GENE MUTATIONS IN APOE, Aβ, PRESINILIN GENES AND ALZHEIMERS DISEASE

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ABSTRACT
Alzheimer disease (AD) is a genetically complex disorder. Mutations in genes ApoE, Presenilin 1 & 2 and amyloid precursor protein may be implicated in the pathogenesis of the disease. In this review, we discuss current advances in AD genetics, implications of the known AD genes, on the clinical diagnosis, treatment and genetic counseling of patients and families with early- and late-onset AD.

KEYWORDS: Alzheimer Disease; ApoE, Presenilin 1 & 2 and Amyloid Precursor Protein.

INTRODUCTION
Alzheimer’s disease (AD), the most common progressive neurodegenerative disease results from irreversible loss of neurons, particularly in the cortex and hippocampus. A major focus of AD research has been to understand the genetic etiology of AD and its relationship to AD neuropathology. The key neuropathological features of AD are abundant neurofibrillary tangles composed of hyperphosphorylated tau protein and senile plaques made of β-amyloid (Aβ). Over 2 decades of genetic research has resulted in a better understanding of the pathway leading to the accumulation of insoluble protein, Aβ. The accumulation of Aβ is considered a central component in the pathogenesis of AD and has been associated with the 3 autosomal dominant, deterministic genes known to be involved in Early Onset Alzheimers Disease (EOAD), Presenilin 1 (PSEN1), Presenilin 2 (PSEN2), and Amyloid Precursor Protein (APP). A fourth gene,
apolipoprotein E (APOE), has been confirmed as a susceptibility factor/risk factor for late onset AD (LOAD). As our understanding of the genes involved in the disease evolves, so may the ability to identify at-risk individuals who would be eligible for early treatment and prevention.

**Genes and Alzheimer's disease**

AD is a progressive neurodegenerative disorder characterized by a gradual destruction of brain cells that results in a progressive decline in mental functions (e.g., memory or ability to learn, reason, make judgments, and communicate), culminating in severe dementia. At advanced stages, this decline leaves the patient unable to carry out daily activities, such as dressing, eating, or personal hygiene. Moreover, the patient’s personality and behavior may change so that he or she becomes more anxious, agitated, suspicious, or even aggressive.

Alzheimer’s disease (AD) is neuropathologically characterized by extracellular senile plaques containing amyloid beta (Aβ) and intracellular neurofibrillary tangles containing hyperphosphorylated tau protein. Mendelian forms of the disease are caused by mutations in the amyloid precursor protein (APP) gene and the presenilin 1 and 2 genes (PSEN1 and PSEN2 respectively). While only apolipoprotein E (APOE) has been clearly identified as a susceptibility gene in the more common form of AD, data from recent genome-wide association studies has implicated several other common risk variants.[1-8] Intracellular neurofibrillary lesions composed of the microtubule-associated protein tau and extracellular amyloid plaques assembled from the Aβ peptide are the pathological hallmarks that characterize Alzheimer’s disease. Unlike amyloid plaques, the spatial and temporal appearance of tau lesions correlates closely with the progression of disease. Therefore, identifying the underlying mechanisms of tau lesion formation is of critical importance for understanding the disease. Mutations in the MAPT gene encoding tau protein lead to neurofibrillary lesions, neurodegeneration, and cognitive decline. Although it depends on the location within the coding sequence, most tau missense mutations have been shown to increase aggregation propensity. In Alzheimer’s disease, tau protein associated with neurofibrillary lesions is mostly found to be hyperphosphorylated. Hyperphosphorylation of tau acts to increase the free tau concentration available for aggregation. Postmortem histopathological examination of amyloid plaques and neurofibrillary tangles in the brain is still the only method to
definitively confirm the diagnosis of AD. Because tau can serve as a surrogate marker for neurodegeneration in AD, detection of tau aggregates is of practical importance for disease.

The AD have two forms: the rare early onset Alzheimer's disease, where first symptoms appear before the age of 65; and the much more common late onset Alzheimer's disease, where typically the first symptoms develop after this age. These two types of Alzheimer's disease generally have different patterns of genetic inheritance.

**Early onset Alzheimer's disease**
This form of Alzheimer's tends to cluster within families, sometimes with several generations affected, in which case it is called familial disease. In some of these cases, early onset Alzheimer's is caused by mutations in one of three genes. These three genes are the amyloid precursor protein gene (APP) and two presenilin genes (PSEN-1 and PSEN-2). People with any of these extremely rare mutations tend to develop Alzheimer's disease in their 30s or 40s.

**Amyloid Precursor Protein**
The amyloid precursor protein gene (APP, OMIM 104,760, chromosome 21q21) encodes a ubiquitously expressed, integral Type I membrane glycoprotein that exists as different alternatively spliced isoforms, with three predominant ones: APP751, APP770, and APP695, the latter being the main isoform found in the brain. The proteolytic processing of APP results in the production of different peptides (including Aβ), after a series of secretase cleavages, and occurs through two mutually exclusive pathways: the amyloidogenic pathway (fundamentally considered as the pathogenic pathway) and the non-amyloidogenic or constitutive pathway. The identification of Aβ as a metabolic product of APP and the reports of AD families harboring APP causative mutations led to the general concept that Aβ is a key player in the development of AD, and that EOAD mutations are influencing the properties or ratios of the different Aβ isoforms in the brain. Dominant mutations in APP are, however, a rare cause of AD with an estimated frequency of 16% of familial EOAD patients. More recently, two mutations in APP (A673V and E693A) have been reported to cause AD only in the homozygous state in families with apparently recessive modes of inheritance.
Presenilin 1 & 2
The presenilin 1 (PSEN1, OMIM 104,311, chromosome 14q24.3) and presenilin 2 (PSEN2, OMIM 600,759, chromosome 1q31-q42) genes have a very similar genetic structure and encode two proteins expressed in a multiplicity of tissues including the brain, with higher levels in the cerebellum and the hippocampus and a primarily neuronal expression.\cite{17, 18} These are highly homologous, sharing an overall amino acid sequence identity of 67%. Hydrophobicity plots predicted these to be integral membrane proteins\cite{18} most likely adopting a transmembrane structure containing nine segments with a hydrophilic intracellular loop region.\cite{19, 20} PSENs are important components of the multimeric gamma-secretase complex and are predominantly located in the endoplasmic reticulum and Golgi compartments, clearly suggesting their involvement in protein processing.\cite{21, 22} The first disease causing mutations in PSEN1 and PSEN2 were identified in 1995.\cite{18, 23} Today, 175 pathogenic mutations and seven variants nonpathogenic or with unclear pathogenicity have been identified in PSEN1. PSEN2 harbors fewer mutations: 14 pathogenic mutations and nine variants nonpathogenic or with unclear pathogenicity. The PSENs mutation range encompasses mainly missense mutations scattered all over the proteins, with some clustering around transmembrane domains.\cite{24, 25}

Late onset Alzheimer's disease
A small but growing number of genes have now been identified which affect - to different degrees – the chances of developing late onset Alzheimer's. The effects of these genes are subtle, with variations acting to increase or decrease the risk of developing Alzheimer's disease, but not directly to cause it. The gene with the greatest known influence on the risk of developing late onset Alzheimer's disease is called apolipoprotein E (APOE). This gene is found on chromosome 19 and comes in three forms, which by convention are named with the Greek letter epsilon (ε): APOE ε2, APOE ε3 and APOE ε4. All individuals have two copies of the APOE gene, and these may be the same as each other or different. Hence each have one of the six possible combinations are possible: ε2/ε2, ε2/ε3, ε3/ε3, ε2/ε4, ε3/ε4 or ε4/ε4. APOE ε4 is associated with a higher risk of Alzheimer's. About a quarter of the general population inherits one copy of the APOE ε4 gene. This increases their lifetime risk of developing Alzheimer's disease by up to four times. About 2 per cent of the population gets a 'double dose' of the APOE ε4 gene - one from each parent. This increases their risk of developing Alzheimer's disease by about 10
times or more. However, even then, they are not certain to develop Alzheimer's. About 60 per cent of the population has a 'double dose' of the APOE ε3 gene and is at 'average' risk. Up to half of this group develops Alzheimer's disease by their late 80s. The APOE ε2 form of the gene is mildly protective against Alzheimer's: people with it are slightly less likely to develop the disease. In the general population, 11 per cent has one copy of APOE ε2 together with a copy of APOE ε3, and one in 200 (0.5 per cent) has two copies of APOE ε2. Recent scientific developments have allowed researchers to test many more genes to see whether there are additional links with Alzheimer's disease. This approach has revealed further genes which are linked to increased risk, called CLU, PICALM, CR1, BIN1, ABCA7, MS4A, CD33, EPHA1 and CD2AP. Variants in these genes are linked to significant differences in risk of Alzheimer's, but their effects are much smaller than for APOE.

ApoE
Circulating ApoE is synthesized mainly in liver and it is associated to lipoproteins, but astrocytes and possibly microglia are important sources for apoE in brain. ApoE has an important function as a regulator of lipid metabolism during development and is involved in the growth and regeneration of injured neurons. The levels of apoE increase after brain lesions which may contribute to the regeneration of neurons.

The mechanisms by which the ε4 allele convey the development of AD are largely unclear but many studies have revealed isoform specific effects on neurodegeneration, formation of NFTs and β-amyloid. Arendt et al. have shown that plastic responses in brain are impaired in ε4 carriers, and in vitro studies have shown that apoE3 promotes neurite outgrowth more than apoε4. ApoE ε4 carriers have also more severe astrogliosis compared to ε4 non-carriers.

Accumulating data suggest that apoE plays a role in the development of neurofibrillary and amyloid pathology. ApoE3 has been shown to bind avidly to microtubule-associated proteins and to promote their polymerization, and apoE4 is claimed to depolymerize microtubules. The stabilizing effect of apoE3 may prevent the abnormal phosphorylation of the tau protein. In addition to the in vitro data, neuropathological studies have shown that the apoE ε4 allele is linked to increased density of NFTs in brain. On the other hand, ApoE is bound strongly to β-amyloid plaques. In vitro studies have indicated that apoE4 promotes β-amyloid aggregation, and apoE3
inhibits the aggregation of β-amyloid.[43] The accumulation of β-amyloid in brain is increased in controls and AD patients carrying the ε4 allele.[44–46] This effect may be based on the augmentation of Aβ40 deposition.[47, 48] In contrast, AD patients carrying the ε2 allele have a decreased amyloid burden.[49] It has also been suggested that the apoE ε4 allele increases vascular β-amyloid deposition.[50] and amyloid accumulation after a traumatic brain injury.[51]

Clinical studies support the significance of apoE in the development of AD. MRI studies have shown that AD patients with at least one ε4 allele have decreased volume in their entorhinal cortex,[52] and hippocampus.[53] In single photon emission computed tomography (SPECT), AD patients homozygous for ε4 allele exhibit the most severe cerebral hypoperfusion[54], and in positron emission tomography (PET) the low baseline metabolism in non-demented ε4 carriers predicts a cognitive decline.[55] In neuropsychological tests, the ε4 allele has been associated with the cognitive decline in subjects with cognitive impairment, but not in cognitively normal individuals.[56]

Despite the strong association between AD and apoE ε4 allele, apoE genotyping is not recommended in the routine diagnosis of AD. The presence of the ε4 allele by itself does not confirm AD, and the disease cannot be excluded if an individual does not carry the risk allele.

In the population based study of Hyman et al.[57] over half of the individuals over 80 years of age carrying two ε4 alleles were cognitively normal. Therefore, apoE genotyping provides only a marginal increase in the confidence of the diagnosis and cannot be used as a diagnostic test for AD.[58]

**Diagnosis of Alzheimer disease**

The clinical diagnosis of AD, based on signs of slowly progressive dementia and findings of gross cerebral cortical atrophy on neuroimaging, is correct about 80 –90% of the time. The association of the APOE ε4 allele with AD is significant; however, APOE genotyping is neither fully specific nor sensitive. APOE genotyping may have an adjunct role in the diagnosis of AD in symptomatic individuals and a limited role at this time in predictive testing of asymptomatic individuals. Three forms of EOAD caused by mutations in one of three genes (APP, PSEN1, and PSEN2) are recognized. The APP is cleaved by alpha- and gamma-secretases to form the Aβ peptide, which is the primary
component of the extracellular amyloid plaque deposited in AD. Presenilin 1 (PS1) is part of the gamma-secretase complex (and PS2 is a close homolog of PS1). Molecular genetic testing of the three genes is available in clinical laboratories.

Establishing the diagnosis of Alzheimer disease relies on clinical-neuropathologic assessment.[59] Neuropathologic findings on autopsy examination remain the gold standard for diagnosis of AD. The clinical diagnosis of AD (before autopsy confirmation) is correct about 80–90% of the time.[60]

- Clinical signs: slowly progressive dementia,
- Neuroimaging: gross cerebral cortical atrophy[61], fb
- Neuropathologic findings: microscopic extracellular amyloid-β (Aβ) neuritic plaques, intraneuronal neurofibrillary tangles, and amyloid angiopathy at postmortem examination. The plaques should stain positively with Aβ-amyloid antibodies and negative for prion antibodies, which are diagnostic of prion diseases. The numbers of plaques and tangles must exceed those found in age-matched controls without dementia. Aggregation of alpha-synuclein in the form of Lewy bodies may also be found in neurons in the amygdale.[62]

**Treatment**

Treatment is supportive. Each symptom is managed on an individual basis. Assisted living arrangements or care in a nursing home is usually necessary. Drugs that increase cholinergic activity by inhibiting acetylcholinesterase produce a modest but useful behavioral or cognitive benefit in some affected individuals. Antidepressant medication may improve associated depression. The first such drug was tacrine; however, this agent is also hepatotoxic. Newer such drugs with similar pharmacologic action, such as Aricept® (donepezil)[63–65], Exelon® (rivastigmine)[66] and galantamine,[67–69] are not hepatotoxic.

Vitamins and other over-the-counter medications have been used in the treatment of AD.[70] Some, but not all, reports suggest that affected individuals taking 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A reductase inhibitors for hypercholesterolemia have a reduced incidence of dementia.[71-73] Immunization of an AD mouse model with β-amyloid has attenuated the AD pathology and stimulated the search for a possible vaccination approach to the treatment of human AD.[74] A human trial of this approach was halted because of encephalitis in a few subjects.[75-77] Alternative approaches to immunization
therapy have been proposed.\textsuperscript{[78]} Thus far, treatment of symptomatic AD with estrogens has not proven beneficial.\textsuperscript{[79, 80]}

**Perspectives**

There have been huge advances in our understanding of the genetics of AD over the last few years. The discovery of the three EOAD-related genes, APP, PSEN1, and PSEN2, has improved our knowledge of the physiopathology of AD. Ongoing and future large-scale genome-wide association studies and next-generation whole genome or whole exome sequencing hold the promise of unraveling the complexities of the genetic architecture of this disease. This should lead to identification of novel targets for genetic testing, as well as developing preventative and curative therapies for AD.

**REFERENCES**


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