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 (CR1), 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...
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 (Abeta) production (both Abeta 40 and Abeta 42), 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 Abeta 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 Abeta 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 Abeta 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 Abeta peptide.13,14 N-terminal mutations in the Abeta sequence can affect the endosomal/lysosomal cleavage of Abeta, 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 Abeta 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 Abeta 40, and the mutations at codon 716-717 increased the production and secretion of Abeta 42. This study suggests that gamma secretase cleavage might increase the ratio of Abeta 42 to Abeta 40.10-13,18

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 Abeta 42 and 40. In an alternative mechanism, the levels of Abeta 42 and Abeta 40 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.
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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). 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.

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. 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. 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. 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. E2 allele was suggested to be protective against AD. Further, APOE E2 and E3 may participate in neuronal maintenance and repair. A Korean study detected significant correlation between the APOE E4 allele and AD. 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.

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. 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). 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.

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. Animal models have suggested it might be secreted with soluble Abeta. Clusterin can act as a molecular chaperon, which might prevent Abeta oligomerization and fibrilization. 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.

The complement receptor 1 (CR1) gene, located on chromosome 1, encodes the receptor for C3b complement
| Gene | Exon | SNP          | Country/countries                      | References |
|------|------|--------------|----------------------------------------|------------|
| APP  | 17   | Ala692Gly    | The Netherlands, Belgium               | 17,29      |
|      |      | Glu693Gln    | The Netherlands                        | 30         |
|      |      | Glu693Gly    | Arctic, USA                            | 31         |
|      |      | Glu693del    | Japan                                  | 16         |
|      |      | Ala713Thr    | France, Italy, Spain                   | 32         |
|      |      | Thr714Ala    | Iran                                   | 33         |
|      |      | Thr714Ile    | Austria                                | 34         |
| Val175Met | 16 | val715Met    | Britain, France, Korea                 | 21,26,36   |
|      |      | Val715Ala    | Germany, UK                           | 13,36      |
|      |      | lle716Val    | USA, UK                                | 36         |
|      |      | lle716Phe    | Spain                                  | 37         |
|      |      | lle716Thr    | Italy                                  | 14         |
|      |      | Val717le    | UK, Germany, Japan                     | 38,39      |
|      |      | Val717Leu    | USA, Belgium, Germany                   | 40         |
|      |      | lle718Leu    | China, Taiwan                          | 41         |
|      |      | lle720Ser    | China, Taiwan                          | 41         |
|      |      | Val710Gly    | China, Taiwan                          | 41         |
|      |      | Val717Phe    | USA                                     | 42         |
|      |      | Val717Gly    | UK, France                             | 43         |
|      |      | lle723Pro    | Australia                              | 44         |
|      |      | Lys724Asn    | Belgium                                | 45         |
|      | 16   | Asp678Asn    | Japan                                  | 46         |
|      |      | Lys670Asn    | Sweden                                 | 15         |
|      |      | Met671Leu    | Sweden                                 | 15         |
|      |      | Glu682Asn    | Belgium                                | 47         |
| PSEN1| 4    | Ala79Val     | Belgium, Germany                       | 48–50      |
|      |      | Val82Leu     | France                                 | 51         |
|      |      | Met83del     | UK                                     | 52         |
|      |      | lle85Pro     | Japan                                  | 53         |
|      |      | Val89Leu     | Spain                                  | 54         |
|      |      | Cys92Ser     | Italy                                  | 14,55      |
|      |      | Val94Met     | Colombia                               | 56         |
|      |      | Val96Phe     | Japan                                  | 57         |
|      |      | Val97Leu     | China                                  | 58         |
|      |      | Phe105Ile    | France                                 | 59         |
|      |      | Phe105Val    | Spain                                  | 60         |
|      |      | Phe105Leu    | Germany                                | 49         |
|      |      | Leu13Gln     | Germany                                | 42         |
|      |      | Leu13Pro     | France                                 | 61         |
| PSEN1| IVS4  | InsTAC      | USA, UK                                | 50         |
| PSEN1| 5    | Tyr115His    | France                                 | 49         |
|      |      | 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, USA                            | 66         |
|      |      | Glu120Lys    | Denmark, USA                           | 67         |
|      |      | Glu120Gly    | Spain                                  | 60         |
|      |      | Glu120Asp    | USA, France, Israel                    | 51,59      |
|      |      | Asn135Asp    | USA                                     | 68         |
|      |      | Asn135Ser    | Germany, USA                           | 42         |
|      |      | Ala136Gly    | China                                  | 58         |

(Continued)
Table 1 (Continued)

| Gene   | Exon | SNP    | Country/countries          | References |
|--------|------|--------|----------------------------|------------|
| Glu123Lys |      | Met139Val | USA, Finland, Denmark, Germany, | 65,67       |
|        |      |         | Poland, Sweden              |            |
| Met139Lys |      | Met139Thr | France                      | 70         |
| Met139Thr |      | Met139Lys | France, Spain               | 51         |
| Met139Lys | 59   |         | **Korea, USA**              |            |
| Ile143Phe |      | Ile143Thr | France, Japan, Columbia     | 50,56      |
| Ile143Thr |      | Ile143Val | Italy                       | 73         |
| Ile143Val |      | Ile143Met | South Africa                | 14         |
| Ile143Met |      | Ile143Asn | France                      | 59         |
| Met146Leu |      | Met146Val | Italy, USA, France, Canada  | 21,42      |
| Met146Val |      | Met146Ile | Sweden, Canada              | 50         |
| Met146Ile |      | Thr147Ile | France                      | 21         |
| Thr147Ile |      | Leu153Val | France, UK                  | 36         |
| Leu153Val |      | Tyr154Asn | **Japan**                   | 74         |
| Tyr154Asn |      | Tyr154Cys | **UK**                      | 36         |
| InsFl   | 65   |         | **Canada, Italy**           | 50         |
| His163Tyr |      | His163Arg | **Korea, France, Japan**    | 8,26,76    |
| His163Arg | 76   |         | **Korea**                   |            |
| His163Pro | 77   |         | **Korea**                   |            |
| Trp165Gly |      | Trp165Cys | **Japan**                   | 78         |
| Trp165Cys |      | Leu166del | **France**                  | 21         |
| Leu166del |      | Leu166His | **UK**                      | 79         |
| Leu166His | 80   |         | **Italy**                   |            |
| Leu166Pro |      | Leu166Arg | **Germany**                 | 81         |
| Leu166Arg | 82   |         | **Spain**                   |            |
| Ile167del | 36   |         | **UK**                      |            |
| Ile167del | 36   |         | **UK**                      |            |
| Ile168del | 36   |         | **Spain**                   |            |
| Ser169Pro |      | Ser169Arg | **Spain**                   | 82         |
| Ser169Arg |      | Ser169del | **China**                   | 84         |
| Ser169del |      | Ser170Phe | **USA, Italy, Poland**      | 85         |
| Ser170Phe |      | Leu171Pro | **UK, Mexico**              | 36         |
| Leu171Pro |      | Leu173Trp | **France**                  | 21         |
| Leu173Trp | 86   |         | **Italy**                   |            |
| Leu174Met |      | Leu173Phe | **Japan**                   |            |
| Leu174Phe |      | Leu174Arg | **Germany**                 | 87         |
| Leu174Arg | 50   |         | **France, Canada**          |            |
| Phe177Leu |      | Ser178Pro | **Canada**                  | 50         |
| Phe177Ser |      | Gly183Val | **Belgium**                 | 88         |
| Gly183Val | 89   |         | **Japan, UK**               |            |
| Val191Ala |      | Gly206Ser | **Korea, France, Canada**   | 35,50      |
| Gly206Ser | 77   |         | **France**                  |            |
| Gly206Asp |      | Gly206Ala | **Spain, Canada**           | 50         |
| Gly206Ala |      | Gly206Val | **USA**                     | 90         |
| Gly206Val |      | Gly209Arg | **Japan**                   | 91         |
| Gly209Arg | 91   |         | **USA**                     |            |
| Gly209Glu |      | Gly209Val | **USA**                     | 93         |
| Gly209Val |      | Ser212Tyr | **USA**                     | 93         |
| Ser212Tyr | 93   |         | **Canada**                  |            |
| Ile213Leu | 65   |         | **Poland**                  |            |
| Ile213Pro | 57   |         | **Japan**                   |            |
| Ile213Thr | 57   |         | **Spain**                   |            |
| His214Asp | 37   |         | **Spain**                   |            |
| Gene     | Exon | SNP      | Country/countries | References |
|----------|------|----------|-------------------|------------|
| His214Tyr |  | France   |                   | 59         |
| Gly217Arg |  | USA      |                   | 94         |
| Gly217Asp |  | Japan    |                   | 95         |
| Leu219Phe |  | Italy    |                   | 14         |
| Leu219Pro |  | Australia|                   | 96         |
| Gln222Arg |  | Canada   |                   | 50         |
| Gln222His |  | USA      |                   | 97         |
| Gln223Arg |  | Germany  |                   | 98         |
| Leu226Phe |  | Poland, Spain |             | 99         |
| Leu226Arg |  | USA      |                   | 100        |
| Ile229Phe |  | US       |                   | 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      |
| Phe237Ile |  | Japan    |                   | 104        |
| Phe237Leu |  | UK       |                   | 36         |
| Lys239Asn|  | Spain    |                   | 105        |
| Thr245Pro |  | USA      |                   | 106        |
| Ala246Glu |  | Poland, Canada |             | 107        |
| Leu248Arg |  | Spain    |                   | 93         |
| Leu250Val |  | Japan    |                   | 108        |
| Leu250Ser |  | USA, UK  |                   | 67         |
| Tyr256Ser |  | USA      |                   | 97         |
| **Ivs8-Ivs9** |  | **9del** |                   | 14,109     |
| 9del     |  | UK, USA, Japan |             | 110        |
| IVS8 8   |  | c.869-22_869-23ins18 | | 111        |
| 8       |  | France   |                   | 111        |
| Ala260Val|  | Canada, Japan, UK, USA |         | 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      |
| Gly266Ser|  | Japan    |                   | 116,117    |
| Pro267Ser|  | Sweden, UK |                   | 67         |
| Pro267Leu|  | Poland   |                   | 107        |
| Arg269Gly|  | Spain, UK |                   | 118        |
| Arg269His|  | Japan, Spain, UK |             | 26,60      |
| Leu271Val|  | Australia|                   | 119        |
| Val272Ala|  | Spain    |                   | 93         |
| Glu273Ala|  | Japan    |                   | 26         |
| Thr274Arg|  | Canada   |                   | 50         |
| Arg278Thr|  | Australia|                   | 120        |
| Arg278Ser|  | UK       |                   | 121        |
| Arg278Lys|  | Italy    |                   | 122        |
| Arg278Ile|  | UK       |                   | 123        |
| Glu280Ala|  | Japan, Australia, Sweden, Britain | | 120        |
| Glu280Gly|  | France, Sweden, Britain, USA |         | 25,60      |
| Leu282Val|  | Belgium  |                   | 124        |
| Leu282Phe|  | Japan    |                   | 125        |
| Leu282Arg|  | Spain    |                   | 60         |
| Pro284Leu|  | Japan    |                   | 109        |

(Continued)
| Gene   | Exon | SNP          | Country/countries | References |
|--------|------|--------------|-------------------|------------|
| Ala285Val | 9    | Japan, Canada | 126               |
| Leu286Val | 10   | Japan, Canada | 127               |
| Leu286Pro | 11   | Spain        | 128               |
| Thr291Pro |      | France       | 111               |
| Arg358Gln |      | Canada       | 50                |
| Ser365Ala |      | Spain        | 93                |
| Arg377Met | 11   | UK           | 36                |
| Gly378Glu |      | Germany, Japan| 127              |
| Gly378Val |      | Australia    | 36                |
| Leu381Val |      | Japan, Bulgaria | 129            |
| Gly384Ala |      | Japan, Belgium| 26,130           |
| Phe386Ser |      | France       | 59                |
| Ser390Ile |      | France       | 21                |
| Val391Phe |      | France       | 21                |
| Leu392Val | 12   | France, Japan | 21,127           |
| Leu392Pro |      | Italy        | 14                |
| Gly394Val |      | Canada, Italy | 50                |
| Asn405Ser | 12   | Japan        | 131               |
| Ala409Thr |      | Italy        | 102               |
| Cys410Tyr |      | France, Canada| 21               |
| Leu418Phe | 12   | Canada       | 50                |
| Leu420Arg |      | USA          | 132               |
| Leu424Val |      | Spain        | 133               |
| Leu424Pro |      | Bulgaria     | 14                |
| Leu424His |      | France, Poland| 59,99            |
| Leu424Arg |      | Poland       | 107               |
| Ala426Pro |      | USA          | 92                |
| Ala431Glu |      | USA          | 50                |
| Ala431Val | 12   | Japan        | 134               |
| Ala434Cys |      | Canada, USA  | 50                |
| Leu435Phe |      | Canada       | 50                |
| Pro436Ser | 5    | UK           | 72                |
| Pro436Gln |      | The Netherland| 135            |
| Ile439Ser |      | Spain        | 60                |
| T440del  | 4    | Japan        | 29                |
| PSEN2   |      |              |                   |
| Arg71Trp | 5    | Spain        | 37                |
| Ala85Val |      | Spain        | 136               |
| Thr122Pro |      | Germany      | 42,49             |
| Asn141Ile |      | Germany, Canada| 25,42         |
| Val148Ile |      | Spain        | 137               |
| Met174Val | 6    | Spain        | 93                |
| Ser175Cys |      | Italy        | 138               |
| Gin228Leu | 7    | Poland       | 65                |
| Met239Val |      | Italy        | 25                |
| Met239Ile | 12   | 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. © 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 rs381361).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
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: Cys112, Cys158; E3/E3: Cys112, Arg158; E4/E4: Arg12, Arg158 |
| Heterozygous | E2/E3: Cys112, Cys158, Arg158; E2/E4: Cys112, Cys158; E3/E4: Cys112, Arg112, Arg158 |

**Note:** Data from Rihn et al.\(^{143}\)

**Figure 3** Factors involved in late-onset Alzheimer’s disease (AD). **Abbreviations:** Abeta, amyloid beta; APOE, apolipoprotein E.

**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.

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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 CTNNA.\(^{164}\) 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 Tubα 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).173 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 (DAP12), which can be involved in downstream signaling. Functions of TREM2 protein can include the clearance of apoptotic cells and immunosupression.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 binding. 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
double-stranded DNA in polyacrylamide gel containing linearly increasing concentrations of denaturing chemicals.\textsuperscript{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.\textsuperscript{181,182}

**Heteroduplex analysis with Surveyor\textsuperscript{®} 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.\textsuperscript{183–185}

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**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.

**Figure 7** The basic steps of genotyping with Surveyor\textsuperscript{®} Nuclease (Transgenomic, Inc, Omaha, NE, USA). After mixing the polymerase chain reaction ampicons 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. 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). 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.

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. 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.

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. 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.

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. 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.

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. 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.
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. 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.

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The authors declare no conflicts of interest in this work.

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