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Genetics of Alzheimer Disease

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Abstract

Alzheimer disease (AD) is the most common causes of neurodegenerative disorder in the elderly individuals. Clinically, patients initially present with short-term memory loss, subsequently followed by executive dysfunction, confusion, agitation, and behavioral disturbances. Three causative genes have been associated with autosomal dominant familial AD (*APP*, *PSEN1*, and *PSEN2*) and 1 genetic risk factor (*APOEε4* allele). Identification of these genes has led to a number of animal models that have been useful to study the pathogenesis underlying AD. In this article, we provide an overview of the clinical and genetic features of AD.

Keywords

Alzheimer disease, genetics, neurodegeneration

Introduction

Prevalence and Incidence

Alzheimer disease ([AD] OMIM #104300) is the most common irreversible, progressive cause of dementia. It is characterized by a gradual loss of memory and cognitive skills. Alzheimer disease accounts for over 50% of all dementia cases, and it presently affects more than 24 million people worldwide. Moreover, over 5 million new cases of AD are reported each year, and the incidence increases from 1% between the ages of 60 and 70 to 6% to 8% at the age of 85 years or older[1] and is likely to increase as a greater proportion of the population ages.[2]

The prevalence and incidence of AD strongly suggest that age is the most influential known risk factor. Indeed, AD prevalence increases significantly with age, and AD incidence increases from 2.8 per 1000 person years for people between 65 and 69 years to 56.1 per 1000 person years for people who are older than 90 years.[3] Approximately 10% of persons older than 70 years have significant memory loss, and more than half of these individuals have probable AD. An estimated 25% to 45% of persons older than 85 years have dementia.[4] The duration of disease is typically 8 to 10 years, with a range from 2 to 25 years after diagnosis.

The disease is divided into 2 subtypes based on the age of onset: early-onset AD (EOAD) and late-onset AD (LOAD). Early-onset AD accounts for approximately 1% to 6% of all cases and ranges roughly from 30 years to 60 or 65 years. However, LOAD, which is the most common form of AD, is defined as AD with an age at onset later than 60 or 65 years. Both EOAD and LOAD may occur in people with a positive family history of AD. Approximately 60% of EOAD cases have multiple cases of AD within their families, and of these familial EOAD cases, 13% are inherited in an autosomal dominant manner with at least 3 generations affected.[5,6] Early-onset disease can also occur in families with late-onset disease.[4] With the exception of a few autosomal dominant families that are single-gene disorders (see below), most AD cases appear to be a complex disorder that is likely to involve multiple susceptibility genes and environmental factors.[4,7-10] Although the first-degree relatives of patients with late-onset disease have approximately twice the expected lifetime risk of the disease, the pattern of transmission is rarely consistent with Mendelian inheritance.

Clinical Symptoms

Both EOAD and LOAD present clinically as dementia that begins with a gradual decline of memory and then slowly increases in severity until the symptoms eventually become

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incapacitating. An inability to retain recently acquired information is typically the initial presentation, whereas memory for remote events is relatively spared until later. With disease progression, impairment in other areas of cognition (eg, language, abstract reasoning, and executive function or decision making) occurs to varying degrees and typically is associated with difficulty at work or in social situations or household activities. Changes in mood and affect often accompany the decline in memory. Delusions and hallucinations are not typically presenting signs but can present any time during the course of illness. Neurological symptoms that may occur later in the course of illness include seizures, hypertonia, myoclonus, incontinence, and mutism. Death commonly occurs from general inanition, malnutrition, and pneumonia.[4] Treatment of AD with cholinesterase inhibitors and memantine may result in slowing of cognitive decline in patients with mild-to-moderate dementia, but current treatments do not modify the course of illness.[11,12]

Clinical Diagnosis

Currently, the diagnosis of AD is based on clinical history, neurological examination, and neuropsychological tests. The *Diagnostic and Statistical Manual of Mental Disorders* (Fourth Edition [*DSM-IV*]) criteria for diagnosing dementia requires the loss of 2 or more of the following: memory, language, calculation, orientation, or judgment.[13] Another commonly used criteria, the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) Work Group requires the presence of dementia that is documented by clinical examination, deficits in at least 2 cognitive domains, absence of other systemic disorders, progressive worsening of memory for the diagnosis of "probable AD."[14]

The Mini-Mental State Examination (MMSE) is 1 bedside test that can help to evaluate changes in a patient's cognitive abilities. In addition, a diagnosis of probable AD necessitates the exclusion of other neurodegenerative disorders associated with dementia, such as frontotemporal dementia (including frontotemporal dementia with parkinsonism 17 and Pick disease), Parkinson disease, diffuse Lewy body disease, Creutzfeldt-Jakob disease, and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).[15] Discriminating AD from other forms of dementia is usually done through clinical history and neurological examination.[4] In addition, other systemic causes of dementia need to be excluded, especially the treatable forms of cognitive impairment, such as impairment due to depression, chronic drug intoxication, chronic central nervous system infection, thyroid disease, vitamin deficiencies (eg, B_{12} and thiamine), central nervous system angitis, and normal-pressure hydrocephalus.[4]

Neuropathological Diagnosis

A definitive diagnosis of AD requires a clinical assessment of probable AD as well as postmortem confirmation, with the presence of 2 histopathological features: neurofibrillary tangles and amyloid plaques.[16-18] Expert clinicians correctly diagnose AD, 80% to 90% of the time.[19] Even though plaques and tangles are often found in cognitively normal age-matched controls, the density of the plaques and the distribution of neurofibrillary tangles are more severe in patients with AD, according to standardized histological assessments.[16] According to a consensus work group, the following categories are recommended to provide an estimate of the likelihood that AD pathological changes underlie dementia:

1. There is a high likelihood that dementia is due to AD lesions when the postmortem brain shows the presence of both neuritic plaques and neurofibrillary tangles in neocortex (ie, a frequent neuritic plaque score according to CERAD [Consortium to Establish a Registry for Alzheimer's Disease[20] and a stage V/VI according to Braak and Braak.[21]

2. There is an intermediate likelihood that dementia is due to AD lesions when the postmortem brain shows moderate neocortical neuritic plaques and neurofibrillary tangles in limbic regions (ie, CERAD moderate and Braak and Braak stage III/IV).

3. There is a low likelihood that dementia is due to AD lesions when the postmortem brain shows neuritic plaques and neurofibrillary tangles in a more limited distribution and/or severity (ie, CERAD infrequent and Braak and Braak stage I/II).[22] The precise pathogenic mechanisms that are responsible for the development of these changes remain unknown.

The major component of amyloid plaques, found in AD brain, is amyloid-β (Aβ).[23,24] The most common form of Aβ in humans is 40 amino acids long and is called Aβ40. A 42-amino-acid–long fragment, Aβ42, is less abundant than Aβ40 and differs only in that it has 2 additional amino acid residues at the C-terminus. The Aβ42 fragment is associated with AD.[25] Amyloid-β is derived from the amyloid precursor protein (APP) after cleavage by secretases. First, α-secretase (nonneurotoxic "normal" cleavage) or β-secretase (potential neurotoxic "abnormal" cleavage) cleaves APP, and a second cleavage of the β-secretase product, by γ-secretase, cleaves APP further to produce Aβ[26-32] (Figure 1). Depending on the point of cleavage by γ-secretase, 2 main forms of Aβ are produced consisting of either 40 or 42 amino acid residues (Aβ40 or Aβ42). The proportion of Aβ40 to Aβ42 that is formed is particularly important in AD because Aβ42 is far more prone to oligomerize and form fibrils than the more abundantly produced Aβ40 peptide. Indeed, although it appears that the production of Aβ isoforms is a normal process of unknown function, in a small number of individuals, an increased proportion of Aβ42 appears sufficient to cause EOAD.[18,33]

Neurons bearing neurofibrillary tangles are another frequent finding in AD brains,[34,35] and the temporal and spatial appearance of these tangles, which contain hyperphosphorylated tau, more closely reflects disease severity than does the presence of amyloid plaques.[36,37] Tangles are formed by
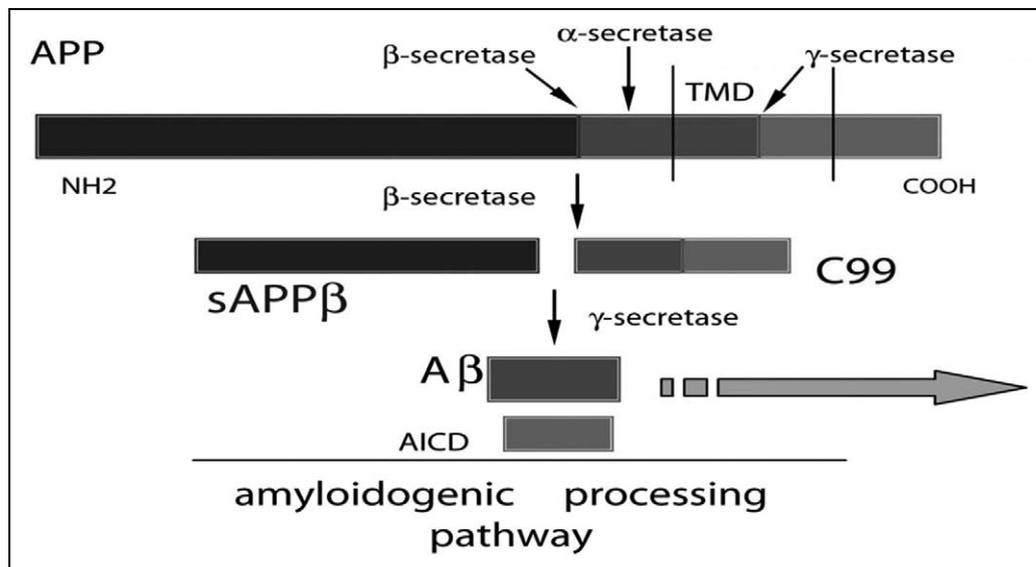
Figure 1. Amyloidogenic processing of the amyloid precursor protein (APP) and generation of the β-amyloid peptides. The APP protein can be cleaved by 3 different secretases: α, β, or γ. Subsequent to "normal" α-secretase cleavage, sAPPα is produced and released into the extracellular space and the C83 peptide remains in the cell membrane (panel B). Subsequent to β-secretase cleavage, sAPPβ is produced and released into the extracellular space and the C99 peptide remains in the cell membrane Subsequent to β-secretase cleavage, the C99 peptide is "abnormally" cleaved by γ-secretase to yield an Aβ peptide and the AICD peptide. Scale is approximate. Aβ indicates amyloid-β peptide; AICD, amyloid precursor protein intracellular domain; sAPPβ, soluble fragment amyloid-β peptide; TMD, transmembrane domain. Large arrow represents accumulation of plaques or amyloid plaque deposition.

Table 1. Alzheimer Disease (AD) Genes[a]

AD Loci	Gene Symbol	Gene Name	Chromosome	Inheritance
AD1	APP	Amyloid precursor protein	21q21	Autosomal Dominant
AD2	APOE	Apolipoprotein E	19q13.32	Sporadic
AD3	PSEN1	Presenilin 1	14q24.2	Autosomal Dominant
AD4	PSEN2	Presenilin 2	1q42.13	Autosomal Dominant

[a] Alzheimer disease genes are located on 4 different chromosomes and are associated with autosomal dominant or sporadic inheritance.

hyperphosphorylation of a microtubule-associated protein known as tau, causing it to aggregate in an insoluble form. However, neurofibrillary tangles are also found in other disorders, such as frontotemporal dementia and progressive supranuclear palsy. Moreover, these tangles are not necessarily associated with the cognitive dysfunction and memory impairment that is typical of AD and mutations in the gene that encodes the tau protein (*MAPT*), a main component of neurofibrillary tangles, have not been genetically linked to AD.[24]

Genetics of AD

Introduction

Overall, more than 90% of patients with AD appear to be sporadic and to have a later age at onset of 60 to 65 years of age (LOAD).[38] Although twin studies support the existence of a genetic component in LOAD, no causative gene has been yet identified. Indeed, the only gene that has been consistently found to be associated with sporadic LOAD, across multiple genetic studies, is the apolipoprotein E (*APOE*) gene[39-43]

(Table 1). However, many carriers of the *APOE* risk allele (ε4) live into their 90s, which suggests the existence of other LOAD genetic and/or environmental risk factors that have yet to be identified. To this end, several unreplicated genetic variants have been reported, and these findings suggest that there may be 5 to 7 major LOAD susceptibility genes.[4,44,45] For a catalog of candidate gene association studies, please refer to the AlzGene online database (http://www.alzforum.org/res/com/gen/alzgene/default.asp).

Genes Associated With Autosomal Dominant AD

Although several hundred families carry one of the following mutations, they account for less than 1% of cases.

AD1: Amyloid precursor protein (APP)

Inheritance and clinical features. In the 1980s, Kang and colleagues purified both plaque and vascular amyloid deposits and isolated their 40-residue constituent peptide (Aβ), which subsequently led to the cloning of the APP type I integral
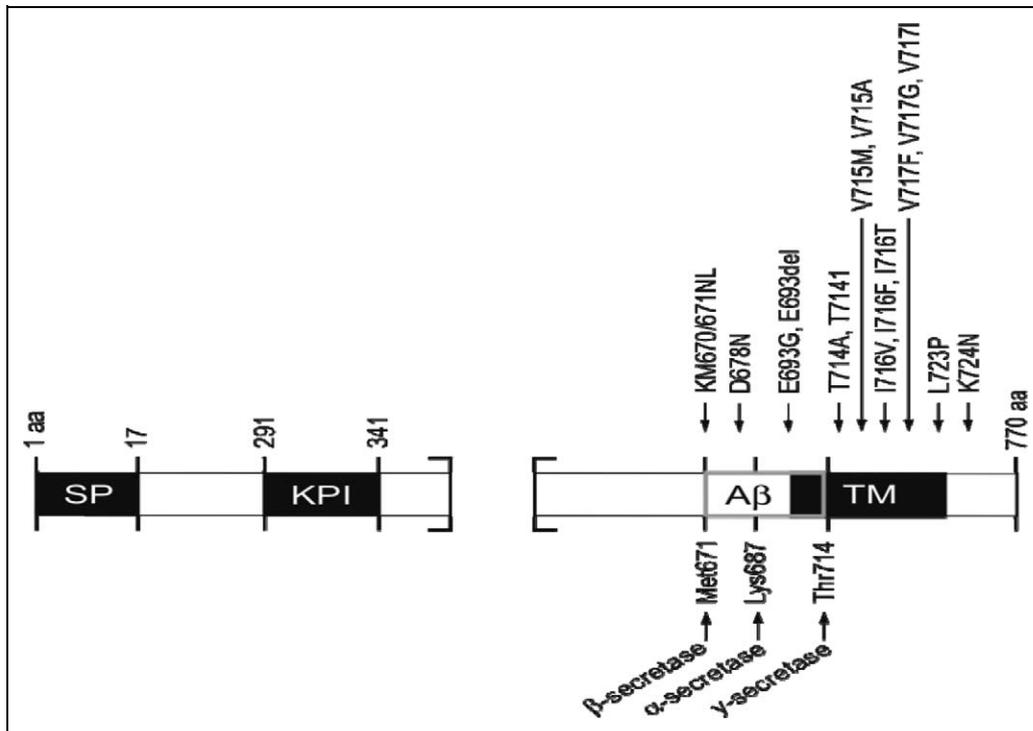
Figure 2. Amyloid precursor protein (*APP*) structure and mutations. Thus far, over 32 different *APP* missense mutations have been identified, of which a few are shown. SP indicates signal peptide; KPI, Kunitz protease inhibitor domain; Aβ, amyloid-β; TM, transmembrane domain. Scale is approximate.

membrane glycoprotein from which Aβ is proteolytically derived.[46] The *APP* gene was then mapped to chromosome 21q, which accounted for the observation that patients with Down syndrome (trisomy 21) develop amyloid deposits and the neuropathological features of AD when in their 40s.[24,47-49]

Since then, over 32 different *APP* missense mutations have been identified in 85 families. Interestingly, most of these mutations are located at the secretase cleavage sites or the *APP* transmembrane domain on exons 16 and 17 (Figure 2). Information regarding *APP* mutations are available in the NCBI database and the Alzheimer Disease Mutation Database (www.molgen.ua.ac.be/ADMutations).[50] Mutations within *APP* account for 10% to 15% of early-onset familial AD (EOFAD),[4,51-53] appear to be family specific, and do not occur within the majority of sporadic cases with AD. The majority of these EOFAD mutations are in or adjacent to the Aβ peptide sequence (Figure 2), the major component of amyloid plaques[54,55] Most cases[56] containing *APP* mutations have an age of onset in the mid-40s and -50s.

Gene location and structure. Sequences encoding *APP* were first cloned by screening complementary DNA (cDNA) libraries.[46] The initial full-length cDNA clone encoded a 695-amino-acid protein (APP695)[40] and consisted of 18 exons. The *APP* gene, located on chromosome 21q21, is alternatively spliced into several products, named according to their length in amino acids (ie, APP695, APP714, APP751, APP770, and APP563) and expressed differentially by tissue type, whereby the 3 isoforms that are most relevant to AD are restricted to the central nervous system (APP695) or expressed in both the peripheral and central nervous system (CNS) tissues (APP751 and APP770).[46,57-63]

Gene function and expression. Amyloid precursor protein is a type-I integral-membrane protein[46] that resembles a signal-transduction receptor. It is expressed in many tissues and concentrated in the synapses of neurons. Its primary function is not known, though it has been implicated in neural plasticity[64] and as a regulator of synapse formation.[65] Amyloid precursor protein is synthesized in the endoplasmic reticulum, posttranscriptionally modified in the Golgi (N- and O-linked glycosylation, sulfation, and phosphorylation), and transported to the cell surface via the secretory pathway. Amyloid precursor protein is also endocytosed from the cell surface and processed in the endosomal−lysosomal pathway.[66,67] Amyloid precursor protein and its by-product Aβ have been found to be translocated inside mitochondria and implicated in mitochondrial dysfunction.[68-70]

Proteolysis of APP by α-secretase or β-secretase leads to the secretion of soluble fragment amyloid-α peptide (sAPPα) or soluble fragment amyloid-β peptide (sAPPβ). This proteolysis generates C-terminal fragments of 10 and 12 kDa, respectively, which are inserted into the membrane. These fragments can be cut by γ-secretase to extracellularly release the Aβ peptide[71] and intracellularly release a cytoplasmic fragment identified as amyloid precursor protein intracellular domain[72] ([AICD] see Figure 1). The majority of EOFAD mutations alter this

processing of *APP* in such a way that Aβ42 levels are changed relative to other Aβ isoform levels.[73,74] The function of these *APP* proteolytic fragments is still unclear.

The missense *APP* "Swedish" mutations (*APP*SW, *APP*K670N, and M671L) and the "London" mutations (*APP*LON and *APP*V717I) are examples of *APP* mutations that lead to increased Aβ production and development of AD.[75,76] Transgenic mouse models of such *APP* mutations (PDAPP, Tg2576, APP23, TgCRND8, and J20) have been developed.[77] Each of these mouse models have different APP expression levels and different neurorological abnormalities.[77,78] For example, the Tg2576 mouse model that carries the "Swedish" mutation has high *APP* levels, high Aβ levels, and cognitive disturbances[79] that are progressive and start as early as 6 months of age.[80]

Genetic variation. Amyloid precursor protein transcripts have been identified in which exons 7, 8, and 15 are alternatively spliced. APP695, the predominant isoform in neurons,[81] contains exon 15 but excludes exons 7 and 8. The APP751 and APP770 isoforms both encode Kunitz-type protease inhibitor (KPI)-containing forms of APP,[58-60,82] are present in both peripheral tissue and neurons, and contain exons 16 and 17 but exclude exons 7, 8, and 15. The gene region that encodes the portion of APP that is cleaved to produce the Aβ peptide is located within exons 16 and 17 of the APP770 splice variant[83] (see Figure 2). Other splice variants have been observed, with missing exon 15 in various combinations with exons 7 and 8; these splice variants are referred to as L-APPs.[82,84] A number of studies have indicated that alternative splicing of exons 7 and 8 is modulated in brain during aging and possibly during AD.[84-89] Even though the function of APP and its various splice variants is unknown, differential expression of these splice variants between tissues may imply functional differences. It is important to note that although most of the described splice variants contain Aβ-encoding sequences, 2 additional rare transcripts, APP365 and APP563, do not, implicating additional functionally important variability in APP isoform function.[62,90]

The first described and best characterized *APP* mutation (V717I) is located within the transmembrane domain near the γ-secretase cleavage site[75] (Figure 2). Other substitutions have since been identified at this site, and several groups have reported the V717I mutation in families who are unrelated to the initial London family. Many other *APP* mutations have since been identified, especially near the γ-secretase cleavage site, and the bulk of these mutations have been associated with modulation of Aβ levels. For example, a C-terminal L723P mutation was identified in an Australian family; this mutation is reported to generate an increase of Aβ42 peptide levels in CHO cells.[91] The majority of EOFAD mutations alter processing of APP in such a way that the relative level of Aβ42 is increased by increasing Aβ42, decreasing Aβ40 peptide levels, or increasing Aβ42 while decreasing Aβ40 peptide levels.[73,74]

AD3: Presenilin 1 (PSEN1)

Inheritance and clinical features. Linkage studies[92] established the presence of an AD3 locus on chromosome 14, and positional cloning led to the identification of mutations in the presenilin 1 (*PSEN1*) gene, which encodes a polytopic membrane protein.[93] Presenilins are major components of the atypical aspartyl protease complexes that are responsible for the γ-secretase cleavage of APP.[94,95] Mutations in *PSEN1* are the most common cause of EOFAD. Indeed, *PSEN1* missense mutations account for 18% to 50% of autosomal dominant EOFAD cases.[96] Over 176 different *PSEN1* mutations have been identified in 390 families. *PSEN1* mutations appear to increase the ratio of Aβ42 to Aβ40, thereby resulting in a change in function that leads to reduced γ-secretase activity.[97] It has been suggested that deposition of Aβ42 may be an early preclinical event in *PSEN1* mutation carriers.[98]

Defects in *PSEN1* cause the most severe forms of AD, with complete penetrance and an onset occurring as early as 30 years of age. However, there is a wide variability in age of onset as other *PSEN1*-associated AD cases have a mean age of onset greater than 58 years. *PSEN1*-associated AD is an autosomal dominant neurodegenerative disorder characterized by progressive dementia and parkinsonism, notch signaling modulation, and Aβ intracellular domain generation.[18,99] There is also considerable phenotypic variability in patients with *PSEN1*-associated AD, including some patients who develop spastic paraparesis and other atypical AD symptoms. Some of these variable clinical phenotypes have been associated with specific mutations. Neuropathological studies often confirm the clinical diagnosis of AD with measurement of amyloid plaque and Braak stage (as described above) as well as other neurodegenerative changes in other brain areas according to the presence of specific *PSEN1* mutations.[100,101] For example, researchers found that the clinical and neuropathologic features of a Greek family with a *PSEN1* mutation (N135S) included memory loss when the family members were in their 30s, as well as variable limb spasticity and seizures. On neuropathological examination of these family members, the diagnosis of AD was confirmed, and there was also histological evidence of corticospinal tract degeneration.[101] Moreover, a *PSEN1* mutation (I143M) that lies in a cluster in the second transmembrane domain of the protein has been described in an African family with an age at onset in the early 50s and a short duration of illness for 6 to 7 years. These family members were diagnosed on autopsy with severe AD pathology characterized by neuronal loss, abundant Aβ neuritic plaques and neurofibrillary tangles, and degeneration extending into the brain stem.[102]

Gene location and structure. *PSEN1* is located on chromosome 14q24.2. It consists of 12 exons that encode a 467-amino-acid protein that is predicted to traverse the membrane 6 to 10 times; the amino and carboxyl termini are both oriented toward the cytoplasm.[103]

Gene function and expression. *PSEN1* is a polytopic membrane protein that forms the catalytic core of the γ-secretase complex.[94,104] γ-secretase is an integral membrane protein typically found at the cell surface, but it may also be found in the Golgi, endoplasmic reticulum, and mitochondria.[94,105]
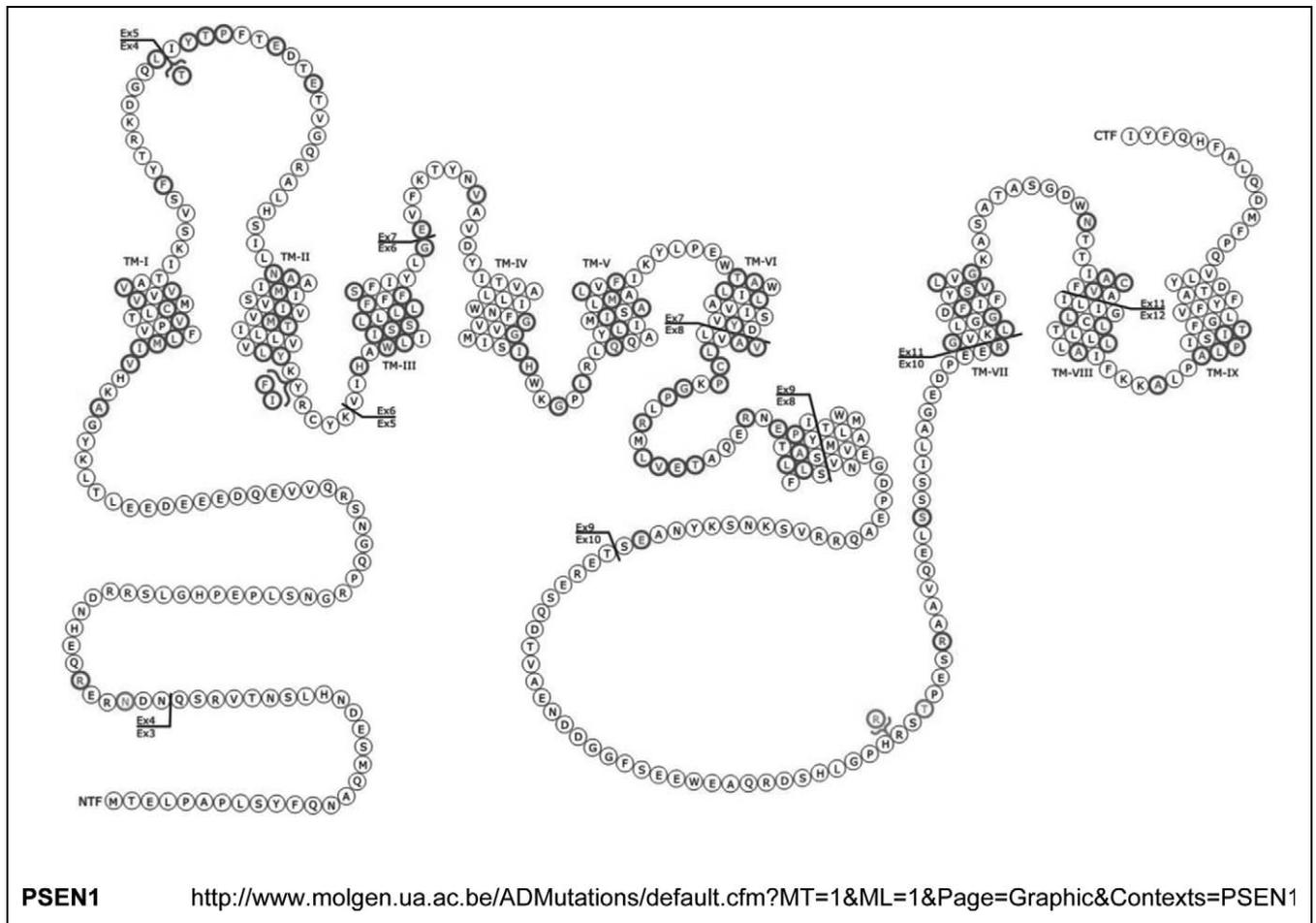
Figure 3. Presenilin 1 (*PSEN1*) structure and mutations. Thus far, there have been at least 123 mutations in the *PSEN1* gene described, of which a few are shown. For a more complete list of *PSEN1* mutations, see http://www.molgen.ua.ac.be/ADMutations. TM indicates transmembrane domains. Scale is approximate.

PSEN1, nicastrin (Nct), anterior pharynx defective 1 (Aph-1), and presenilin enhancer 2 (PSENEN) are required for the stability and activity of the γ-secretase complex.[106-110] This complex cleaves many type-I transmembrane proteins, including APP and Notch,[94,111] 2 proteins in the hydrophobic environment of the phospholipid bilayer of the membrane.[109]

PSEN1 knockout (KO) mice are not viable,[112] but a conditional *PSEN1* KO mouse model, where the loss of the gene is limited to the postnatal forebrain, found that KO mice exhibited mild cognitive impairments in long-term spatial reference memory and retention.[113] These findings suggest that presenilins play a role in cognitive memory. Moreover, knockin mice with missense mutations of the endogenous murine *PSEN1* have high Aβ42 levels and perform poorly on the object recognition test.[114,115] Double *PSEN1/APP* transgenics have been developed, and these transgenics suggest that *PSEN1*, *APP*, and mutations within these genes play a role in the production of Aβ.[78,116]

Genetic variation. To date, there have been 176 *PSEN1* mutations reported (Figure 3). A comprehensive list of *PSEN1* mutations is available through the NCBI database (http://

www.molgen.ua.ac.be/ADmutations). The majority of these mutations are missense mutations. These missense mutations cause amino acid substitutions throughout the PSEN1 protein and appear to result in a relative increase in the ratio of Aβ42 to Aβ40 peptides; this increase seems to occur through increased Aβ42 production, decreased Aβ40 production, or alternatively, a combination of increased Aβ42 production and decreased Aβ40 production.[73] For example, individuals that carry the *PSEN1*-L166P mutation can have an age at onset in adolescence, and in vitro studies indicate that this mutation induces exceptionally high levels of Aβ42 production as well as impaired notch intracellular domain production and notch signaling.[100]

AD4: Presenilin 2 (PSEN2)

Inheritance and clinical features. A candidate gene for the chromosome 1 AD4 locus was identified in 1995 in a Volga German AD kindred with a high homology to the AD3 locus (*PSEN1*); this gene was later named presenilin 2 (*PSEN2*).[51,117,118] In contrast to the mutations in the *PSEN1* gene, missense mutations in the *PSEN2* gene are a rare cause of EOFAD, at least in

PSEN2 http://www.molgen.ua.ac.be/ADMutations/default.cfm?MT=1&ML=1&Page=Graphic&Contexts=PSEN2
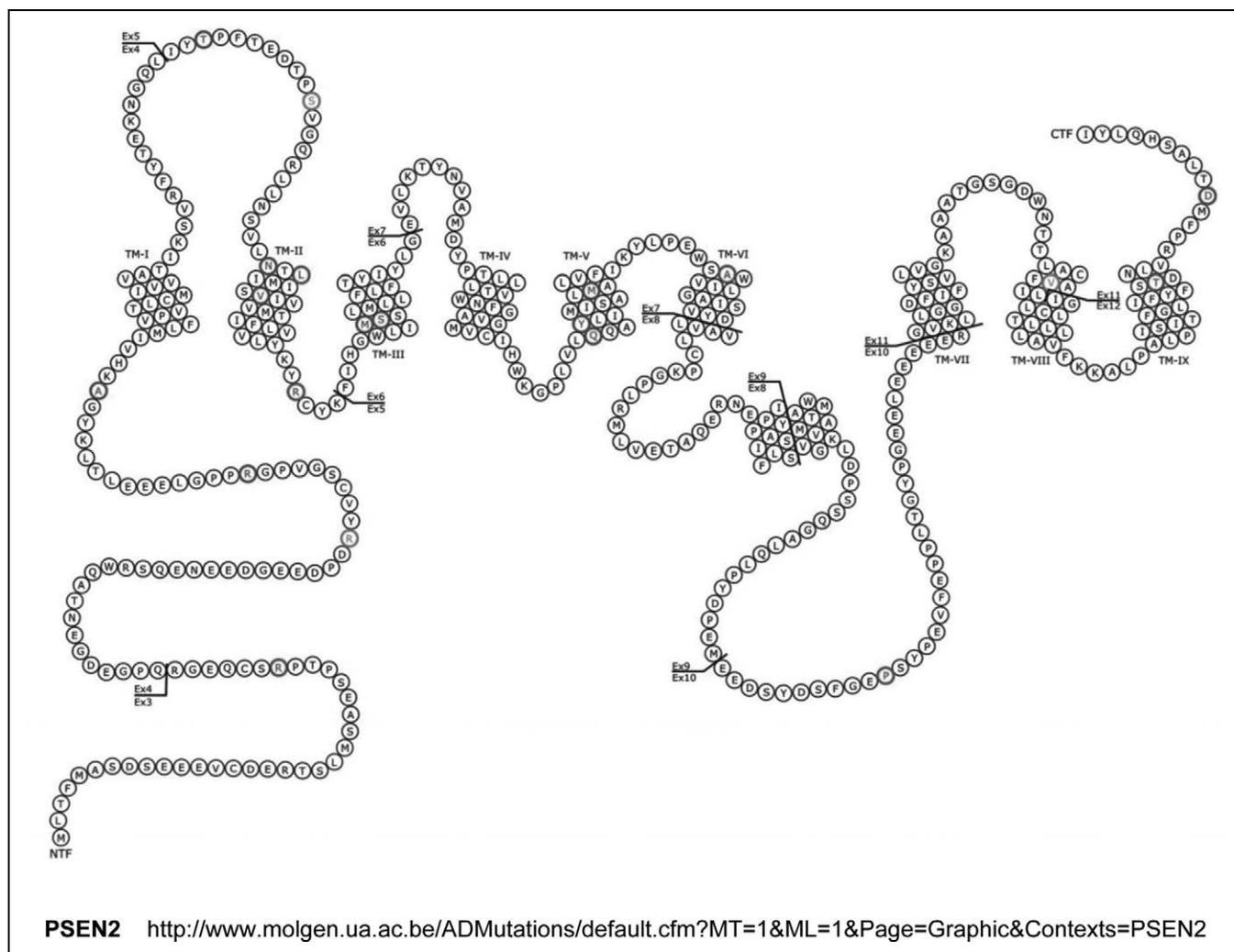
Figure 4. Presenilin 2 (*PSEN2*) structure and mutations. Thus far, there have been at least 16 mutations in the *PSEN2* gene described, of which a few are shown. For a more complete list of *PSEN2* mutations, see http://www.molgen.ua.ac.be/ADMutations. TM indicates transmembrane domains. The V393M novel mutation was most recently found in 1 case (Lindquist et al).[126] Scale is approximate.

Caucasian populations. The clinical features of PSEN2-affected families appear to differ from the clinical features of PSEN1-affected families in that the age of onset in these family members is generally older (45-88 years) than for some family members with *PSEN1* mutations (25-65 years). Furthermore, the age of onset is highly variable among PSEN2-affected members of the same family; whereas in families with *PSEN1* mutations, the age of onset is generally quite similar among affected family members, and it is even similar among people from different families with the same mutation.[5,51,93,117] Missense mutations in the *PSEN2* gene may be of lower penetrance than in the *PSEN1* gene and therefore may be subject to the modifying action of other genes or environmental influences.[51,119]

Gene location and structure. The *PSEN2* gene is located on chromosome 1 (1q42.13) and was identified by sequence homology and then cloned.[117,118] *PSEN2* has 12 exons and is organized into 10 translated exons that encode a 448-amino-acid peptide. The PSEN2 protein is predicted to consist of 9 transmembrane

domains and a large loop structure between the sixth and seventh domains (Figure 4). *PSEN2* also displays tissue-specific alternative splicing.[117,118,120-122]

Gene function and expression. Like *PSEN1*, *PSEN2* has been described as a component of the atypical aspartyl protease called γ-secretase, which is responsible for the cleavage of Aβ.[94,95] *PSEN2* is expressed in a variety of tissues, including the brain, where it is expressed primarily in neurons.[123] *PSEN2*-associated mutations have been reported to increase the ratio of Aβ42 to Aβ40 (Aβ42/Aβ40) in mice and humans,[73,97] indicating that presenilins might modify the way in which γ-secretase cuts APP.

Amyloid precursor protein processing at the γ-secretase site has been reported to be differentially affected by specific presenilin mutations. For example, *PSEN1*-L166P mutations cause a reduction in Aβ production whereas *PSEN1*-G384A mutations significantly increase Aβ42. In contrast, PSEN2 appears to be a less efficient producer of Aβ than PSEN1.[25] The

functions and biological importance of presenilin splice variants are poorly understood, but it appears that differential expression of presenilin isoforms may lead to differential regulation of the proteolytic processing of the APP. For example, aberrant *PSEN2* transcripts lacking exon 5 appear to increase the rate of production of Aβ peptide,[124] whereas naturally occurring isoforms without exons 3 and 4 and/or without exon 8 do not affect production of Aβ.[125]

Genetic variation. Mutations in PSEN2 are a much rarer cause of familial AD than are *PSEN1* mutations; *PSEN2* mutations have been described in 6 families, including the Volga-German kindred where a founder effect has been demonstrated,[50,51,117,118] whereas *PSEN1* mutations have been found in 390 families. To date, as many as 14 *PSEN2* mutations have been identified. One of the first mutations to be identified was a point mutation located within the second transmembrane domain that resulted in the substitution of an isoleucine for an asparagine at residues 141 (N141I).[118] Most recently, a V393M mutation located within the seventh transmembrane domain has been described[126] (Figure 4). A comprehensive list of *PSEN2* mutations is available through the NCBI database (http://www.molgen.ua.ac.be/ADmutations).

Genes Associated With Risk in Sporadic AD

AD2: APOE

Inheritance and clinical features. The *APOE* gene has been associated with both familial late-onset and sporadic late-onset AD in numerous studies of multiple ethnic groups. The *APOE* ε4 genotype is associated with higher risk of AD,[127] earlier age of onset of both AD[128] and Down syndrome (where there is an additional copy of chromosome 21 carrying the *APP* gene),[129] and a worse outcome after head trauma[130] and stroke, both in humans[131] and in transgenic mice expressing human *APOE* ε4.[132] The frequency of the *APOE* ε4 allele varies between ethnic groups, but regardless of ethnic group, *APOE* ε4− carriers are more frequently found in controls and *APOE* ε4+ carriers are more frequently found in patients with AD.[39-41,133-142]

Gene location and structure. The *APOE* gene is located on chromosome 19q13.2 and consists of 4 exons that encode a 299-amino-acid protein. The *APOE* gene is in a cluster with other apolipoprotein genes: *APOC1*, *APOC2*, and *APOC4*. The *APOE* ε4 loci are located within exon 4 of the gene. The 3 *APOE* ε4 alleles (ε2, ε3, and ε4) are defined by 2 single nucleotide polymorphisms, rs429358 and rs7412, which encode 3 protein isoforms (E2, E3, and E4). The most frequent apoE isoform is apoE3, which contains cysteine and arginine at amino acid positions 112 and 158. In contrast, these positions contain only cysteine residues in apoE2 and only arginine residues in apoE4 (Figure 5). The cysteine-arginine substitution affects the 3-dimensional structure and the lipid-binding properties between isoforms. In apoE4, the amino acid substitution results in the formation of a salt bridge between an arginine in

position 61 and a glutamic acid in 255; whereas apoE3 and apoE2 bind preferentially to high-density lipoproteins (HDLs), this changed structure causes the apoE4 isoform to bind preferentially to very-low-density lipoproteins (VLDLs).[143]

Gene function and expression. The mechanisms that govern apoE toxicity in brain tissue are not fully understood. Some proposed mechanisms include isoform-specific toxicity, *APOE* ε4-mediated amyloid aggregation, and *APOE* ε4-mediated tau hyperphosphorylation.[144]

The *APOE* polymorphism is unique to humans and has been proposed to have evolved as a result of adaptive changes to diet.[145,146] It is known that apoE plays an important role in the distribution and metabolism of cholesterol and triglycerides within many organs and cell types in the human body.[143] Individuals carrying *APOE* ε4 have higher total and LDL cholesterol.[147] Moreover, in vitro neurons have a cholesterol uptake that is lower when lipids are bound to apoE4 compared to apoE2 and apoE3,[148] and apoE4 appears to be less efficient than the other isoforms in promoting cholesterol efflux from both neurons and astrocytes.[149]

As the major apolipoprotein of the chylomicron in the brain, apoE binds to a specific receptor and works through receptor-mediated endocytosis to rapidly remove chylomicron and VLDL remnants from circulation; this process is essential for the normal catabolism of triglyceride-rich lipoprotein constituents.[150] In the brain, lipidated apoE binds aggregated Aβ in an apoE isoform-specific manner, with apoE4 being much more effective than the apoE3 isoform. Researchers have also proposed that the more efficient binding process of apoE4 enhances the deposition of the Aβ peptide.[151]

Brain cells from *APOE* knockout (*APOE*−/−) mice are more sensitive to excitotoxic and age-related synaptic loss,[152] and Aβ-induced synaptosomal dysfunction in these mice is also enhanced compared to control animals.[153] When human apoE isoforms (apoE3 and apoE4) are expressed in *APOE*−/− mice, the expression of apoE3, but not apoE4, is protective against age-related neurodegeneration[152] and Aβ toxicity.[153] In addition, astrocytes from *APOE* −/− mice that express human apoE3 release more cholesterol than those expressing apoE4; this suggests that apoE isoforms may modulate the amount of lipid available for neurons. Other studies report apoE-specific effects on Aβ removal from the extracellular space, whereby the apoE3 isoform has a higher Aβ-binding capacity than apoE4.[154,155] Animal and in vitro models show that, in the brain, astrocytes and microglia are the main producers of secreted apoE.[156,157] ApoE secretion in human primary astrocytes can be reduced by a combination of cytokines[158]; whereas under stress conditions, neurons appear to be the main producers of apoE.[159,160] In a rodent model, moderate injury has been shown to induce enhancement of apoE levels in clusters of CA1 and CA3 pyramidal neurons,[161] and in another rodent model, apoE levels have been shown to increase in response to peripheral nerve injury.[162]

In addition, individuals carrying *APOE* ε4 have higher amyloid and tangle pathology[163] and an increase in
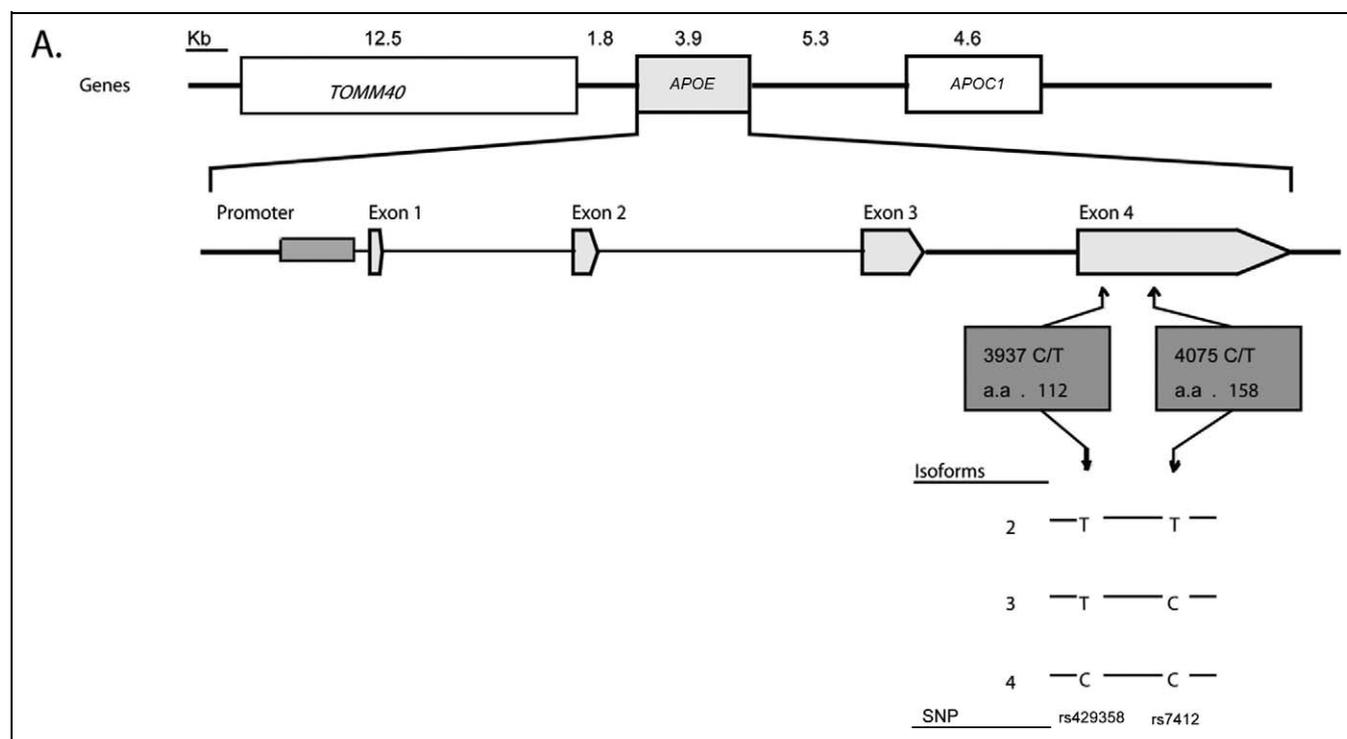
Figure 5. Apolipoprotein E (*APOE*) structure and single nucleotide polymorphisms (SNPs). The general protein structure of *APOE* is shown. The 2 SNPs in exon 4 and corresponding protein locations are shown (rs429358 and C112R; and rs7412 and R158C). The 3 *APOE* ε4 alleles (ε2, ε3, and ε4) are defined by 2 SNPs, rs429358 and rs7412, with ε2 defined by nucleotides T-T; ε3 defined by T-C, and ε4 defined by C-C, respectively.

mitochondrial damage[164] compared to those carrying other *APOE* polymorphisms.

Genetic variation. The gene dose of *APOE* ε4 is a major risk factor for AD, with many studies reporting an association between gene dose, age at onset,[165] and cognitive decline.[166] After age 65, the risk of AD among individuals with a family member with AD increases depending on the number of ε4 alleles present in the affected individual. Risks to family members with the *APOE* 2/2 and 2/3 genotypes are nearly identical at all ages to risks for family members with the *APOE* 3/3 genotype. Among family members with *APOE* 3/3, the lifetime risk of AD by age 90 can be as much as 3 times greater than the expected risk found in *APOE* ε4 carriers, suggesting that factors other than *APOE* contribute to AD risk. In addition, a 44% risk of AD by age 93 among family members of *APOE* 4/4 carriers indicates that as many as 50% of people having at least 1 ε4 allele do not develop AD. There also appears to be a gender modification effect because the risk to male family members with *APOE* 3/4 is similar to that for the *APOE* 3/3 carriers but is significantly less than the risk for the *APOE* 4/4 carriers, whereas among female family members, the risk for the *APOE* 3/4 carriers is nearly twice that for the *APOE* 3/3 carriers.[134-142]

Summary

Alzheimer disease is characterized by an irreversible, progressive loss of memory and cognitive skills that can occur in rare familial cases as early as the third decade. Currently, there is no cure for AD, and treatments only manage to slow down the progression of AD in some patients.[11,12] The early-onset familial forms of AD have an autosomal dominant inheritance linked to 3 genes: *APP, PSEN1,* and *PSEN2,* whereas the most common sporadic form of AD, which occurs after the age of 60, has thus far been consistently, across numerous studies, associated with only 1 gene, the *APOE* gene. The mechanistic contribution of these genes in AD pathogenesis has been studied extensively, but the specific biology involved in the progression of AD remains unclear, suggesting that AD is a genetic and environmentally complex disease.

Genetic Testing and Counseling

APOE ε4 is most highly associated with AD for individuals with a family history of dementia, and this association is highest for individuals that carry 2 *APOE* ε4 alleles (ε4/ε4 genotypes). The ε4/ε4 genotype is uncommon, and although the ethnicity of the population may alter the expected prevalence, the genotype occurs in about 1% of normal Caucasian controls. In contrast, the ε4/ε4 genotype occurs in nearly 19% of familial LOAD populations. *APOE* ε4-associated risk is also found in African Americans and Caribbean Hispanics.[167,168] Women with an *APOE* ε4/ε4 genotype have a 45% probability of developing AD by age 73,[169] whereas men have a 25% risk of developing AD by that age.[170] Alzheimer disease risk is also lower for individuals with only 1 *APOE* ε4 allele (by age 87) or no *APOE* ε4

allele (by age 95).[170] Approximately 42% of persons with LOAD do not have an *APOE* ε4 allele.[171] Thus, the absence of the *APOE ε4* allele does not rule out a LOAD diagnosis. First-degree relatives of a person with LOAD have a cumulative life-time risk of approximately 20% to 25%, whereas the risk in the general population is 10.4%.[141,172] It is still not known whether the age of onset of a patient with LOAD changes the risk to first-degree relatives. However, the number of additional affected family members most likely increases the risk in close relatives.[173] Given the low predictive value of the *APOE ε4* allele, the general consensus is that *APOE* genetic testing has limited value in asymptomatic persons for predicting AD risk.[174,175] Family history may, therefore, be a better predictor of LOAD risk.[173]

In contrast, EOFAD, with an age at onset before 60 to 65 years old, has an autosomal dominant mode of inheritance in which 20% to 70% of cases are estimated to have a *PSEN1* mutation, 10% to 15% of cases are estimated to have an *APP* mutation, and *PSEN2* mutations are rare.[174,176] Indeed, approximately 60% of patients with EOAD have another known affected family member. The remaining 40% of patients with EOAD may lack a family history because of an early death of a parent, failure to recognize the disorder in family members, or, very rarely, a de novo mutation.[5] If the parent of a patient with EOAD has a mutant allele, then the risk to the patient's sibling of inheriting the mutant allele is 50%. The child of a patient with EOAD who carries a mutation (*APP*, *PSEN1*, or *PSEN2*) has a 50% chance of transmitting the mutant allele to each of their children.

Testing of asymptomatic adults who are at risk for EOAD caused by mutations in the *PSEN1*, *PSEN2*, or *APP* genes is available clinically. However, genetic testing results for at-risk asymptomatic adults can only be interpreted after the disease-causing mutation has first been identified in the affected family member. It should be emphasized that testing of asymptomatic at-risk individuals with nonspecific or equivocal symptoms is predictive not diagnostic. In addition, obtaining results from genetic testing can affect an individual's personal relationships as well as their emotional well-being, and it may even cause depression. The principal arguments against testing asymptomatic individuals during childhood are that the testing then removes their choice to know or not know this information, it raises the possibility of stigmatization within the family and in other social settings, and it could have serious educational and career implications. Thus, the general consensus is that individuals who are at risk for adult-onset disorders should not be tested during childhood. Prenatal genetic testing for mutations in the *PSEN1* gene is possible if the *PSEN1* allele has been identified in an affected family member first. Preimplantation genetic diagnosis is also available for families that have a disease-causing mutation. However, parental requests for prenatal or preimplantation genetic testing of adult-onset diseases are rare.[4]

In this article, we have reviewed the genetics of AD. Further molecular genetic investigations should clarify the roles of additional known genes in the pathogenesis of both common sporadic as well as rare familial forms of AD. Already, investigations of the normal and aberrant functions of Aβ protein and ApoE has provided insight into the underlying mechanisms for AD. Such research will continue to provide new strategies for therapeutic interventions.

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