Review Article

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New genetic players in late-onset Alzheimer's disease: Findings of genome-wide association studies

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Late-onset Alzheimer's disease (LOAD) or sporadic AD is the most common form of AD. The precise pathogenetic changes that trigger the development of AD remain largely unknown. Large-scale genome-wide association studies (GWASs) have identified single-nucleotide polymorphisms in multiple genes which are associated with AD; most notably, these are *ABCA7*, bridging integrator 1(*B1N1*), triggering receptor expressed on myeloid cells 2 (*TREM2*), *CD33*, clusterin (*CLU*), complement receptor 1 (*CRI*), ephrin type-A receptor 1 (*EPHA1*), membrane-spanning 4-domains, subfamily A (*MS4A*) and phosphatidylinositol binding clathrin assembly protein (*PICALM*) genes. The proteins coded by the candidate genes participate in a variety of cellular processes such as oxidative balance, protein metabolism, cholesterol metabolism and synaptic function. This review summarizes the major gene loci affecting LOAD identified by large GWASs. Tentative mechanisms have also been elaborated in various studies by which the proteins coded by these genes may exert a role in AD pathogenesis have also been elaborated. The review suggests that these may together affect LOAD pathogenesis in a complementary fashion.

Key words Alzheimer's disease - genome-wide association study - Heart and Aging Research in Genomic Epidemiology - LOAD - single nucleotide polymorphism - Translational Genomics Research Institute

Introduction

The first case of Alzheimer's disease (AD) was described more than 100 years ago, but the precise pathogenetic changes leading to the development of AD are still a matter of considerable controversy. Based on the age of onset and heredity, AD is classified into early-onset AD (EOAD), late-onset AD (LOAD) and familial AD. LOAD or sporadic AD is the most common form of AD, accounting for about 90 per cent of cases and usually occurring after the age of 65 yr[1]. Neurofibrillary tangles of phosphorylated tau protein and senile plaques composed of amyloid β (Aβ)-protein are the two characteristic pathological hallmarks of AD; however, there exists controversy in how well these correlate with AD phenotype as some AD brains on post-mortem examination reveal minimal plaques and tangles[2].

The protein apolipoprotein E (ApoE) is the only well-established genetic risk factor for LOAD. The *APOE* gene consists of four exons and three introns, with a total of 3597 base pairs, and is mapped to chromosome 19. ApoE is polymorphic with three major isoforms, ApoE2, ApoE3 and ApoE4. High frequency of the *APOE4* allele is found in patients with AD than in the

general population[3]. ApoE4 is known to inhibit neurite outgrowth, disrupt neuronal cytoskeleton[4], stimulate tau phosphorylation and cause neurodegeneration[5]. However, neither is the *APOE4* variant present in all AD cases nor is it absolutely essential for AD pathogenesis[6]. Multiple rare mutations in the amyloid precursor protein gene (*APP*), *PSEN1* gene and *PSEN2* gene cause early-onset AD[7]. However, a large case-control study (3940 cases and 13,373 controls) reported that common variants in these genes were not likely to make strong contributions to susceptibility for LOAD[8].

Recent efforts have been focussed on conducting genome-wide association studies (GWASs) to identify newer risk genes for LOAD. Multi-stage meta-analytic reports by different groups documented the association of single-nucleotide polymorphisms (SNPs) in 10 genes with AD; these being *ABCA7,* bridging integrator 1 gene (*BIN1*), triggering receptor expressed on myeloid cells gene (*TREM*), *CD33,* clusterin gene (*CLU*), complement receptor 1 gene (*CR1*), ephrin type-A receptor 1 gene (*EPHA1), CD2AP,* membrane-spanning 4-domains, subfamily A (*MS4A*) gene cluster and phosphatidylinositol binding clathrin assembly protein gene (*PICALM*)[9-12].

In 2009, Lambert *et al*[13] published an open letter of two-stage GWAS performed on AD subjects and controls. The three-city study identified two new susceptibility loci: *CLU* and *CR1*. They also detected evidence for the association of *PICALM* with AD[13]. A collaborative consortium from Europe and the USA [European AD Initiative 1 (EADI 1)] also performed a GWAS over 16,000 individuals with AD and controls. They identified two novel loci *CLU* and *PICALM*, significantly associated with AD. They also observed one more associated locus *BIN1*[14]. In 2010, Seshadri *et al*[15] performed a three-stage analysis of GWAS data to identify additional loci associated with LOAD. In their gene discovery phase, they concluded that *BIN1* showed association with AD in GWAS. They also confirmed the association of two reported loci; *CLU* and *PICALM* with LOAD[15]. Hollingworth *et al*[10] undertook a combined analysis of four independent genome-wide studies- GERAD1, TGEN1, ADNI and EADI1 - to identify new susceptibility loci of AD. Their data provided significant evidence for the association of *ABCA7*, *MS4A* gene cluster with AD at stage one. In stage two, they observed association of more suggestive loci; *CD33* and *EPHA1* with AD[10]. To identify newer susceptibility loci for AD, the AD Genetic Consortium (ADGC) group conducted a three-staged association study on AD patients and provided compelling evidence for the association of *MS4A4A*, *EPHA1* and *CD33* with AD. They also replicated previous associations of *CR1*, *CLU* and *PICALM* with LOAD[11]. Advances in sequencing techniques of entire genomes identified rare variants in those patients, in whom linkage analysis cannot be done. *TREM2* is one of the variants that increase the risk of AD[12].

Fig. 1 gives a schematic representation of the multiple research groups who worked to find new susceptibility genes for LOAD and also the different loci which affect LOAD pathogenesis.

Alzheimer's disease (AD) pathogenesis as the cumulative effect of multiple genetic risk factors

Large-scale GWASs have identified SNPs in ten genes: *ABCA7*, *BIN1*, *TREM2*, *CD33*, *CLU*, *CR1*, *EPHA1*, *MS4A*, *CD2AP* and *PICALM* which may participate in the pathogenesis of AD by several functional pathways that are affected[9-12]. These genes may be categorized on the bases of their involvement in cellular pathways:

(*i*) Immune response and inflammation: *CR1*, *MS4A* family, *EPHA1*, *CD33*, *TREM*[9,10,12].
(*ii*) Lipid (cholesterol) metabolism: *CLU* and *ABCA7*[9,10].
(*iii*) Endocytosis and synaptic function: *PICALM*, *BIN1*, *CD2AP* and *EPHA1*[9,10].

It is hypothesized that these gene SNPs identified by GWAS influence their respective interconnected cellular processes to cause AD. The exact pathogenesis of AD is still unclear, and it is possible that not all of the above processes are deranged in each case of LOAD. Either of the three may dominate in or solely contribute to LOAD in individual patients. Further, the exact links between the pathways still need to be worked out. However, the common pathways through which these act are widely believed to be the amyloidogenic pathway and the tau hyper-phosphorylation pathway[1].

Fig. 2 represents how the various genetic risk factors may be interconnected and contribute to LOAD risk by ultimately inducing amyloid and hyper-phosphorylated tau protein accumulation.

Functional significance of new genetic loci associated with LOAD

Genes associated with lipid metabolism: *CLU* codes for the secretory hetero-dimeric 75-80 kDa CLU also known as apolipoprotein J[16]. This gene encodes a 2 kb mRNA which translates into a 449 amino

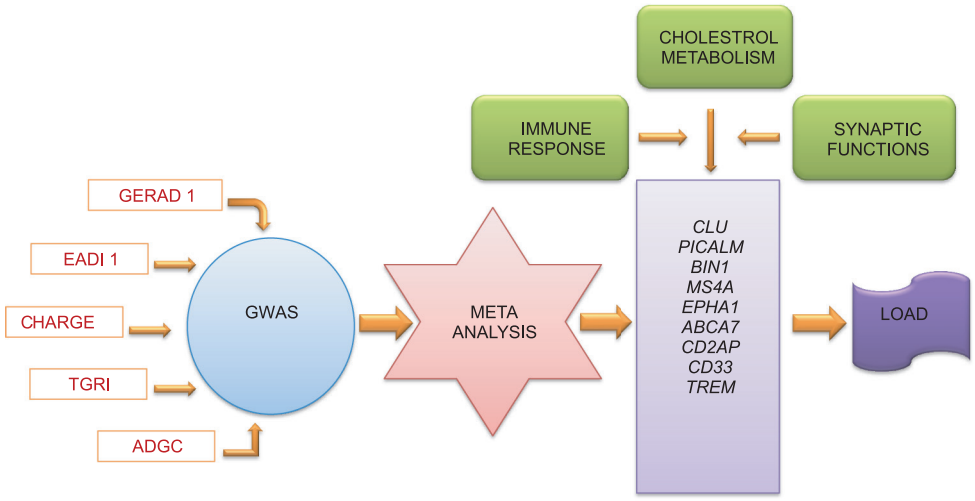

Fig. 1. Schematic representation of multiple organizations who worked to find new genome-wide association study loci and how different loci are connected with each other. The gene loci found as a result of meta-analyses belong to three broad functional categories: immune response, synaptic function and cholesterol metabolism. GWAS, genome wide association studies; GERAD1, genetic and environmental risk for Alzheimer's disease consortium 1; EADI1, European Alzheimer's disease initiative 1; CHARGE, Cohorts for Heart and Aging research in genomic epidemiology; TGRI, Translational Genomics Research Institute; ADGC, Alzheimer's disease genetic consortium; LOAD, late onset Alzheimer's disease.

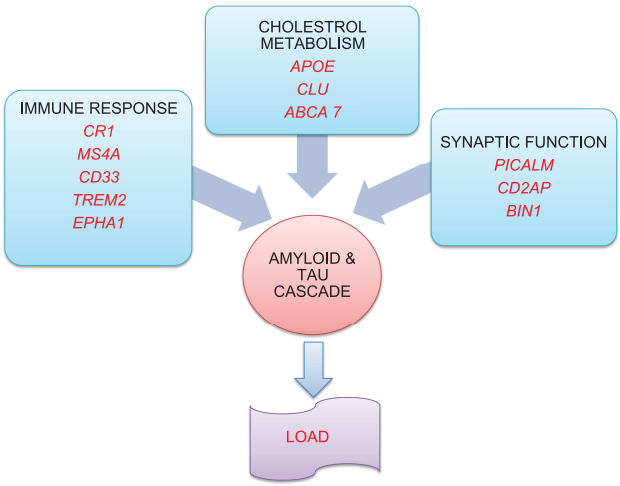

Fig. 2. Interconnected responsible pathways to cause amyloid and tau accumulation. Gene involved in AD pathogenesis can be broadly grouped into 3 categories; immune response (CR1, MS4A, TREM2, CD33, EPHA1), cholesterol metabolism (APOE, CLU, ABCA7), synaptic function (PICALM, CD2AP, BIN1). The cumulative effect of all these genes is manifested through the final common pathway of amyloid and tau cascade.

acid primary polypeptide chain[17]. CLU is a highly conserved chaperone protein that is found in the cell cytosol under some stress conditions[18]. It is expressed in most mammalian tissues[19], and has been reported to be involved in neurodegeneration and hypoxic-ischaemic neuronal death[20]. Elevated level of CLU has been found in post-mortem AD brains and also in the brains of ApoE4 carriers[21]. CLU is involved in the regulation of Aβ. This has been demonstrated in guinea pig brain perfusion model where apolipoprotein J interacts with the soluble form of Aβ in a specific and reversible manner and forms complexes in the brain, facilitating the transport of soluble Aβ across the blood-brain barrier[22]. In transgenic mouse model (clu^- and clu^+), it has been seen that Aβ deposits in clu^- mice are significantly reduced as compared to clu^+ which indicates that CLU has a role in Aβ fibril formation and neurotoxicity[23]. Plasma CLU level was reported to be associated with rapid clinical progression in AD, suggesting its possible use as a biomarker of AD[24]. GWASs found a significant negative association [odds ratio (OR)=0.86] between an SNP within the *CLU*, rs11136000 and the risk of having AD[14]. This association was found in both APOE4 carriers and non-carriers[15].

ABCA7 is a member of the superfamily of ATP-binding cassette (ABC) transporters, which transport various molecules across extra- and intra-cellular membranes. These transporters are divided into eight distinct subfamilies. *ABCA7* is a member of the ABC1 subfamily[25]. This gene codes for a membrane protein which is expressed in the myelolymphatic tissues, brain and trachea[26]. Analysis of isolated foetal human brain cells has shown that microglia express the highest level of *ABCA7* mRNA[27]. This gene is also involved in AD pathogenesis[28]. It regulates the phagocytosis of apoptotic cell debris inside the brain. Protein products of these loci bind with APOA1 and contribute to

the apolipoprotein-mediated phospholipid efflux mechanism in cells[29].

In stage 1 meta-analysis of *GERAD1*, *TGEN1*, *ADNI* and *EADI1*, evidence was found for the positive association (OR=1.22) of SNP of *ABCA7* (rs3764650) with AD. This has further been proven in stages 2 and 3 meta-analysis[10]. Another SNP variant of *ABCA7*, *i.e.* rs3752246, was found to be associated with AD in stage 2 meta-analysis (OR=1.17). However, association of rs3764650 with *ABCA7* expression was not observed[30].

Genes associated with inflammatory response: *CD33* is located on chromosome 19q13.3 in humans and codes for the 67kDa CD33 protein[31]. CD33 belongs to the sialic acid-binding immunoglobulin-like lectins (Siglecs) family[32]. It is expressed in microglial cells in the human brain[33]. The Siglecs family mediates cell-cell interaction through glycan recognition[34]. They also play an important role in the regulation of functions of innate and adaptive immune cell systems[35]. CD33 is expressed by haematopoietic and phagocytic cells and participates in adhesion processes of human primary immune cells[36]. It appears to inhibit the production of pro-inflammatory cytokines [such as interleukin-1β, tumour necrosis factor alpha (TNF-α)] by monocytes[37]. Being an inhibitory receptor in immune response, it also regulates cell growth and survival and also induces apoptosis[38].

CD33 inhibits Aβ clearance in LOAD[39]. It has been seen that levels of CD33-positive microglial cells are increased in brains of AD patients, and play a direct role in the progression of AD. The *CD33* SNP rs3865444, which confers protection against AD, has been seen to be associated with reductions in both *CD33* expression and insoluble Aβ42 levels in AD brain[33]. Various SNPs of *CD33* such as rs3826656 and rs3865444 are found to be associated with AD[40].

CR1 found on chromosome 1q32 codes for the complement regulatory protein, CR1 or CD35 which is expressed widely on a number of blood cells[41] and can also be found dissolved in the blood plasma[42]. CR1 induces phagocytosis by forming a complex with C3b/C4b. Extracellular domain of CR1 is composed of long homologous repeats (LHRs). Genetic duplications and deletions result in increased number of LHR regions, which result in the formation of four co-dominant alleles of *CR1*. Frequencies of the four alleles vary only slightly between populations[43]. The increased number of LHRs means that the larger alleles have additional C3b/C4b-binding sites[44].

The classical complement pathway has been long known to play a protective role in AD by acceleration of clearance of the Aβ plaques. Aβ interacts with C1q of the classical complement pathway[45]. This results in the activation of the membrane attack complex comprising C3b/C4b, which results in activation of glial cells[46]. *CR1* helps in this process by providing multiple C3b/C4b-binding sites[47]. Lambert *et al*[13] found an SNP variant of *CR1*, rs6656401 (OR=1.12) with a strong association with LOAD.

EPHA1 also known as eph is located on chromosome 7q34.1. The protein product belongs to the tyrosine kinase receptor family[48] and the ephrin receptor subfamily. The ligand for the EphA receptor is ephrin-A, which is anchored to the cell membrane through a glycosylphosphatidylinositol linkage[49]. Eph receptors and ephrins are expressed in endothelial and epithelial cells[50], and guide the migration of cells during embryonic development and also have a role in cytoskeletal organization of neuronal processes[51]. They play a role in synaptic development and plasticity[52]. Additional roles in apoptosis and inflammation exist[53]. AD patients with an allele of *EPHA1* (A allele) having enhanced rate of cerebral metabolism for glucose in the right lateral occipitotemporal gyrus and inferior temporal gyrus may not have hippocampal atrophy[54]. Combined result of the meta-analysis of the GERAD consortia with the ADGC GWAS shows that the rs11767557 SNP of the *EPHA1* gene is negatively associated with AD (OR=0.90)[10].

MS4A encodes several proteins including CD20. This gene family is further divided into at least 12 subgroups from *MS4A1* to *MS4A12*[55]. CD20 expressed by B-lymphocytes[56] forms a hetero-tetrameric complex on the cell membrane that regulates Ca^{2+} influx downstream[57]. This regulation of calcium signalling may have an important role in neurodegeneration and AD pathogenesis[58]. Several members of this cluster (such as MS4A1, MS4A2 and MS4A4B) have an important role in immunity[59]. MS4A4B appears to have a role in Th1 development, CD8+ memory T-cell function and modulation of regulatory T-cell signalling[60]. MS4A2 mediates interactions with IgE-bound antigens that lead to cellular responses such as the degranulation of mast cells[61].

Meta-analysis data of GWAS by ADGC suggested two SNPs of the *MS4A* gene cluster: rs610932 and rs670139 to be associated with LOAD[10]. Another independent GWAS study on the Spanish population revealed the association of rs1562990 SNP of *MS4A* with AD[62].

TREM2 codes for a membrane glycoprotein, consisting of an extracellular immunoglobulin-like domain and a cytoplasmic tail that is involved in receptor signalling complex along with the DAP12 and TYRO binding proteins[63]. This protein functions in the immune response and may be involved in chronic inflammation[64]. In brain cells, TREM2 is primarily expressed on microglia[65,66]. Microglia stimulate the proliferation of CD4+ T-cells, as well as the secretion of TNF and CCL2[67]. Microglia have phagocytic role on amyloid plaques[68]. In a study, reduced phagocytic activity was found in microglial cells to phagocytose β amyloid fragment of AD brain in *TREM2* knockdown mice in comparison with mice expressing *TREM2*[69]. A rare missense mutation (rs75932628) in the *TREM2* results in an R47H substitution which has been found to confer a significant risk of AD. This may be because of the inability of the brain to clear Aβ toxicity[65].

Genes associated with endocytosis: *PICALM* codes for PICALM which can influence the risk of AD through modulation of APP processing via AP2-dependent clathrin-mediated endocytotic pathways, resulting in changes in Aβ level[70]. PICALM initiates clathrin polymerization at sites of coated pit formation[71]. It was seen in cell culture experiments that clathrin-mediated endocytosis (CME) retrieved full length APP from the cell surface, thus promoting the intracellular accumulation of amyloid[72]. In the endosome, full length APP is cleaved in to Aβ by β-secretase (BACE) and this is released into the brain interstitial fluid. Increased number of endosomes formed by CME drives more APP into the cell[73], resulting in an increase of Aβ production[74]. Synaptic vesicles limit the dispersion of neurotransmitter at the pre-synaptic plasma membrane. It was seen in live cell image of hippocampal neurons that synaptic vesicle containing VAMP2 on surface helped in diffusing neurotransmitters along the axonal membrane[75]. *PICALM* may also be involved in directing the trafficking of VAMP2[76]. The SNP of *PICALM* which has been found to be most significantly protective against LOAD is rs3851179 (OR=0.86)[14].

BIN1 codes for Myc box-dependent-interacting protein 1. It is a nucleo-cytoplasmic tumour suppressor adaptor protein[77]. Isoforms of this protein expressed in the central nervous system are involved in synaptic vesicle endocytosis[78]. The *BIN1*is identified as the most important genetic susceptibility locus in LOAD after *APOE*[79]. Higher *BIN1* expression has been reported to be linked with later age at onset and shorter disease duration[80]. Although the mechanisms are still not fully understood, data suggest that *BIN1* affects AD risk primarily by modulating tau pathology. *BIN1* also affects other cellular functions including endocytosis/trafficking, inflammation, calcium homoeostasis and apoptosis[79]. Seshadri *et al*[15] combined the data from CHARGE, TGEN, EADI1 and GERAD1 groups and analyzed by a three-stage sequential meta-analysis. They reported the association (OR=1.13) of the *BIN1* SNP rs744373 with LOAD[15]. Another independent study- The Washington Heights-Inwood Columbia Aging Project and the Estudio Familiar de Influencia Genetica de Alzheimer study also showed positive associations of the *BIN1* SNP rs7561528 with LOAD in the ε4 carrier state[81].

CD2AP codes for CD2-associated protein which is a scaffolding molecule that regulates the actin cytoskeleton[82]. It plays a role in receptor-mediated endocytosis. *CD2AP* contributes to APP metabolism and subsequent Aβ generation[83]. It regulates the encounter of APP and BACE1 in axonal and dendritic endosomes[84]. GERAD1, EADI1, deCODE and AD-IG GWAS datasets observed independent evidence for the association of *CD2AP* gene loci with AD (OR=1.11 for rs9349407 SNP)[10].

Racial variation of Alzheimer's disease susceptibility genes

Survival after the diagnosis of AD varies amongst different races, ranging from 3 to 9 years. African American and Latino AD patients have better survival than Caucasian patients and genetic background plays an important role in the progression of AD[85]. Most GWASs and replication studies of AD have been done in populations of European descent, and non-European genetic studies of new AD-susceptibility loci are limited. Studies that evaluated the association of *CLU* and *CR1* with AD in Asian populations are limited[86]. Many AD-associated SNPs of *CLU, PICALM* and *BIN1* were not necessarily identical in Caribbean Hispanic individuals compared with a European American data set[81]. Meta-analytic data showed that *CLU*, *PICALM* and *CR1* were associated with LOAD in Caucasians subjects, but a study found that investigated SNPs of *CR1*, *CLU* and *PICALM* were not associated with AD in a Polish population[87]. A study found that in the Korean population, the *PICALM* is the only AD susceptibility loci in addition to *APOE*[88]. ADGC assembled multiple data sets for meta-analysis representing African American older subjects. The data showed another SNP (rs115550680) of *ABCA7* (OR=1.79) was associated with AD in comparison to European ancestry[89].

Potential therapeutic implications of GWAS loci

Novel loci may exert their effects in a number of pathways such as oxidative balance, protein metabolism, cholesterol metabolism and synaptic function[90]. Genes with moderate to large effects on LOAD risk are valuable targets for therapeutic development. Neuroinflammation is both a cause and a consequence of AD and treatment with anti-inflammatory agents is likely to be successful if initiated before the onset of neurological symptoms[91]. Similarly, on the lipid metabolism front, the CLU protein may be targeted to reduce AD risk[92]. Genes associated with endocytosis and synaptic functions are *BIN1, PICALM* and *CD2AP*. Modulating these at the gene-expression level using siRNA or antisense techniques is a valid approach.

New developments

While the present review focuses on the most established gene loci involved in AD pathogenesis as suggested by GWAS, several newer loci have made a foray into the AD scene. Under the supervision and support of International Genomics of AD project, two-stage meta-analysis identified 11 loci which are HLA-*DRB5-DRB1* gene, *SORL1*, *PTK2B*, *SLC24A4*, *ZCWPW1*, *INPP5D*, *MEF2C*, *CELF1*, *NME8*, *CASS4*, *FERMT2* genes, with suggestive evidence of association with AD[93]. The Table represents newer loci involved in AD pathogenesis as suggested by GWAS with tentative pathogenic mechanisms[94].

Table. New susceptibility gene loci of Alzheimer's disease

Genes as risk factors				
Gene	Location	Function	SNP variant	OR
HLA-DRB5-HLA-DRB1	Chromosome 6	Binds with antigen peptide of APC, encoding MHC-II, associated with immune-competence and histocompatibility	rs9271192	1.11
PTK2B	Chromosome 8	Involved in calcium-induced regulation, regulate neuronal activity, involved in the induction of hippocampal CA1 in memory formation, mediates responses to cellular stress	rs28834970	1.10
INPP5D	Chromosome 2	Affecting multiple signaling pathways, interact with CD2AP	rs35349669	1.08
CELF1	Chromosome 11	Involved in mRNA editing, and translation, may affect long-term neuronal viability in Alzheimer's disease	rs10838725	1.08
FERMT2	Chromosome 14	Expressed in the brain, involved in tau-mediated toxicity via protein localization	rs17125944	1.14
Protective genes				
SORL1	Chromosome 11	Gene encoding sortilin-related receptor, expressed in the central nervous system, plays roles in endocytosis and sorting, and formation of beta-amyloid	rs11218343	0.77
SLC24A4-RIN3	Chromosome 14	Role in calcium transport during amelogenesis, involved in iris development, protein that may be connected to tau-mediated pathology	rs10498633	0.91
ZCWPW1	Chromosome 7	Encoding zinc finger, modulates epigenetic regulation, in mice affects brain size and neurites elongation	rs1476679	0.91
MEF2C	Chromosome 5	Mutations associated with severe mental retardation, stereotypic movements, epilepsy and cerebral malformation, essential role in hippocampal-dependent learning, crucial for normal neuronal development, distribution, and electrical activity	rs190982	0.93
NME8	Chromosome 7	Responsible for primary ciliary dyskinesia type 6	rs2718058	0.93
CASS4	Chromosome 20	Involved in actin dynamics, binds to CMS in Drosophila	rs7274581	0.88

SNP, single-nucleotide polymorphism; APC, antigen-presenting cells; MHC, major histocompatibility complex; OR, odds ratio; CMS, cas ligand with multiple SH3 domain; CA1, Cornu Ammonis

Source: Refs 93, 94

Conclusion

GWASs have revealed the association of new gene loci with AD. The first few identified SNPs from GWAS suggest the involvement of different associated pathways with pathogenesis of AD although the exact mechanisms remain unknown. Modification and advancing the research in these pathways may lead to therapeutic intervention for AD. Many of these GWAS loci may serve as biomarkers of AD. The search for additional genetic risk factors requires more large-scale meta-analysis of GWAS and enhanced statistical power as well as replicating these findings at the molecular level. Exciting times await us in AD genetic research and newer paradigms might open in the near future.

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