

REVIEW ARTICLE

Genes associated with Alzheimer Disease

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Abstract

Alzheimer's disease (AD) is one of the neurodegenerative disorders, characterized by gradual loss of memory, decline in other cognitive functions and decrease in functional capacity. Increasing age and a positive family history of dementia are the definite risk factors of the disease. Molecular analysis of families with early onset of AD (EOAD) has made it possible to identify dominantly acting mutations in genes such as amyloid precursor protein precursor protein and presenilin 1 and 2 (PSEN 1 & PSEN 2). However, the etiology of the late onset of AD (LOAD) is less straightforward than EOAD. The availability of novel genetic tools such as high throughput methods for single nucleotide polymorphism (SNP) genotyping, which simultaneously genotype hundreds of thousands of SNPs using a single SNP array, may facilitate the discovery of genetic influences in the disease. These genome-wide association studies have great potential to revolutionize our ability to identify additional genes that contribute to the risk of sporadic AD. It is hoped that the identification of individuals with a high genetic risk of AD will help to develop more rational, cost effective and novel prevention strategies and therapeutic approaches.

INTRODUCTION

Alzheimer disease (AD) is a neurodegenerative disease caused by gradual loss of memory and decrease in functional capacity. In due course of time the patient is unable to look after himself and becomes a burden on the family. AD is an age dependent disorder and its prevalence has been reported to increase with advancing age. About 10% in individuals above 65 years and 50% above 85 years of age suffer from AD. Epidemiological studies have shown that increasing age and a positive family history of dementia are the only definite risk factors for the disease.¹ Having an AD-affected mother confers a greater risk than having an AD-affected father.² Women are known to be at greater risk of developing AD and this has been associated with postmenopausal estrogen decline.³ Cardiovascular disease patients and individuals with previous head injury show higher AD risk than normal controls. A family history of AD in the first degree relatives has been shown to be associated with a fourfold increase in risk in developing the disease, suggestive of a genetic involvement in AD pathogenesis.⁴ This relative risk figure grows higher with increasing numbers of affected first-degree relatives.¹

The characteristic features seen in the brain of AD patients are the presence of a large number of neuritic plaques, neurofibrillary tangles and cerebrovascular amyloid deposits.⁵ Neurofibrillary tangles are not specific to AD and therefore are not considered essential for the diagnosis.⁶ There is an extensive neuronal loss and synaptic changes in the cortex and hippocampus. Individuals with Down syndrome (trisomy 21) in general, and specifically with translocation (21q), are at high risk for developing AD.⁷ Down syndrome individuals with dementia have autopsy findings of typical AD pathologies such as amyloid plaques and neurofibrillary tangles similar to those seen in an aging brain.

GENETICS OF ALZHEIMER DISEASE

AD affects around 15 million people around the world and by 2040 the figure is expected to rise to 80 million.⁸ The etiology entails a large genetic component. The most common form of AD (90%) in the population occurs sporadically and is late in onset, usually occurring after 65 years of age.⁹ Familial AD (FAD), or early onset AD (EOAD)

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accounts for 10% of the cases and manifests itself before the age of 65 years.⁹

The mode of inheritance of AD differs in each type. Only about 10% of FAD cases are inherited in an autosomal dominant pattern. The rest of the FAD as well as LOAD cases have a non-mendelian complex mode of inheritance.¹⁰ Dominantly acting mutations in genes like amyloid precursor protein (APP), presenilin -1 (PSEN1) and presenilin -2 (PSEN2) have been found to be associated with AD.¹¹⁻¹³ These mutations have been reported to cause AD that presents early in life, often before 65 years of age (early-onset AD or EOAD). In contrast, the etiology of the late onset AD (LOAD) is less straight forward than EOAD. Although linkage approaches are difficult to conduct in late life disorders, there have been suggestions that contributory genes might reside on chromosomes 9q and 10q.¹⁴ LOAD does not seem to be the result of single gene mutations but rather the interaction of multiple genetic and environmental risk factors. Variants at only a single gene locus, the apolipoprotein E (APOE) locus on chromosome 19, have been confirmed as modulators of susceptibility to the common late onset of AD. Risk for late-onset AD seems to be modulated by various other genetic variants with relatively low penetrance but high prevalence.¹⁵ Although inheritance of known genes that predispose to AD accounts for only 5-10% of all clinically presented cases the heritability for AD has been estimated to be 79%.¹⁶

A. GENES ASSOCIATED WITH THE LATE ONSET ALZHEIMER DISEASE

1. Apolipoprotein E (APOE)

The apolipoprotein E (apoE denotes protein, APOE denotes gene) is a lipid transport protein in the brain. In humans, three different alleles $\Sigma 2$, $\Sigma 3$ and $\Sigma 4$ exist giving rise to three common isoforms, E2, E3 and E4, with frequencies of 7%, 78% and 15% respectively.¹⁷ It seems that apoE has a number of roles in the central nervous system and these are being elucidated using transgenic mice. APOE has been reported to mediate neuronal protection, repair and remodeling via a number of mechanisms which include antioxidant effects, interactions with estrogen and modulation of synptodendritic proteins. APOE $\Sigma 4$ allele is a major risk factor for AD and also underlies a genetic susceptibility to the effects of several forms of brain injury.^{18,19} Teasdale *et al* carried out a prospective clinical study of APOE $\Sigma 4$

allele bearing patients who sustained a head injury.²⁰ These individuals had a poor initial response to the injury and a poorer clinical recovery than non-APOE $\Sigma 4$ individuals. A history of head injury and possession of APOE $\Sigma 4$ each increase the chance of developing AD in later life by almost three fold.²¹ $\Sigma 2$ allele seems to be slightly protective against AD, whereas APOE $\Sigma 3$ is intermediate in risk.²² Having one or two APOE $\Sigma 4$ alleles increases the risk of LOAD and also lowers the average age of onset with a gene dosage effect. Meta-analysis shows that the risk of AD increases by three times in heterozygotes and by 15 times in homozygotes.²³ Undoubtedly, there is clear clinical evidence that APOE genotype has an important role to play in the pathophysiology of AD, yet the molecular mechanisms for the disease promoting effect has been difficult to pinpoint. It has been suggested that the formation of insoluble amyloid from soluble A β is promoted by APOE $\Sigma 4$, which acts as a pathological chaperone. Transgenic mice with human APOE $\Sigma 4$ develop a greater amyloid burden in brain in comparison with mice co-expressing APOE $\Sigma 3$. However, the close relationship between APOE and Alzheimer disease risk is highlighted by the observation that transgenic mice over-expressing familial AD mutation on an APOE -/- (null background) show much less amyloid deposition in comparison with those on very low to normal APOE +/- (wild type) background.²⁴ Many studies have shown that APOE $\Sigma 4$ allele accounts for most of the genetic risk in sporadic AD.²⁵ Thus, the contribution of other candidate genes seems to be minor.⁹

In addition to APOE tri-allele polymorphism, a genetic variant of the APOE promoter has also been proposed to be implicated in the pathogenesis of sporadic Alzheimer's disease.²⁶ Based on the data obtained from transfection assays, A/T polymorphism of the APOE promoter has been reported where the A-491 allele is associated with higher constitutive APOE promoter activity than T-491 allele.²⁶

2. Dynamin (DNM)

Another gene, dynamin 2 (DNM2), has been found to be associated with LOAD in a Japanese population. However, the association has been reported to be especially significant in non-APOE- $\Sigma 4$ carriers.²⁷ In non-APOE- $\Sigma 4$ carriers two SNPs have been reported to be associated with LOAD. β -amyloid, which is deposited in the AD brain interacts with dynamin 1 gene. Dynamin 2 gene is homologous to dynamin 1 and is located on

chromosome 19p13.2 where a susceptibility locus has been detected by linkage analysis. Expression of dynamin 2 as well as dynamin 1 is down regulated by β -amyloid in hippocampal neurons²⁸, suggestive of the involvement of dynamin proteins in the cascade of neurodegeneration caused by β -amyloid.²⁸ Dynamin binding protein (DNMBP) gene located on chromosome 10 has also been associated with LOAD.²⁹ However, the mechanism by which the DNMBP gene causes the disease is not clear. Researchers have observed a decrease in the expression of hippocampal DNMBP mRNA but it is not clear whether the decrease in the DNMBP expression is the cause or outcome of AD.

B. GENES ASSOCIATED WITH EARLY ONSET ALZHEIMER DISEASE

1. Amyloid precursor protein (APP)

The first clue pointing to the involvement of chromosome 21 in AD came from the observations that Down syndrome patients develop the clinical and pathological features of AD if they live over 30 years. The gene coding for the amyloid β precursor protein (β APP) was isolated and localized on chromosome 21 in the region 21q11.2-q21.3.³⁰ This discovery helped researchers establish association between APP gene and AD. The APP is a transmembrane protein which bears a long extracellular N-terminal segment and a short intracellular C-terminal tail. APP is cleaved by β - and γ -secretase in a sequential manner, to yield $A\beta$ peptides (including $A\beta_{40}$ and $A\beta_{42}$). The $A\beta$ peptides are the major components of the amyloid plaques found in AD patients. In 1990, Frangione and colleagues, after sequencing the exons 16 and 17 encoding the $A\beta$ domain, revealed the first pathogenic mutation in APP³¹, which caused hereditary cerebral hemorrhage with amyloidosis in a Dutch family.³¹ These two exons were subsequently sequenced in EOAD families which led to the discovery of the first EOAD mutation.³² Currently 27 mutations have been described in the APP gene and these mutations are clustered near the $A\beta$ sequence on APP.³³

The APP gene contains 18 exons and the alternative splicing of exons 7, 8 and 15 gives eight different transmembrane isoforms. The APP isoforms, which bear exon 15, are mainly expressed by neurons, and are more amyloidogenic and release much more $A\beta$ in comparison to non-neuronal APP isoforms.

The first mutations discovered in familial AD were the missense mutations in APP and these mutations have been found to cluster near the β - and γ -cleavage sites that release $A\beta$ from APP. The location of these mutations is suggestive of a disease mechanism favoring amyloidogenic (producing $A\beta$) over non-amyloidogenic APP catabolism by γ -secretase (a toxic gain of function). The missense mutations promote $A\beta_{40}$ or $A\beta_{42}$ (or both) generation. The pathogenic APP mutations within $A\beta$ sequence result in a much greater $A\beta$ /amyloid burden within blood vessels in addition to parenchymal amyloid deposits found in AD.³⁴

The transgenic mice for human mutant APP gene have been generated and these developed age dependent behavioral decline in learning and memory tasks as well as progressive central nervous system (CNS) degeneration, and also $A\beta$ amyloid accumulation similar to human AD.³⁵ However, neurofibrillary tangles and neuronal loss do not develop in these mice and thereby these are partial AD-like models of the human disease.³⁶

There is increasing evidence that variants in the promoter of the APP gene could up-regulate the APP gene expression leading to amyloid beta protein accumulation and thereby contribute to the development of Alzheimer's disease. A study in Chinese Han population showed APP promoter polymorphisms increase APP expression which in turn was associated with the development of sporadic AD.³⁷ Recently, it has been reported that not only mutations but also the duplication of APP locus causes autosomal dominant early-onset AD with cerebral amyloid angiopathy (CAA).^{33,38} These researchers found abundant parenchymal and vascular deposits of amyloid- β peptides in the brains of individuals with APP duplication in comparison to the healthy subjects. The duplications were detected using Quantitative Multiplex PCR of short fluorescent fragments (Q-MPSF). To further strengthen the view that these duplications were present in affected subject, fluorescence in situ hybridization (FISH) was performed on peripheral blood lymphocytes from two affected subjects, which showed three signals in interphase nuclei and a larger-sized signal on one copy of chromosome 21. Such an observation was consistent with a segmental duplication involving the APP locus.³⁸ Therefore, not only mutations but also the gene dosage alterations may be involved in the etiology of the neurodegenerative disorders like AD.

2. Presenilin 1 & Presenilin 2 (PSEN-1 & PSEN-2)

None of the early onset familial AD pedigrees had mutation in APP, indicating the involvement of other genes.¹ These families have been shown to bear mutations in two other genes namely presenilin 1 (PSEN-1 or presenilin dementia) and presenilin 2 (PSEN-2). These genes have been found to be located on chromosome 14¹² and chromosome 1¹³ respectively. Rare early onset familial forms of AD mostly show mutations in PSEN-1. Studies involving transgenic mice with mutant human PSEN-1 and APP genes showed accelerated amyloid deposition in brain compared to transgenic mice expressing only mutant human APP. Obviously, PSEN-1 mutations result in increased generation of A β 42 from APP. The increased ratio of A β 42/A β 40 suggests that the mutations alter the position of the γ -secretase cleavage of APP.³⁹ PSEN-1 mutations might also have some other detrimental effects in promoting AD pathologies, such as increasing susceptibility of neurons to apoptosis.

The PSEN-1 gene contains 10 protein-coding exons and 2 to 3 additional exons encoding the 5'-untranslated region. Alternative splicing of exon-8 in this gene has been reported.^{12,13} The major RNA transcripts from PSEN-1 gene are 2.7 and 7.5 kb and these are expressed in different regions of the human brain, skeletal muscle, kidney, pancreas, placenta and heart. The PSEN-1 is a serpentine protein consisting of 467 amino acids with nine transmembrane domains. The protein is localized in the nuclear envelope, endoplasmic reticulum and Golgi apparatus in mammalian cells.⁴⁰ More than 41 mutations in the PSEN-1 gene have been identified and most of them have been found to lie in exon 5 and 8.⁴¹ Although the majority of the mutations are missense mutations, with the change of a single amino acid, some of these are deleterious. The deleterious mutations in these two gene clusters are related to different ages of disease onset. Patients having mutations in exon 8 have higher mean age of onset than those with mutations in exon 5.

The PSEN-2 gene contains 10 protein-coding exons and two other exons encoding the 5'-untranslated region. The PSEN-2 is also a serpentine protein having 448 amino acids with 6-9 transmembrane domains. The PSEN-2 has a structure similar to PSEN-1, but the mutations are located in different codons from that of the PSEN-1.

In the in-vitro cell lines, cells transfected with PSEN-1 or PSEN-2 gene mutations show

deposition of A β 42. Transgenic mice bearing human PSEN-1 mutation have twice as much soluble mouse A β 42 in their brains compared with normal mice.^{42,43}

Knocking of both PSEN-1 and PSEN-2 genes eliminated γ -secretase cleavage completely.⁴⁴ Therefore, Presenilins are essential for this proteolytic function^{45,46}, the disruption of which might also contribute to familial AD pathogenesis.⁴⁷

3. Presenilin enhancer-2 (PSENEN-2)

Presenilin enhancer-2 (PSENEN) is a fundamental component of the γ -secretase protein complex involved in β -amyloid precursor protein (β APP) processing, a critical step in the amyloidegenic pathway responsible for triggering AD. The gene (OMIM-172341) on chromosome 19q13.12 is 1.4 kb in length and is composed of 4 exons. The first exon is non-coding. The protein encoded by the gene bears 101 amino acids and depicts a U-shaped structure. It has been reported that PSENEN stabilizes presenilin N- and C-terminal domains after their autocatalytic activation and that C-terminal has been reported to be essential in the interaction with the γ -secretase complex. PSENEN also regulates apoptosis in zebra fish neuronal system and embryo development.^{48,49}

A novel PSENEN coding mutation (S73F) has been reported in a woman with complaints of memory loss and family history of AD. The biochemical effects of this mutation on A β (1-42) generation have been studied using skin primary fibroblasts cultured from the mutation bearing members of this kindred. The pathogenic role for this substitution is not clear and therefore, further studies are required to evaluate its role in the development of AD.⁵⁰

4. Tau

The characteristic feature of AD is the degradation of selected population of nerve cells that develop filamentous inclusions prior to degeneration. These neuronal inclusions are made of the microtubule-associated protein 'tau' in a hyperphosphorylated state. The abundant tau inclusions are not limited to AD only, but are also characteristic of frontotemporal dementias, progressive supranuclear palsy and corticobasal degenerations. The discovery of mutations in the tau gene linked to chromosome 17 (FTDP-17) in familial frontotemporal dementia, has thrown light on AD mechanisms.⁵¹ Tau is a phosphoprotein found mainly in neurons in the peripheral and

central nervous system, where it is associated with microtubule binding and assembly in axons that are necessary for axoplasmic transport.⁵²

The major physiological function of tau is to promote microtubule assembly and to bind to microtubules thus stabilizing it. A number of tau isoforms are produced from a single gene by alternative mRNA splicing. Six tau isoforms ranging from 352 to 441 amino acids in length have been found to be expressed in adult human brain. These isoforms differ from each other by the presence or absence of three exons, and the longest human brain tau isoform has 11 exons.^{53,54} There are three to four tandem repeats of 31/ 32 amino acids located in the carboxy-terminal half, each containing a characteristic Pro-Gly-Gly-Gly motif. Experiments with tau proteins expressed in *E. coli* have shown that it is the carboxy terminal repeats and some adjoining sequences which constitute microtubule binding domains.⁵⁵ Known mutations have been found to either reduce ability of tau to interact with microtubules or cause an overproduction of tau isoforms with aberrant microtubule binding repeats. These lead to the assembly of tau in AD brain.

C. OTHER GENES ASSOCIATED WITH ALZHEIMER DISEASE

5, 10-Methylenetetrahydrofolate reductase

5, 10-Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in homocysteine metabolism. A common polymorphism C677T in the gene encoding this enzyme has been shown to be associated with raised homocysteine concentrations. Recent studies have focused on the homocysteine levels in blood in relation to cognitive impairment and AD. The mean plasma homocysteine level has been found to be significantly higher in patients with AD.⁵⁶ Three case control studies have also correlated AD with high homocysteine levels.⁵⁷⁻⁵⁹

Cell division cycle 2 gene (cdc2 or P34)

Many studies have suggested linkage between AD and two markers i.e. D10S1225 and D10S583 present on chromosome 10, which are suggestive of the presence of an important susceptibility gene for AD on the long arm of the chromosome 10.^{60,61}

The cell division cycle 2 (cdc2 or P34) gene is located on chromosome 10q21.1, which is approximately 2Mbp away from the marker

D10S1225, which has been shown to be linked to the causation of AD.⁶¹ Cdc2 is a protein kinase which is involved in the regulation of cell cycle and also in neuronal differentiation.⁶² Cdc2 has been reported to be involved in abnormal phosphorylation of tau in AD and it also phosphorylates APP at Thr 668 and β -amyloid at Ser 26.⁶³⁻⁶⁵ Johansson *et al* sequenced coding exons, flanking intronic sequences and the promoter region of the Cdc2 gene and found three new single nucleotide polymorphisms (SNPs). Homozygosity for one of the SNPs (Ex6+71/D) was found to be more frequent in both AD and frontotemporal dementia, suggesting that Ex6+71 allele is associated with these two types of dementia.⁶⁶ However, further studies are warranted to confirm these findings.

Matrix metalloproteinase-9 (MMP-9)

AD is characterized by the accumulation of extracellular A β deposits which are generated by the proteolytic cleavage of APP. The accumulation of A β peptide is the key to disease pathogenesis. Most of the studies on its catabolism suggest the involvement of metalloproteinases such as neprilysin, endothelium converting enzyme or matrix metalloproteinases (MMP) in the degradation of the amyloid peptide.⁶⁷ MMP-9 belongs to a wide family of zinc dependent proteinases which have been implicated in several diseases of the cardiovascular as well as the nervous systems. It has been reported that AD patients show an increased expression of MMP levels; in particular MMP-9.⁶⁸ The latent form of MMP-9 can be processed proteolytically to an active form by serine proteases such as elastase or cathepsin G and also by superoxide anions. In fact the enzyme was predominantly found in the latent or proenzyme form in the proximity of extracellular amyloid plaques.⁶⁹ Further, it has been shown that the active enzyme could process A β , and the major cleavage site is Leu34-Met-35 chemical bond inside the transmembrane domain of the peptide suggesting a protective role of MMP-9 in dementia through degradation of A β .⁶⁹ The gene encoding MMP is located on chromosome 20q11.2-q13.2. Common C/T polymorphism occurs at position-1562 of the gene. The genotype CC leads to promoter low-activity while CT and TT genotypes lead to the high activity. Pollanen *et al*⁷⁰ and Helbeeque *et al*⁷¹ have reported a small protective effect of the MMP-9 promoter polymorphism on the risk of dementia in the individuals of a French Caucasian

population, which did not bear APOE ϵ 4 allele. Of course, the study needs to be replicated in other well characterized populations.

SORL1

Recent studies have shown that misdirected protein transport might contribute to the development of AD, particularly in older people. A multi institutional team linked a gene called SORLA or LR11, which is thought to be involved in regulating protein movements through the cell. Mutations in SORL1 lead to a decrease in the protein product of the gene which increases the risk of developing the disease. Moreover, when the SORL1 protein is lacking, the APP protein is trafficked off to the compartments in cell where the enzymes BACE and PSEN-I snip out and release neurotoxic β -amyloid. If confirmed, the SORL 1 will be added to the list of genes responsible for developing AD and this might lead to better ways of identifying and also possibly treating those who are at risk of developing the disease.⁷²

The genes associated with the AD and the cascades of events leading to clinical manifestations of AD have been summed up in Figures 1 and 2 respectively.

GENOME-WIDE ASSOCIATION STUDIES

An extension of the classical patient-control studies are genome wide association studies (GWAS). These provide a non-bias approach to examine the effect of genetic variation in a specific trait. A number of genome wide association studies in AD have been reported. However, most of these studies were underpower in detecting genetic variants with modest effects because of limited sample size. One of the first convincing genome wide association study has demonstrated a variant in the X-linked gene PCDH11X to be associated with LOAD in female homozygote carriers.⁷³ Recently two independent GWAS have convincingly established three additional genetic risk factors for LOAD.^{74,75} The strongest association signal (by a wide margin) in both these studies has been found at APOE. In addition to this, both the studies report genome-wide significant association for rs11136000, located in the clusterin (CLU) gene. Lambert *et al.*⁷⁴ also found a genome-wide significant association for a linkage disequilibrium block within the boundaries of the complement component (3b/4b) receptor 1 (CR1) gene. However, this SNP did not meet genome-wide significance in the study of Harold

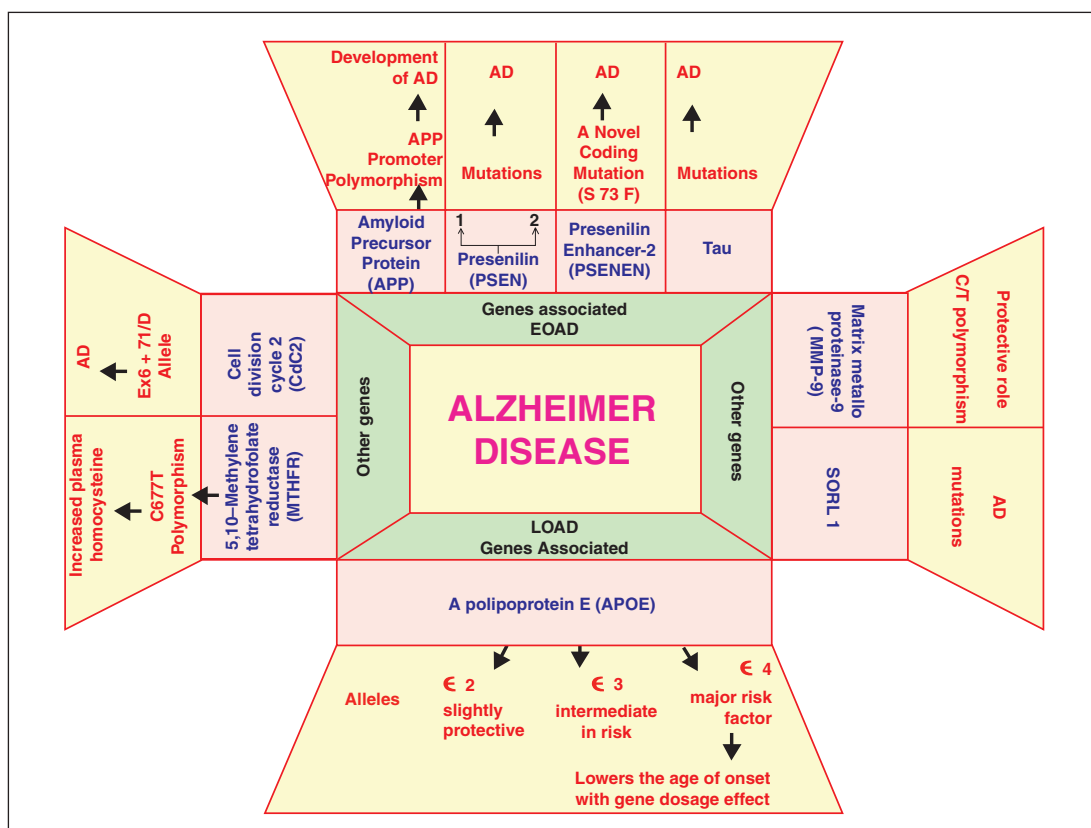


Figure 1. Genes associated with Alzheimer disease

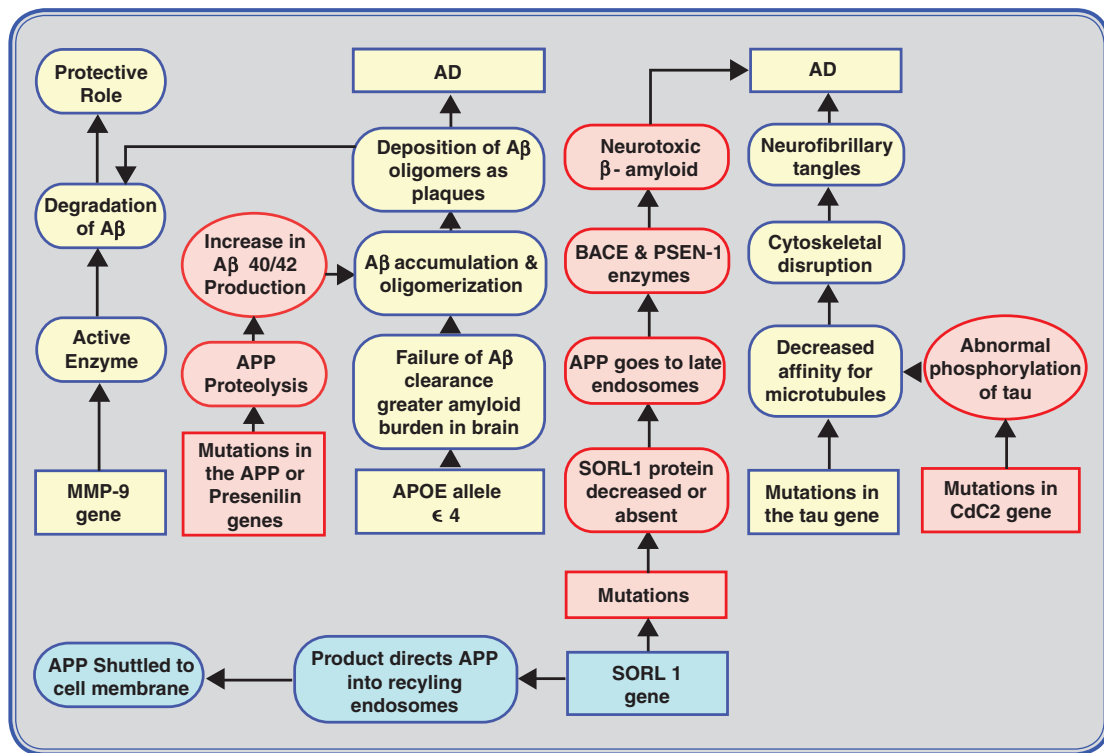


Figure 2. Cascades of events leading to clinical manifestations of Alzheimer disease

*et al.*⁷⁵, who found a genome-wide association for rs3851179 in the phosphatidylinositol-binding clathrin assembly protein gene (PICALM). In short, these studies convincingly report the associations of genetic variants at *CLU*, *PICALM* and *CR1* to *LOAD*.

CONCLUDING REMARKS

AD ranks fourth as the cause of death. It is a progressive, irreversible brain disorder with no known cure. Genetic causes of *AD* are known but only for a small proportion of familial *AD* patients. As far as the majority of sporadic *AD* patients are concerned, genetic causal factors are still unknown. There is a lack of understanding of pathophysiology of the disease and there are no early detectable biomarkers for sporadic *AD*. Mitochondrial mutations seem to be critical for the “initiation” of late onset of *AD* disease and therefore mitochondrial mutations might turn out to be good markers of *AD*. Three genome-wide association analyses, using case-control designs have detected highly significant association at *APOE* locus.⁷⁶⁻⁷⁹ Recently two genome-wide association studies detected highly significant associations of genetic variants at *CLU*, *PICALM* and *CR1* to *LOAD*.^{74,75} *LOAD* probably results

from the combined effects of variations in a number of genes as well as environmental factors.

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