–brain barrier transcytosis and clearance.<sup>127</sup> PICALM also plays a role in autophagy and tau clearance.<sup>128</sup> Treusch et al<sup>129</sup> revealed that PICALM modulates A $\beta$ -induced toxicity in a yeast model by impairing endocytosis. Morgen et al<sup>130</sup> showed that synergistic interaction of PICALM (rs3851179) and APOE  $\epsilon$ 4 allele was associated with declined cognitive function and brain atrophy in AD.

### EPHA1

EPHA1, located on chromosome 7q34, is a member of the tyrosine kinase family and plays a crucial role in synapse formation and development of the nervous system.<sup>131</sup> EPHA1 has high affinity for membrane-bound ligand, ephrins-A1, which regulates bidirectional signaling to adjacent cells via synapse formation.<sup>132</sup> EPHA1 is widely expressed in differentiated epithelial cells and lymphocytes. EPHA1 SNPs rs11771145

and rs11767557 were documented to be associated with decreased LOAD risk.<sup>6,38,72</sup> Recently, Wang et al<sup>133</sup> demonstrated that EPHA1 (rs11771145) genetic variant reduced LOAD risk by functional modification of hippocampus, lateral occipitotemporal, and inferior temporal gyri in AD. EPHA1 is implicated in immune function, synaptic plasticity, chronic inflammation, and cell membrane processes.

## Other genes implicated in LOAD

Recent meta-analysis of GWASs identified additional genes implicated in LOAD: HLA-DRB5/HLA-DRB1, INPP5D, MEF2C, CASS4, PTK2B, NME8, ZCWPW1, CELF1, FERMT2, SLC24H4-RIN3, and DSG2<sup>6,37–39</sup> (Table 2). Functions of most of these genes are unknown or poorly characterized. These genes may be involved in different possible pathways.<sup>134</sup> HLA-DRB5/HLA-DRB1, INPP5D, and MEF2C are involved in immune response and inflammation. NME8, CELF1, and CASS4 are implicated in cytoskeletal function and axonal transport. PTK2B is involved in hippocampal synaptic function. ZCWPW1 is an epigenetic regulator. FERMT2 is involved in tau pathology and angiogenesis. SLC24H4-RIN3 has possible cardiovascular risk.

### HLA-DRB5/HLA-DRB1

HLA-DRB5/HLA-DRB1 is situated on chromosome 6p21.3, and it is a member of the major histocompatibility complex class II. HLA-DRB5/HLA-DRB1 plays an important role in immune response and histocompatibility.<sup>135</sup> HLA-DRB5/HLA-DRB1 is expressed on microglia in the brain. An association of HLA-DRB1 rs9271192 with LOAD was reported in a meta-analysis of 74,046 individuals.<sup>38</sup> HLA-DRB1/DRB5 polymorphism was associated with multiple sclerosis.<sup>136</sup> Recent GWASs have also shown association between rs3129882 polymorphism in intron 1 of HLA-DRA gene and Parkinson's disease.<sup>137</sup> HLA-DRB5/HLA-DRB1 is also implicated in antigen presentation, inflammation, and complement system.

### INPP5D

INPP5D is located on chromosome 2q37.1, and it encodes the SH2-containing inositol 5-phosphatase 1 (SHIP1). INPP5D, also known as SHIP1, is a key regulator of cytokine signaling. The SNP rs35349669, near INPP5D, is associated with increased LOAD risk.<sup>38</sup> In addition to the hematopoietic compartment, SHIP is also present in osteoblasts, mature granulocytes, and macrophages. Recently, Chen et al<sup>138</sup> found that inhibitor of INPP5D can be used in acute lymphoblastic leukemia to overcome drug resistance. Diseases such as

cancer, inflammatory diseases, diabetes, atherosclerosis, and AD may be treated with the help of SHIP modulation.<sup>139</sup> SHIP is implicated in multiple signaling pathways, such as phosphatidylinositol 3-kinase (PI3K) pathway, and PI3K is central to signal transduction pathway.

## MEF2C

MEF2C is located on 5q14.3, and it encodes a member of the MADS box transcription enhancer factor 2. MEF2C is involved in myogenesis, neurogenesis, and development of multiple organ system. A GWAS in 74,046 individuals revealed that SNP rs190982 near MEF2C is associated with increased LOAD risk.<sup>38</sup> MEF2C mutations are associated with severe mental retardation, seizure, cerebral malformation, and stereotypic movements.<sup>140</sup> In addition to hematopoietic role, MEF2C regulates B cell proliferation. MEF2C is a regulator of adipose metabolism in vertebrates, and it is a core transcriptional component of the innate immune response of the adult fly.<sup>141</sup>

## CASS4

CASS4 is located on chromosome 20q13.31, and it is also known as HEPL. CASS4 is widely expressed in the lung and spleen, and CASS4 mRNA expression is highest in leukemia cell lines.<sup>142</sup> Although the exact function of CASS4 is unknown, it appears to play an important role in focal adhesion kinase regulation, cellular adhesion, cell migration, and motility.<sup>142</sup> In a meta-analysis of 74,046 individuals, CASS4 SNP rs7274581 has been associated with decreased LOAD risk.<sup>38</sup> Recently, CASS4 was associated with neuritic plaques burden and neurofibrillary tangles in brains with AD.<sup>143</sup> The SNPs rs6024870 and rs16979934 near CASS4 were identified as AD risk alleles in GWASs in LOAD.<sup>134,144</sup> CASS4 plays a vital role in inflammation, calcium signaling, and microtubule stabilization, suggesting that CASS4 might be associated with AD pathogenesis.<sup>145</sup>

## PTK2B

PTK2B (also known as PYK2, Cak2 $\beta$ , or RAFTK) is located on chromosome 8p21.1, and it encodes a cytoplasmic protein tyrosine kinase, which is involved in calcium-induced regulation of ion channels and activation of the MAP kinase signaling pathway.<sup>146</sup> PTK2B is highly expressed in the central nervous system. SNP in PTK2B (rs28834970) was associated with LOAD risk in 74046 individuals from diverse ethnicities.<sup>38</sup> SNP-SNP interaction between rs28834970-rs6656401 (PTK2B-CR1) and rs28834970-rs6656401 (PTK2B-CD33) was associated with increased LOAD risk.<sup>147</sup> Recently, Kaufman et al demonstrated that downstream signaling from

Fyn to the AD risk gene product, Pyk2, was blocked by Fyn kinase inhibitor, AZD0530, in Alzheimer mice. Fyn kinase inhibitions improved the learning and memory impairment in Alzheimer mice.<sup>148</sup> There is a strong interaction between calcium homeostasis and NEDD9 and PTK2B.<sup>145</sup> Hence, PTK2B might be of immense value to investigate its functional role in the context of AD.

## NME8

NME8 is located on chromosome 7p14.1, and it encodes a protein with an N-terminal thioredoxin domain and C-terminal nucleoside diphosphate kinase domains. NME8 is member of NM23 family, and it is involved in proliferation and differentiation of neuronal cells.<sup>149</sup> SNP (rs2718058) adjacent to NME8 was associated with decreased LOAD risk.<sup>38</sup> Liu et al<sup>150</sup> found that there is positive correlation between NME8 (rs2718058) genetic variants and AD pathological features such as cognitive decline, the elevated tau levels in CSF, and the hippocampus atrophy. NME8 has also been associated with primary ciliary dyskinesia and knee osteoarthritis.<sup>151,152</sup>

## ZCWPW1

Zinc finger CW (zf-CW) domain consists of 60 amino acids. Although the function of zf-CW domain is unknown, it might be regarded as a new histone modification reader. zf-CW domain is found in methylation or demethylation enzymes and several other proteins.<sup>153</sup> ZCWPW1 is located on chromosome 7q22.1, and it may be involved in epigenetic regulation by histone modification.<sup>153</sup> Rosenthal et al<sup>144</sup> reported genome-wide significant SNPs (ZCWPW1/rs1476679) associated with LOAD. Protective LOAD allele SNP rs1476679 at ZCWPW1 affects binding of CTCF and RFX3 in K562 by acting expression quantitative trait loci for GATS, PILRB, and TRIM4.<sup>143</sup> Recently, ZCWPW1/PILRB locus mapping showed that SNP rs1476679 was associated with decreased level of PILRB and LOAD risk in one group, while in another group with increased levels of PILRB and no association with LOAD.<sup>154</sup>

## CELF1

CELF1, located on chromosome on 11p11, is a member of the CELF/BRUNOL protein family that is implicated in the regulation of pre-mRNA alternative splicing, mRNA editing, and mRNA translation.<sup>155</sup> The SNP rs10838725 located in an intron of the CELF1 gene was associated with LOAD risk.<sup>144</sup> Recent studies have shown that CELF1 is overexpressed in many human malignant tumors, and it is also upregulated in glioma and promotes glioma cell proliferation.<sup>156,157</sup> The role of CELF1 in myotonic dystrophy

type 1 (DM1), cytoskeletal function, and axonal transport was also reported.<sup>158</sup> LOAD susceptible gene CELF1 mediates tau toxicity in drosophila.<sup>159</sup>

## FERMT2

FERMT2 (also known as kindlin-2, KIND2) is located on chromosome 14q22.1, and it regulates integrin activation by binding to the integrin  $\beta$  cytoplasmic tail via a C-terminal domain.<sup>160</sup> Kindlin-2 was involved in the progression of non-small-cell lung cancer, breast cancer, and gastric cancer.<sup>161–163</sup> Kindlin-2 is also essential for myogenesis and angiogenesis.<sup>164,165</sup> The SNP rs17125944 located in FERMT2 gene was associated with LOAD risk.<sup>134</sup> Study in flies showed that tau toxicity is modulated by altered expression of CELF1 and FERMT2, suggesting a potential role of FERMT2 in AD pathogenesis.<sup>159</sup>

## SLC24A4/RIN3

SLC24A4 (also known as NCKX4) is located on chromosome 14q32.12, and it encodes a member of the potassium-dependent sodium/calcium exchanger. SLC24A4 is associated with neural development, hair color, and skin pigmentation.<sup>166,167</sup> SNP rs10498633 located at intron of SLC24A4 is associated with LOAD risk.<sup>134</sup> Yu et al<sup>168</sup> found the association of SLC24A4 with methylation, and brain DNA methylation has a role in the pathology of AD. RIN3 is located near SNP rs10498633 of SLC24A4. Interaction of RIN3 and BIN1 plays an important role in endocytic pathway.<sup>169</sup> SLC24A4 is also involved in lipid metabolism.<sup>170</sup>

## DSG2

DSG2 is located on chromosome 18q12.1, and it encodes calcium binding transmembrane glycoprotein desmoglein. Desmoglein-2 expression is abundant in epithelial cells and cardiomyocytes. SNP rs8093731 at DSG2 reached genome-wide significance for LOAD in stage 1 but was not replicated in stage 2.<sup>38</sup> Mutations in DSG2 have been associated with arrhythmogenic right ventricular cardiomyopathy and sudden unexplained death.<sup>171</sup> Alterations in the expression of desmogleins have been reported for a variety of cancers. There is reduced expression of DSG2 in pancreatic cancer and overexpression of DSG2 in squamous cell carcinomas of the skin.<sup>172,173</sup>

## Novel sequencing technologies and rare variants

GWASs successfully identified many common variants with low penetrance, not the rare variants associated with LOAD. However, advances in sequencing techniques such

as whole-exome sequencing and whole-genome sequencing have proven to be very powerful techniques to identify novel genes with low-frequency variants associated with both EOAD and LOAD. Using novel sequencing technologies, the following genes have been identified: TREM2, PLD3, UNC5C, AKAP9, and ADAM10<sup>104,107,174</sup> (Table 2).

## PLD3

PLD3 is located at chromosome 19q13.2, and it encodes a nonclassical PLD3 protein associated with the endoplasmic reticulum. PLD3 is highly expressed in brain (such as frontal, temporal, and occipital cortices and hippocampus), and it may be involved in neurotransmission, cell differentiation, epigenetic modifications, and signal transduction.<sup>175</sup> Whole-exome sequencing performed by Cruchaga et al<sup>175</sup> found that the rare missense variant V232M (rs145999145) in exon 7 of PLD3 was associated with increased risk of LOAD. PLD1 and PLD2 have been associated with A $\beta$  pathology; little is known about the actions of PLD3 in AD. Cruchaga et al<sup>175</sup> revealed that PLD3 may be involved in APP processing and is overexpressed in brain tissue from AD patients.<sup>174</sup> Satoh et al<sup>176</sup> found that accelerated accumulation of PLD3 on neuritic plaques in AD brains may be associated with AD pathology. Compared with control brains, PLD3 is expressed at lower level in neurons from AD brains. Decreased expression of PLD3 is associated with increase in extracellular A $\beta$ 42 and A $\beta$ 40.<sup>174</sup>

## UNC5C

UNC5C gene is located on chromosome 4q22.3, and it is highly expressed in the adult hippocampus and cerebellum neurons. Whole-genome sequencing and whole-exome sequencing in European ancestry revealed the rare coding variant T835M (rs137875858) in netrin receptor UNC5C gene that showed significant association with LOAD.<sup>177</sup> A study by Wetzel-Smith et al<sup>177</sup> revealed that neurons with overexpression of T835M UNC5C are more prone to A $\beta$ -mediated cell death, and T835M carriers with enhanced expression of UNC5C in the hippocampus are at increased risk of AD. In Chinese Han population, four rare variants were detected near rs137875858, of which rs137875858 variant showed association with AD.<sup>178</sup> Hunkapiller et al<sup>179</sup> showed that protein structure and death domain are affected by overexpression of UNC5C variant T835M, and T835M carriers are more vulnerable to LOAD.

## APP rare variant A673T

Jonsson et al<sup>25</sup> performed whole-genome sequencing in Icelandic population, and they found that a rare APP A673T

substitution (rs63750847) confers protection against LOAD. This variant was found to be more common in healthy elderly controls than in AD and was associated with decreased cognitive decline among elderly subjects without a diagnosis of AD, suggesting the protective role of rare APP A673T variant in LOAD.<sup>25</sup> A673T variant reduces BACE1 cleavage of APP in primary neurons, resulting in decreased production and aggregation of A $\beta$  in A673T carrier.<sup>180</sup> In contrast to other Nordic populations, the A673T variant appears to be extremely rare in Danish and Asian population.<sup>181,182</sup>

## AKAP9

AKAP9 is located on chromosome 7q21-q22, and it encodes a member of the AKAP family. AKAP9 is alternatively spliced into many isoforms that localize to cellular compartments such as centrosome and the Golgi apparatus. Logue et al<sup>183</sup> conducted whole-exome sequencing in African American cases and identified two rare variants (rs144662445 and rs149979685 within AKAP9) associated with AD. AKAP9 is expressed in the hippocampus, cerebellum, and the cerebral cortex and interacts with numerous signaling proteins from multiple signal transduction pathways. Recently, Venkatesh et al<sup>184</sup> found that deletion of AKAP9 in mice resulted in marked alterations in the organization of microtubules affecting blood–testis barrier function and subsequent germ cell development during spermatogenesis. Mutation in AKAP9 was also associated with long QT syndrome-11 and congenital long QT syndrome type 1 might be genetically modified by AKAP9.<sup>185</sup>

## ADAM10

ADAM10 is located on chromosome 15q22, and it is ubiquitously expressed in a wide range of cell types, such as the neuroblastoma, kidney cell line, and the hepatocytes. Kim et al<sup>186</sup> conducted genotyping and targeted sequencing in 995 cases and 411 controls of European American origin and revealed that rare variant rs2305421 was associated with AD. However, Cai et al<sup>187</sup> found that rare coding variation in ADAM10 was not associated with sporadic AD. Suh et al<sup>188</sup> found two rare mutations (Q170H and R181G) related to LOAD in ADAM10 and demonstrated that ADAM10 mutations increased plaque load and A $\beta$  levels and affect hippocampal neurogenesis in Tg2576 AD mice.

## Challenges, controversies, and future insights

Despite years of research, much of the heritability of AD remains unexplained. Currently, most of the known genes

are unable to explain a large proportion of the heritability for LOAD. Linkage studies have not been effective in elucidating the genetic basis of LOAD due to small sample size and vulnerability to locus heterogeneity. Candidate gene studies have not been successful in replicating genes consistently due to small sample size, locus heterogeneity, and false-positive results. Due to poor understanding of roles of encoded proteins, underlying biology, and effects of risk variants in AD pathogenesis, no therapeutic interventions have been developed to treat AD. Although mouse knockout models have been used to study normal biology and the pathology of AD, the AD field as a whole has struggled to develop drugs and potential novel targets from mouse models into effective therapies.

Recent studies have raised questions regarding the validity of the amyloid hypothesis that has driven drug development strategies for AD for >20 years but has not been unequivocally disproven, despite multiple clinical failures.<sup>189,190</sup> They concluded that the amyloid hypothesis may be failing therapeutically and suggest we expand our view of pathogenesis beyond A $\beta$  and tau pathology for the understanding of AD pathogenesis.

Experience from other complex diseases suggests that genes emerging from several studies require further validation via genetic replication and through functional assessment by *in vitro* and *in vivo* approaches to uncover common genetic factors for AD. NGS can be used to detect rare and structural variations in candidate regions. Novel approach can be developed to reassess the GWAS. Use of endophenotype including cerebral spinal fluid biomarker data, quantitative neuropathology measures, and phenotypes may be powerful tools for the identification of genetic factors that influence AD risk and plausible biological pathways. Future research should be focused on identifying low effect size and rare variants that may explain missing heritability. In future, deep resequencing can be used for an effective and accurate approach to identify novel rare variants associated with AD, which might provide further insights into the underlying mechanisms of disease. It is evident that epistatic interaction is involved in complex diseases such as AD, and this should not be ignored. Furthermore, novel risk genes for AD can be identified by gene profiling. Additionally, missing AD heritability can be addressed with the development of more sophisticated statistical analyses.

## Conclusion

AD is a complex neurodegenerative disease with a strong genetic component. AD is a major public health problem, and



currently, there is no treatment to slow down the progression of AD. With the advance of molecular genetics, many studies are widely performed in search of the genes responsible for both autosomal dominant forms of AD and sporadic AD. Linkage studies revealed APP, PSEN1, PSEN2, and APOE as AD genes. GWASs have identified >20 loci associated with AD risk. The majority of genes associated with AD roughly cluster within three pathways: lipid metabolism, inflammatory response, and endocytosis. Sequencing technologies have enabled the identification of rare disease variants, such as PLD3, TREM2, UNC5C, AKAP9, and ADAM10. Future studies will likely use more sophisticated methods of GWAS and whole-genome sequencing to reveal more risk loci for AD. Hence, a better understanding of genetic complexity of AD and the elucidation of possible molecular mechanisms of neurodegeneration in AD are the cornerstone for the development of effective prevention and treatment strategies for AD.

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## Disclosure

The authors report no conflicts of interest in this work.

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