

The genetics of Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is a complex and heterogeneous neurodegenerative disorder, classified as either early onset (under 65 years of age), or late onset (over 65 years of age). Three main genes are involved in early onset AD: *amyloid precursor protein (APP)*, *presenilin 1 (PSEN1)*, and *presenilin 2 (PSEN2)*. The *apolipoprotein E (APOE)* E4 allele has been found to be a main risk factor for late-onset Alzheimer's disease. Additionally, genome-wide association studies (GWASs) have identified several genes that might be potential risk factors for AD, including *clusterin (CLU)*, *complement receptor 1 (CRI)*, *phosphatidylinositol binding clathrin assembly protein (PICALM)*, and *sortilin-related receptor (SORL1)*. Recent studies have discovered additional novel genes that might be involved in late-onset AD, such as *triggering receptor expressed on myeloid cells 2 (TREM2)* and *cluster of differentiation 33 (CD33)*. Identification of new AD-related genes is important for better understanding of the pathomechanisms leading to neurodegeneration. Since the differential diagnoses of neurodegenerative disorders are difficult, especially in the early stages, genetic testing is essential for diagnostic processes. Next-generation sequencing studies have been successfully used for detecting mutations, monitoring the epigenetic changes, and analyzing transcriptomes. These studies may be a promising approach toward understanding the complete genetic mechanisms of diverse genetic disorders such as AD.

Keywords: dementia, amyloid precursor protein, presenilin 1, presenilin 2, *APOE*, mutation, diagnosis, genetic testing

Introduction

Alzheimer's disease (AD) is a complex and heterogeneous neurodegenerative disorder. Several genetic and environmental factors and gene interactions may be involved in the disease's occurrence and progression.¹ Experiments have been performed with mono- and dizygotic twins to estimate the role of genetics in AD, the environmental influences, and the disease heritability. Variation in age of onset, neuropathological patterns, and disease duration may be possible due to genetic-environmental interactions.²⁻⁴ AD can be categorized into two subtypes: early onset and late onset. As a polygenic disorder, several additional genes might be potential risk factors for AD. Many single-nucleotide polymorphisms (SNPs) have been identified and confirmed to be associated with AD. The majority of recent studies in the genetics of AD have focused on the identification of novel risk-factor genes and mutations.^{2,5,6}

Early onset Alzheimer's disease

Occurrence of familial Alzheimer's disease (FAD) represents the minority (5%–10%) of all AD cases. Familial early onset Alzheimer's disease (EOAD) can be characterized by

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the Mendelian inheritance pattern; however, EOAD patients have also been reported without any family history (termed “sporadic EOAD”). Three genes are considered the main risk factors for EOAD: *amyloid precursor protein (APP)*, *presenilin 1 (PSEN1)*, and *presenilin 2 (PSEN2)*; Figure 1). Mutations in these genes might result in alteration of amyloid beta (Aβ) production (both Aβ40 and Aβ42), leading to apoptosis of the neurons and dementia.^{6–9} Figure 2 presents a timeline of AD onset according to age.^{5,10}

The *APP* gene is located on chromosome 21. Triplication of chromosome 21 results in the triplication of the *APP* gene, which might enhance APP expression and Aβ accumulation. Down syndrome patients have been reported to develop AD pathology (deposition of senile plaques and neurofibrillary tangles) earlier than those without Down syndrome.¹¹ These findings suggest that overexpression of APP might be related to AD pathology. The *APP* gene contains 19 exons for encoding the APP protein. The Aβ peptide is encoded by exons 16 and 17. Following transcription and alternative splicing, at least five isoforms of APP protein were identified, which contain the Aβ peptide sequence.¹² However, *APP* seems to be a very rare risk factor for AD, as 21 and three mutations were described at exon 17 and 16, respectively. Most of the pathogenic *APP* mutations were located near the cleavage sites of alpha, beta, and gamma secretase enzymes, which suggests they might be involved in the onset of AD through altering the proteolysis of the Aβ peptide.^{13,14} N-terminal mutations in the Aβ sequence can affect the endosomal/lysosomal cleavage of Aβ, and might alter the beta secretase cleavages.^{12,15} Mutations near the cleavage site of alpha secretase (Glu693Lys, Glu693Gly, Glu693del, Asp694Asn) might change the processing of APP, in enhancing the proteolytic resistance of Aβ peptide.^{16,17} De Jonghe et al studied the *APP* mutations near the gamma secretase cleavage site.¹³ Missense mutations at codon 714–715 of *APP* decreased the secretion of Aβ40, and the mutations at codon 716–717 increased the production and secretion of

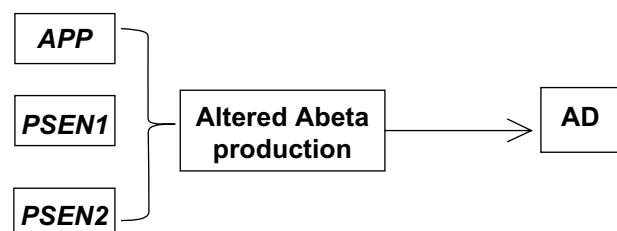


Figure 1 The *amyloid precursor protein (APP)*, *presenilin (PSEN) 1*, and *PSEN2* genes involved in early onset Alzheimer's disease (AD).

Abbreviation: Aβ, amyloid beta.

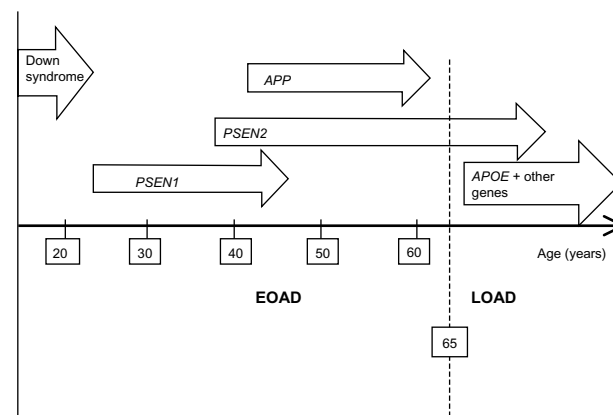


Figure 2 The age onset of Alzheimer's disease (AD), depending on the different involvement of genes. The symptoms of dementia can occur at an earlier age in Down syndrome patients than in AD patients without trisomy.

Abbreviations: APOE, apolipoprotein E gene; APP, amyloid precursor protein gene; EOAD, early onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; PSEN1/2, presenilin 1/2 gene.

Aβ42. This study suggests that gamma secretase cleavage might increase the ratio of Aβ42 to Aβ40.^{10–13,18}

Linkage analyses (1996) identified two highly homologous genes – *PSEN1* and *PSEN2* – that might be involved in the onset of AD.^{19,20} The structures of *PSEN1* and *PSEN2* are similar, with a homology of 67%. Both of them contain 12 exons with ten coding exons (exons 3–12) for a protein of ~450 amino acids. Presenilin 1 (PS1) and presenilin 2 (PS2) proteins are transmembrane (TM) proteins with at least seven TM domains.¹⁹ The function of presenilins was first described by Wolfe et al, who proposed that two transmembrane aspartate (257 and 385) residues in PS1 are critical in gamma secretase activity.²⁰ Most AD risk-factor mutations have been detected in *PSEN1* (approximately 30%–70% of early onset FAD), which is located on chromosome 14. More than 180 mutations were found in *PSEN1* in association with FAD, but they might be involved in sporadic AD or LOAD.¹⁴ Patients with *PSEN1* mutations might develop AD symptoms in their 40s or early 50s, with a few cases occurring in persons in their late 30s and early 60s. Several missense mutations in *PSEN1* can increase the production of Aβ42 and 40. In an alternative mechanism, the levels of Aβ42 and Aβ40 might be increased and decreased, respectively.²¹

PSEN2, on chromosome 1, is another risk-factor gene for AD, especially EOAD among a very small European population. The most well-known group with dementia from *PSEN2* mutation is families with Volga German ancestry. AD arising from *PSEN2* mutations can be highly variable, and may occur between the ages of 40 and 75 years.^{5,21,22} The first *PSEN2* mutation in AD patients was described in 1995.^{5,23–25} Patients with *PSEN2* mutation have not been reported in Korea,

the People's Republic of China, or Japan, but silent mutations have been detected in Japan.²⁶ A few *PSEN2* mutations, such as Leu143His or Arg143His, have not been associated with any neurodegenerative phenotype.²⁷ Two *PSEN2* mutations, Arg62His and Arg71Trp, may be involved in breast cancer, although the pathomechanism is not clear.²⁸ Table 1 summarizes all mutations described in *APP*, *PSEN1*, and *PSEN2* genes that may be involved in AD progression.

Late-onset Alzheimer's disease

In late-onset Alzheimer's disease (LOAD), several genes have been described as potential risk factors, but nongenetic factors may also be involved in the disease's progression (Figure 3).⁹ The *APOE* gene, located on chromosome 19, is an important genetic risk factor for LOAD, and its importance has been validated from population studies. Apolipoprotein E (ApoE) protein is the major cholesterol carrier in the brain, which can be involved in neuronal maintenance and repair. ApoE binds to several receptors on the cell surface, which are involved in lipid delivery and transport, glucose metabolism, neuronal signaling, and mitochondrial function. Normally, ApoE binds to Abeta peptide and play a role in its clearance.¹⁴¹

Two polymorphic sites, located at codon 112 and 158, have been described in the human *APOE* gene. At least three main variations of the *APOE* gene have been identified, called "E2," "E3," and "E4" alleles. E3 was defined as a normal allele with Cys at codon 112 and Arg at codon 158. Two other *APOE* alleles have been described, the E2 and E4 alleles, which carry Arg158Cys and Cys112Arg polymorphisms, respectively.^{142,143} Six different genotypes can be distinguished with the following combinations: homozygous – E4/E4, E3/E3, and E2/E2 – and heterozygous – E2/E3, E2/E4, and E3/E4 (Table 2). E3 is the most common variant (77%), while E2 (8%) and E4 (15%) alleles have been detected less frequently. Higher frequencies of the E4 allele have been found among AD patients, and increased risk of AD can be found in patients with both homo- and heterozygous alleles.¹⁴¹ The pathogenic nature of the E4 allele might be associated with the structural change of ApoE protein. ApoE protein has two major functional domains: a 22 kDa N-terminal and a 10 kDa C-terminal domain, connected by a hinge region. The E4 allele can promote domain interactions through the altered orientation of Arg61 in the N-terminal domain. Arg112 can interact with the Glu255 in the C-terminal domain, resulting in structural changes to ApoE protein, neuronal death, and neurodegeneration. Mouse experiments revealed that the

mutation of Arg61 to Thr, or of Glu255 to Ala, may reduce the domain interactions.^{144–148} Figure 4 shows the differences between the E3 and E4 alleles.

The prevalence of the E2 allele has been found to be significantly lower in individuals with dementia.¹⁴⁸ E2 allele was suggested to be protective against AD.¹⁴⁵ Further, *APOE* E2 and E3 may participate in neuronal maintenance and repair.¹⁴⁵ A Korean study detected significant correlation between the *APOE* E4 allele and AD.¹⁴⁹ Genotyping analysis was performed in a group of AD patients and healthy individuals (controls). The allele and genotype frequency were compared using chi-square and Fisher's exact tests. The frequency of the *APOE* E4 allele in the EOAD and LOAD groups was significantly higher than in the control group. However, the study failed to find any difference in the E2 allele between AD patients and controls. These findings suggest that the E2 allele might not play a protective role against AD in Korea.¹⁴⁹

Genome-wide association studies (GWASs) have identified novel genes that might be associated with LOAD. Recently, SNP arrays have been developed and used for the analysis of several genes and SNPs. GWASs have been successfully applied to complex polygenic disorders, such as diabetes and macular degeneration.^{150,151} Several papers have been published on the association between AD and different genes or alleles. Bertram et al have created a publicly available, constantly updated, database summarizing the potential genes that may be related to AD (<http://www.alzgene.org>).¹⁵² Systematic meta-analyses were performed for each polymorphism with all genotype data described for them. At least three case-control samples were tested. This database collected all potential genes that may be involved in AD onset, thus is a powerful tool to further the understanding of AD genetics. Additionally, it may be considered a model for tracking gene candidates in other polygenic disorders.^{152,153}

Clusterin (*CLU*) is a major inflammatory-related apolipoprotein (Apolipoprotein J; ApoJ) that is expressed in all mammalian tissues. Clusterin may play a protective role against apoptosis, cell damage, or oxidative stress. Clusterin expression has been found to be upregulated in the brains of AD patients.¹⁵⁴ Animal models have suggested it might be secreted with soluble Abeta. Clusterin can act as a molecular chaperon, which might prevent Abeta oligomerization and fibrillization.¹⁵¹ GWASs have determined a strong association between *CLU* mutations (located on chromosome 8) and LOAD. Additionally, a significant association has been found between the *APOE* E4 allele and *CLU* mutations.^{154,155}

The *complement receptor 1* (*CRI*) gene, located on chromosome 1, encodes the receptor for C3b complement

Table I The known Alzheimer's disease risk-factor mutations in *APP* and *PSEN1*–2

Gene	Exon	SNP	Country/countries	References	
APP	17	Ala692Gly	The Netherlands, Belgium	17,29	
		Glu693Gln	The Netherlands	30	
		Glu693Gly	Arctic, USA	31	
		<u>Glu693del</u>	<u>Japan</u>	16	
		Ala713Thr	France, Italy, Spain	32	
		Thr714Ala	Iran	33	
		Thr714Ile	Austria	34	
		Val715Met	Britain, France, Korea	21,26,36	
		Val715Ala	Germany, UK	13,36	
		Ile716Val	USA, UK	36	
		Ile716Phe	Spain	37	
		Ile716Thr	Italy	14	
		<u>Val717Ile</u>	<u>UK, Germany, Japan</u>	38,39	
		Val717Leu	USA, Belgium, Germany	40	
		<u>Ile718Leu</u>	<u>China, Taiwan</u>	41	
		<u>Leu720Ser</u>	<u>China, Taiwan</u>	41	
		<u>Val710Gly</u>	<u>China, Taiwan</u>	41	
		Val717Phe	USA	42	
		Val717Gly	UK, France	43	
		Leu723Pro	Australia	44	
		Lys724Asn	Belgium	45	
		16	<u>Asp678Asn</u>	<u>Japan</u>	46
			Lys670Asn	Sweden	15
			Met671Leu	Sweden	15
			Glu682Asn	Belgium	47
	PSEN1		4	Ala79Val	Belgium, Germany
		Val82Leu		France	51
		Met83del		UK	52
		<u>Leu85Pro</u>		<u>Japan</u>	53
		Val89Leu		Spain	54
Cys92Ser		Italy		14,55	
Val94Met		Colombia		56	
<u>Val96Phe</u>		<u>Japan</u>		57	
<u>Val97Leu</u>		<u>China</u>		58	
Phe105Ile		France		59	
Phe105Val		Spain		60	
Phe105Leu		Germany		49	
Leu113Gln		Germany		42	
Leu113Pro		France		61	
PSEN1	IVS4	InsTAC	USA, UK	50	
PSEN1	5	Tyr115His	France	49	
PSEN1		Tyr115Cys	Canada, Belgium, UK	48	
Thr116Asn		Denmark, France, Italy	37,60		
Thr116Ile		France, Italy	60,62		
Pro117Ala		France, USA	63		
Pro117Ser		USA	64		
Pro117Arg		Poland, Spain	60,65		
Pro117Leu		Poland, Italy, USA	66		
Glu120Lys		Denmark, USA	67		
Glu120Gly		Spain	60		
Glu120Asp		USA, France, Israel	51,59		
Asn135Asp		USA	68		
Asn135Ser		Germany, USA	42		
<u>Ala136Gly</u>		<u>China</u>	58		

(Continued)

Table 1 (Continued)

Gene	Exon	SNP	Country/countries	References
		<u>Glu123Lys</u>	Japan	69
		Met139Val	USA, Finland, Denmark, Germany, Poland, Sweden	65,67
		Met139Lys	France	70
		Met139Thr	France, Spain	51
		Met139Ile	Korea, USA	71
		Ile143Phe	UK	72
		<u>Ile143Thr</u>	France, Japan, Columbia	50,56
		Ile143Val	Italy	73
		Ile143Met	South Africa	14
		Ile143Asn	France	59
		Met146Leu	Italy, USA, France, Canada	21,42
		Met146Val	Sweden, Canada	50
		Met146Ile	Denmark, UK, Sweden	36,50
		Thr147Ile	France	21
		Leu153Val	France, UK	36
		<u>Tyr154Asn</u>	Japan	74
		Tyr154Cys	UK	36
		InsFI	Canada, Italy	50
	6	His163Tyr	Sweden, UK	75
		His163Arg	Korea, France, Japan	8,26,76
		His163Pro	Korea	77
		<u>Trp165Gly</u>	Japan	78
		Trp165Cys	France	21
		Leu166del	UK	79
		Leu166His	Italy	80
		Leu166Pro	Germany	81
		Leu166Arg	Spain	82
		Ile167del	UK	36
		Ile168del	UK	36
		Ser169Pro	Spain	82
		<u>Ser169Leu</u>	Japan	83
		<u>Ser169del</u>	China	84
		Ser170Phe	USA, Italy, Poland	85
		Leu171Pro	UK, Mexico	36
		Leu173Trp	France	21
		<u>Leu173Phe</u>	Japan	86
		Leu174Met	Italy	14
		Leu174Arg	Germany	87
		Phe177Leu	France, Canada	50
		Phe177Ser	Canada	50
		Ser178Pro	Canada	50
		Gly183Val	Belgium	88
	7	<u>Glu184Asp</u>	Japan, UK	89
		Val191Ala	Spain, Africa, USA	37
		Gly206Ser	Korea, France, Canada	35,50
		Gly206Asp	France	59
		Gly206Ala	Spain, Canada	50
		Gly206Val	USA	90
		<u>Gly209Arg</u>	Japan	91
		Gly209Glu	Canada	50
		Gly209Val	USA	92
		Ser212Tyr	USA	93
		Ile213Leu	Canada	50
		Ile213Pro	Poland	65
		<u>Ile213Thr</u>	Japan	57
		His214Asp	Spain	37

(Continued)

Table 1 (Continued)

Gene	Exon	SNP	Country/countries	References
		His214Tyr	France	59
		Gly217Arg	USA	94
		<u>Gly217Asp</u>	<u>Japan</u>	95
		Leu219Phe	Italy	14
		Leu219Pro	Australia	96
		Gln222Arg	Canada	50
		Gln222His	USA	97
		Gln223Arg	Germany	98
		Leu226Phe	Poland, Spain	99
		Leu226Arg	USA	100
		Ile229Phe	UK	36
		Ala231Thr	France, Canada	21,50
		Ala231Val	Belgium	48
		Met233Val	USA	101
		Met233Thr	France, Australia, Korea	21,35,50
		Met233Leu	Italy	102
		Met233Ile	France	103
		Leu235Val	UK	36
		Leu235Pro	France	21,50
		<u>Phe237Ile</u>	<u>Japan</u>	104
		Phe237Leu	UK	36
		Lys239Asn	Spain	105
		Thr245Pro	USA	106
		Ala246Glu	Poland, Canada	107
		Leu248Arg	Spain	93
		<u>Leu250Val</u>	<u>Japan</u>	108
		Leu250Ser	USA, UK	67
		Tyr256Ser	USA	97
	lvs8-lvs9	<u>9del</u>	<u>UK, USA, Japan</u>	14,109
		9del	Finland	110
	IVS8	c.869-22_869-23ins18	France	111
	8	<u>Ala260Val</u>	<u>Canada, Japan, UK, USA</u>	25,36,112
		Val261Leu	Spain	60,113
		Val261Phe	Canada	50
		Leu262Phe	Sweden	114
		Cys263Arg	Italy	115
		Cys263Phe	UK, Belgium	36
		Pro264Leu	France, USA	21,59
		<u>Gly266Ser</u>	<u>Japan</u>	116,117
		Pro267Ser	Sweden, UK	67
		Pro267Leu	Poland	107
		Arg269Gly	Spain, UK	118
		<u>Arg269His</u>	<u>Japan, Spain, UK</u>	26,60
		Leu271Val	Australia	119
		Val272Ala	Spain	93
		<u>Glu273Ala</u>	<u>Japan</u>	26
		Thr274Arg	Canada	50
		Arg278Thr	Australia	120
		Arg278Ser	UK	121
		Arg278Lys	Italy	122
		Arg278Ile	UK	123
		<u>Glu280Ala</u>	<u>Japan, Australia, Sweden, Britain</u>	120
		Glu280Gly	France, Sweden, Britain, USA	25,60
		Leu282Val	Belgium	124
		<u>Leu282Phe</u>	<u>Japan</u>	125
		Leu282Arg	Spain	60
		Pro284Leu	<u>Japan</u>	109

(Continued)

Table I (Continued)

Gene	Exon	SNP	Country/countries	References
PSEN2	9	<u>Ala285Val</u>	Japan, Canada	126
		<u>Leu286Val</u>	Japan, Canada	127
		Leu286Pro	Spain	128
		Thr291Pro	France	111
	10	Arg358Gln	Canada	50
		Ser365Ala	Spain	93
	11	Arg377Met	UK	36
		<u>Gly378Glu</u>	Germany, Japan	127
		Gly378Val	Australia	36
		<u>Leu381Val</u>	Japan, Bulgaria,	129
		<u>Gly384Ala</u>	Japan, Belgium	26,130
		Phe386Ser	France	59
		Ser390Ile	France	21
		Val391Phe	France	21
		<u>Leu392Val</u>	France, Japan	21,127
		Leu392Pro	Italy	14
		Gly394Val	Canada, Italy	50
	12	<u>Asn405Ser</u>	Japan	131
		Ala409Thr	Italy	102
		Cys410Tyr	France, Canada	21
		Leu418Phe	Canada	50
		Leu420Arg	USA	132
		Leu424Val	Spain	133
		Leu424Phe	Bulgaria	14
		Leu424His	France, Poland	59,99
		Leu424Arg	Poland	107
		Ala426Pro	USA	92
		Ala431Glu	USA	50
		<u>Ala431Val</u>	Japan	134
		Ala434Cys	Canada, USA	50
		Leu435Phe	Canada	50
		Pro436Ser	UK	72
		Pro436Gln	The Netherlands	135
		Ile439Ser	Spain	60
		<u>T440del</u>	Japan	29
	4	Arg71Trp	Spain	37
		Ala85Val	Spain	136
	5	Thr122Pro	Germany	42,49
		Asn141Ile	Germany, Canada	25,42
	6	Val148Ile	Spain	137
		Met174Val	Spain	93
		Ser175Cys	Italy	138
	7	Gln228Leu	Poland	65
		Met239Val	Italy	25
	12	Met239Ile	Germany	139
		Thr430Leu	Spain	82
		Asp439Ala	Spain	82,140

Notes: Underlined mutations were discovered in Asia; **emboldened** mutations were discovered in Korea. Reproduced from Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. *Hum Mutat.* 2012;33(9):1340–1344.¹⁴ © 2012 Wiley Periodicals, Inc.

Abbreviations: APP, amyloid precursor protein; PSEN, presenilin; SNP, single-nucleotide polymorphism.

protein. CR1 and C3b can be involved in Abeta clearance and in the prevention of Abeta aggregation. Risk-factor mutations for LOAD have been found in *CR1* (rs6656401 and rs3818361).¹⁵⁵ The functional role of *CR1* mutations in AD pathogenesis is not determined yet, and further studies are needed to find out the effect in Abeta deposition.^{155,156}

Phosphatidylinositol binding clathrin assembly protein (*PICALM* or *CALM*), located on chromosome 11, may be a putative LOAD risk-factor gene. *PICALM* can play a role in APP endocytosis and Abeta generation. Additionally, its overexpression may increase Abeta cleavage and aggregation.¹⁵⁷ Harold et al found strong association between

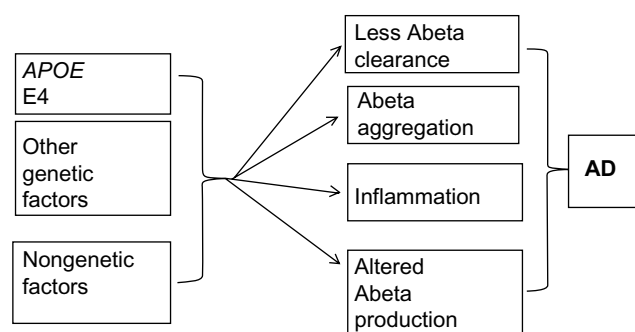


Figure 3 Factors involved in late-onset Alzheimer's disease (AD).

Abbreviations: Abeta, amyloid beta; APOE, apolipoprotein E.

two polymorphisms in *PICALM* and *LOAD*. Rs561655 is located within a transcription factor-binding site, and a silent mutation, rs592297, may be involved in the alternative splicing.¹⁵⁸ Other SNPs in *PICALM* have also been suggested to be involved in *LOAD*, such as rs3851179 and rs541458.¹⁵⁸

Sortilin-related receptor (SORL1) on chromosome 11q23-24 may be involved in Abeta recycling. The under-expression of *SORL1* can increase Abeta generation. Intronic polymorphisms, located near the 3' end of the *SORL1* coding region, might be associated with AD.^{159,160}

A poly-T repeat (rs10524523) was identified in exon 6 of the *translocase of outer mitochondrial membrane 40 homolog (TOMM40; chromosome 19)* gene that can be associated with an earlier age of onset of *LOAD* in patients with *APOE* E3/E3 and E3/E4 alleles. Cruchaga et al suggested that *TOMM40* and other mitochondrial enzymes might be involved in the onset of *LOAD*.¹⁶¹

Bridging Integrator 1 (BIN1; chromosome 2) is a tumor suppressor gene that can be involved with protein for vesicle trafficking. Mutations in *BIN1* may be associated with autosomal recessive centronuclear myopathy. *Caenorhabditis elegans* experiments have suggested that *BIN1* protein might have a role in trafficking APP, ApoE proteins, and Abeta through the endolysosomal pathways, thus *BIN1* mutations may be a putative risk factor for *LOAD*.¹⁶²

Table 2 The six genotypes of the apolipoprotein E (*APOE*) gene

Alleles	Polymorphisms	
Homozygous	E2/E2	Cys 112, Cys 158
	E3/E3	Cys 112, Arg158
	E4/E4	Arg112, Arg158
Heterozygous	E2/E3	Cys112, Cys158, Arg158
	E2/E4	Cys112, Cys158, Arg112, Arg158
	E3/E4	Cys112, Arg112, Arg158

Note: Data from Rihn et al.¹⁴³

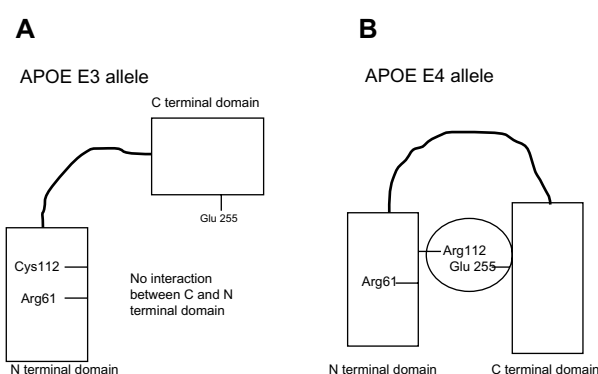


Figure 4 The difference between apolipoprotein E (*APOE*) protein E3 allele (A) and *APOE* E4 allele (B). The pathomechanism of the *APOE* E4 allele could be based on the interaction between Arg112 and Glu255.

Notes: Reproduced with permission from Mahley RW, Huang Y. Alzheimer disease: multiple causes, multiple effects of apolipoprotein E4, and multiple therapeutic approaches. *Ann Neurol*. 2009;65(6):623–625.¹⁴⁴ Copyright © 2009 American Neurological Association. Reproduced with permission from Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2006;103(15):5644–5651.¹⁴⁶ Copyright (2006) National Academy of Sciences, USA.

The low-density lipoprotein receptor-related protein 6 (*LRP6*) gene on chromosome 12 is expressed as a co-receptor for Wnt signaling. Defects in Wnt signaling have been validated as risk factors for neurodegenerative disorders such as schizophrenia, autism, and AD. Wnt signaling proteins, such as beta-catenin or glycogen synthase kinase 3 beta, can form complexes with presenilins, which suggests they might play an important role in Abeta processing and neurotoxicity. Genetic linkage studies have suggested an association between *LOAD* and chromosome 12. Polymorphisms in *LRP6* might result in abnormalities in plasma ApoE catabolism and in Wnt signaling.¹⁶³

The *cadherin-associated protein alpha 3 (CTNNA3)* gene located on chromosome 10 encodes alpha-T catenin, which can be involved in AD pathogenesis by binding to beta-catenin and interacting with PS1. Miyashita et al identified seven putative *LOAD* risk-factor polymorphisms located at intron 9 of *CTNNA3*.¹⁶⁴ Polymorphisms in *CTNNA3* have shown significant association with *LOAD* in female patients, who carried the *APOE* E3 allele, but not the E4.^{164,165}

Growth factor receptor-bound protein 2-associated-binding protein 2 (*GAB2*) molecules are intracellular docking or scaffolding molecules. *GAB2* can be involved in several signal transduction processes, associated with cell growth, survival, differentiation, and apoptosis. *GAB2* might play a role in the suppression of Tau phosphorylation and in neurofibrillary tangles (NFTs) formation. Reiman et al detected six SNPs in *GAB2* (chromosome 11) which might be associated with *LOAD*.¹⁶⁶ Interaction was found between *GAB2* haplotypes and the *APOE* E4 allele.^{166–168}

Dynamin-binding protein (DNMBP) or Tuba protein plays a role in the transport of dynamin to the actin regulatory proteins. A Belgian study found a significant association between two SNPs (rs3740057 and rs10883421) in the 3' region of the *DNMBP* (chromosome 10) gene and LOAD.¹⁶⁹

The *A disintegrin and metalloproteinase domain-containing protein 10* (*ADAM10*; chromosome 15) gene encodes the major brain alpha secretase. Alpha secretase cleavage can prevent Abeta formation and aggregation, and increase Abeta clearance. In vitro and in vivo studies have shown that two mutations (Gln171Gly and Arg181Gly) in the pre-domain region of *ADAM10* may be associated with AD.¹⁷⁰

ATP-binding cassette transporter A7 (*ABCA7*), located on chromosome 19, is a recently discovered potential risk factor for AD. *ABCA7* protein, which is highly homologous to *ABCA1*, may be involved in the synthesis and transport of high-density lipoprotein cholesterol and generate phospholipid and cholesterol efflux from the cells. It can also play a key role in sterol homeostasis and in the host defense system.^{171,172} The two variants (rs3752246 and rs3764650) in *ABCA7* have been suggested to be associated with LOAD.¹⁷¹ Rs3764650 is located in intron 13, and rs3752246 is a missense mutation in exon 32 (Gly1527 Ala).¹⁷¹ Recent findings have revealed an additional SNP (rs115550680) that might be involved in LOAD in African-Americans. Since *ABCA7* plays a role in the lipid metabolism as well as in APP transport, mutations in *ABCA7* gene might be involved in LOAD.¹⁷³

Recent GWASs have revealed that triggering receptor expressed on myeloid cells 2 (*TREM2*), located on chromosome 6 can be involved in AD, especially in LOAD. *TREM2* is a member of immunoglobulin family, and it contains a single variable domain. *TREM2* is located on the membrane of several immune cells, such as macrophages and dendritic cells. Its main ligand is DNA clamp loader is Replication Factor C-activating protein of 12 kilodaltons (DAP12), which can be involved in downstream signaling. Functions of *TREM2* protein can include the clearance of apoptotic cells and immunosuppression.¹⁷⁴ In an Icelandic population, a rare variant (Arg47His) has been suggested to increase the risk of impairment in inflammation, leading to LOAD.¹⁷⁵ Other variants located in exon 2 have been shown higher percentage in AD patients, such as Glu33X or Asp87 Asn. AD, associated with *TREM2* can be associated with chronic brain inflammation with aberrations in microglial phagocytosis or inflammatory pathways.¹⁷⁶

Cluster of differentiation 33 (CD33; chromosome 19) is a 67 kDa transmembrane glycoprotein that is expressed on

the surface of myeloid progenitor cells, mature monocytes, and macrophages. It can function as a lectin, a carbohydrate-binding protein, which inhibits cellular activity. The *CD33* locus is related to altered monocyte function, which suggests it can be involved in innate immunology, leading to AD progression. Rs3865444 can be associated with elevated CD33 expression, leading to cognitive decline and AD. Mutations in *CD33* can be associated with disturbances in myeloid function and amyloid pathology, thus may be involved in the progression of early AD.¹⁷⁷

Methods of detecting mutation

PCR-based methods can be performed for monitoring the mutations in the AD risk factor genes (Figure 5).¹⁷⁸ Genomic DNA can be extracted from total blood, buffy coat (white blood cells), bone marrow, or cell cultures, using a specific extraction kit. DNA should be amplified by specific primers, designed for the AD risk-factor genes such as *APP*, *PSEN1*, *PSEN2*, and *APOE*.^{6-8,22,26} Several mutation detection methods have been developed, such as restriction fragment length polymorphism (RFLP), single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and heteroduplex analysis. RFLP is based on the recognition of a specific cleavage site and can be used for genetic mapping and linkage analysis. To identify the polymorphisms in the PCR products, the amplicons should be sequenced.¹⁷⁸

Methods based on the conformational changes of single-stranded DNA

DGGE is a rapid, commonly used method for mutation detection. The technology is based on the mobility of

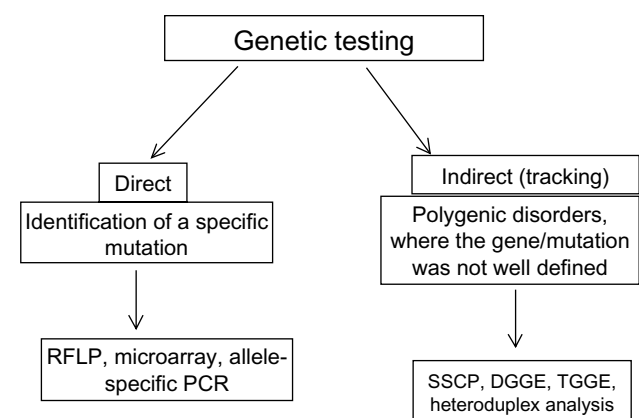


Figure 5 Polymerase chain reaction (PCR)-based genetic methods.

Abbreviations: DGGE, denaturing gradient gel electrophoresis; RFLP, restriction fragment length polymorphism; TGGE, temperature gradient gel electrophoresis; SSCP, single-strand conformation polymorphism.

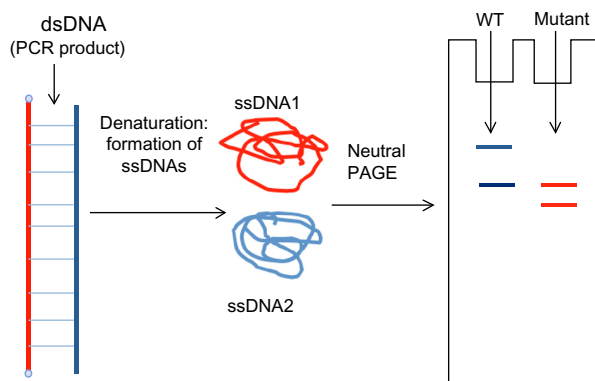


Figure 6 The single-strand conformation polymorphism process. After denaturation of the polymerase chain reaction (PCR) product, the conformation of single-stranded DNA (ssDNA) could be different, resulting in altered mobility in polyacrylamide gel. **Abbreviations:** dsDNA, double-strand DNA; PAGE, polyacrylamide gel electrophoresis; WT, wild type.

double-stranded DNA in polyacrylamide gel containing linearly increasing concentrations of denaturing chemicals.^{179,180} SSCP is a simple PCR-based mutation detection method. The mobility of double-stranded PCR fragments depends on the size of the DNA, since the polymorphisms might result

in the altered mobility of single-stranded DNA by changing its conformation (Figure 6). The PCR products should be denatured by heat and formamide, followed by neutral polyacrylamide gel electrophoresis.^{181,182}

Heteroduplex analysis with Surveyor[®] Nuclease

Surveyor Nuclease (Transgenomic, Inc, Omaha, NE, USA) is a plant (celery) endonuclease that cleaves double-stranded DNA at mismatch sites, including SNPs, insertions, and deletions. A novel PCR-based mutation detection method has been developed by Transgenomic. The process has four main steps: 1) amplification of target DNAs from patients and healthy controls; 2) hybridization of normal DNA with the DNA of the patient; 3) digestion of homo- and heteroduplexes by Surveyor Nuclease; and finally, 4) separation of cleavage products by standard gel electrophoresis or high-pressure liquid chromatography (Figure 7). This method may be promising in molecular diagnosis, and it has been successfully used for the identification of genetic-based disorders.^{183–185}

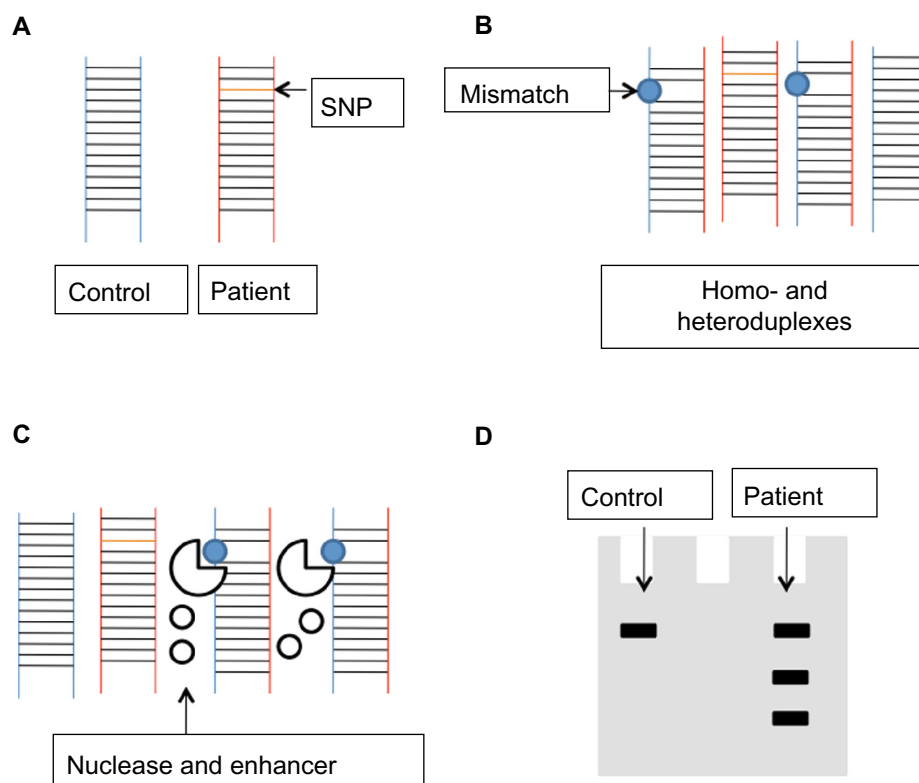


Figure 7 The basic steps of genotyping with Surveyor[®] Nuclease (Transgenomic, Inc, Omaha, NE, USA). After mixing the polymerase chain reaction amplicons of healthy control and patient (A), hybridization should be performed, resulting in homo- and heteroduplex formation (B). Treatment with Surveyor Nuclease cleaves the DNA at the mismatch site (C). Cleavage products can be separated by electrophoresis (D). **Abbreviation:** SNP, single-nucleotide polymorphism.

APOE genotyping

Allele-specific, multiplex PCR has been developed for *APOE* genotyping, with common and specific inner primers for polymorphism detection at codons 112 and 158. The agarose electrophoresis pattern can show the homozygous and heterozygous genotypes of E2, E3, and E4 alleles.¹⁸⁶ Various kits have been designed for *APOE* PCR genotyping. One of the most frequently used kits is the LightCycler® ApoE Mutation Kit by Roche Diagnostics (Basel, Switzerland).¹⁸⁷ PCR-RFLP is a widely used, simple and fast method for *APOE* genotyping. The genomic DNA should be amplified with specific primers, followed by *HhaI* digestion. The samples can be separated in 8% polyacrylamide (PAGE) gel, and visualized with fluorescent dye.¹⁸⁸

Future insights into AD genetics: from GWASs to next-generation sequencing (NGS)

Since AD is a genetically heterogeneous disorder, GWASs have been performed for identification of novel disease risk-factor loci. Several genes and mutations have been tested to find association with disease-related phenotypes, such as changes in biomarker levels and/or neuropathology.¹⁸⁹ Sanger sequencing is a widely used technology, but it has limitations in terms of cost, speed, and efficacy. High-throughput or NGS technologies are recent hot topics in genomic research of animals and humans. NGS technologies included sequencing by synthesis, ligation, or hybridization; single-molecule sequencing; nanopore sequencing; and colony sequencing. NGS technologies provide fast and cost-effective sequencing strategies that can be used in various genetic applications; for example, in high-throughput mutation detection, small RNA detection, or the monitoring of epigenetic changes. The most well-known NGS technologies have been developed by Illumina (and Solexa, Inc, purchased by Illumina in 2007; San Diego, CA, USA), Helicos BioSciences (Cambridge, MA, USA), ABI/SOLiD, and 454 Life Sciences (a subsidiary of Roche; Branford, CT, USA) and use a single-molecule template for mutation detection with cloning-free approaches.^{190,191}

Jin et al performed pooled DNA sequencing with *APP*, *PSEN1*, *PSEN2*, *progranulin (PGRN)* and *microtubule-associated Tau protein (MAPT)* genes that was applied in a large population for monitoring rare human-specific mutations.¹⁹² Samples were collected from selected groups of patients and pooled in complex mixtures with negative control samples (validated as wild-type alleles). The mixes

were then sequenced by NGS analyzers. The sequencing data were mapped back to the sample and to the control as reference. The pooled sequencing analysis detected *PGRN* and *MAPT* mutations in patients with clinically diagnosed AD. These findings show that the clinical phenotype of amnesic frontotemporal dementia and that of AD may be similar, and the overlapping symptoms can result in difficulties in the disease diagnosis. Complex genetic analysis might improve the diagnosis of neurodegenerative disorders.^{192,193}

It has been suggested that the development of the human brain depends on the level of transcription. Alterations in transcription regulation are responsible for the unique gene expression patterns in the brain. Aging is the main risk factor for AD, but normal aging itself can result in only a low degree of neuronal loss. Alternative splicing and gene expression may be involved in AD pathogenesis. Microarrays are widely used for transcriptome analysis, but their accuracy might be limited because of mistakes in hybridization. Transcriptome studies have been performed in animals, various cell lines, cells derived from AD patients, and in postmortem brain tissues. Twine et al performed a whole-transcriptome analysis in different regions of an AD brain.¹⁹⁴ Illumina RNA-Seq analysis was used for whole-transcriptome profiling. This study provided a possible insight into the changes in gene expressions and alternative splicing. NGS can produce digital signals directly from the complementary DNA, decrease the risk for false-positive data, and correspond to the existing genomic sequence.^{194,195}

Conclusion

AD is the most common form of senile dementia, but it can sometimes be difficult to distinguish heterogeneous neurodegenerative disorders, such as frontotemporal dementia, dementia with Lewy Bodies, Parkinson's disease, and Creutzfeldt-Jakob disease.⁵ AD is a complex disorder, so several genes on different chromosomes could be involved in its onset. Finding the potential genes involved in AD progression is an essential step in molecular diagnosis. Genetic testing should be important to understand the mechanisms and pathways leading to neurodegeneration and disease symptoms. It is believed that disease-modifying therapies are more likely to be effective in the earlier stages of AD, especially before the clinical symptoms appear. Genetic testing in the family members of patients should also be important to predict the risk for disease onset in the future. Using disease markers with genetic testing together may provide more effective disease diagnosis. In addition, the discovery of novel genes may provide more information on AD-related pathways.^{9,25,196,197}

Genetic analysis can improve the differential diagnosis of neurodegenerative dementias. Standard Sanger sequencing is still a widely used technology, but can be costly and time consuming. NGS technologies offer a faster, less expensive approach, not only for mutation detection but also for transcriptome analysis or epigenetics.¹⁹⁸ Several loci have been identified that might be involved in both familial and sporadic forms of neurodegenerative disorders. Understanding the complete genetic mechanisms of AD can provide additional information about the pathological mechanisms of neurodegeneration. GWASs and NGS studies may improve the prevention and treatment of AD.¹⁹⁹

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Disclosure

The authors declare no conflicts of interest in this work.

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