Molecular genetics of Alzheimer's Disease

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Abstract

Alzheimer's Disease (AD) is the most common neurodegenerative disorder that leads to dementia in the elderly. Besides environmental factors, age is the most common and effective risk factor for AD development. In more than 90 per cent of cases AD develops after the age of 65 years (late-onset AD, LOAD) the majority being sporadic. But there is enough of evidence that AD has a genetic etiology. The molecular mechanism that leads to AD is yet unclear, but, to date three genes are identified that when mutated cause autosomal dominant early-onset AD (EOAD). These are β-Amyloid Precursor Protein (APP), Presenilin 1 (PSEN1) and Presenilin 2 (PSEN2). In addition, more than 100 genes have been associated with LOAD, as risk factors. However, only the E4 allele of the Apolipoprotein E gene (APOE) is accepted as the single most common genetic determinant of susceptibility to AD. Many researchers have attempted to find chromosomal locations either by linkage or by association studies. Full genome screens revealed many different loci on different chromosomes, but only some of them yielded positive results in at least three independent studies. The association of genes other than APP, PSENs and APOE with AD needs further investigation in different populations.

Key Words: Alzheimer's Disease, APP, presenilin, APOE, neurodegeneration

Alzheimer Hastalığı'nın moleküler genetiği

Özet


Anahtar Sözcükler: Alzheimer Hastalığı, APP, presenilin, APOE, nörodejenerasyon
Alzheimer's Disease (AD), a neurodegenerative disorder, is the most common cause of dementia in the elderly. The disease is clinically characterized by a gradual and progressive decline in intellectual functions such as deficits of recent memory and language, associated with visuospatial and attention problems. Besides, psychosis, depression, agitation and anxiety are common symptoms (Cummings, 1995). Following an extended period of loss of personality and cognition, the symptoms become increasingly distressing for the patient and for those who care for her/him. The disease often results in a state of complete dependency. Following diagnosis, the course of the disease varies considerably from a few years to over 20 years, with a mean survival of about 2-8 years according to age of onset (Wolfson, 2001; Larson et al., 2004). The patient dies because of intercurrent disease, and patients with AD have reduced survival compared to older persons without dementia.

In the AD brain, senile plaques and neurofibrillary tangles are the major pathological findings. Besides, there is a massive neuronal and dendritic loss, particularly in the temporal, parietal and entorhinal cortex, the hippocampus and amygdala (McKhann et al., 1984), resulting in a decreased activity of the enzymes, mainly choline acetyltransferase (Mirra et al., 1991). Atrophy also results in cortical thinning, and enlargement of the lateral cerebral ventricles.

Previous head injury, depression, low educational level, atherosclerosis and exposure to aluminum are found to be risk factors for AD (Breteler et al., 1992; Van Duijn, 1996; Hofman et al., 1997). Besides these factors, age is the most common and effective risk factor for AD development. AD is usually classified according to its age of onset. When the disease occurs before the age of 65, it is called early onset (EOAD) or “presenile AD”, while late onset (LOAD) or “senile AD” occurs after the age of 65. There are no prominent clinical or patho-logical differences between the EOAD and LOAD cases, except the age of onset. The prevalence of AD increases with age. It affects less than one per cent of 60-64 years olds, 7-10 percent of 65-74 years old and up to 40 per cent of those over ages 85 (Breteler et al., 1992; Hebert et al., 2003).

In aged, but not in young animals, chronic brain hypoperfusion results in cerebral microvascular pathology which includes pre- and post-synaptic changes, abnormalities in protein synthesis, dysregulation in energy metabolism, reduction of cerebral glucose utilization, vascular innervation especially the cholinergic breakdown, structural damage to capillaries in the cerebral cortex and visuospatial memory deficits (de la Torre and Fortin, 1994; de la Torre, 1997a; de la Torre, 1997b). AD brains reveal similar biochemical and structural changes as in experimentally induced aging animals. Regional cerebral hypoperfusion is one of the earlier clinical findings in all forms of AD. A variety of disorders with different etiologies that impair or diminish cerebral hypoperfusion are reported to be risk factors for AD (Claus, 1998). It is suggested that advanced aging in the presence of a vascular risk factor can create a critically attained threshold of cerebral hypoperfusion that triggers disturbances in regional brain microcirculation and impairs the delivery of energy substrates needed for normal brain cell function, resulting in metabolic, cognitive and tissue pathology that characterize AD (de la Torre, 2000).

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The majority of AD cases are late onset and sporadic. However, there is enough of evidence that genetic factors also play a role in the etiology of AD. A positive family history of dementia is one of the most consistent risk factors (Ott et al., 1998). The frequently used criterion of a positive family history of dementia, however, does not necessarily indicate genetic susceptibility. Clinically there is no difference between familial AD (FAD) and sporadic AD (Duara et al., 1993; Lippa et al., 1996), however, patients with familial AD may have more rapid disease progression and an earlier onset than sporadic AD (Duara et al., 1993).

In most of the families with multiple AD cases the inheritance pattern of AD is unclear. However, in a small number of pedigrees, the disease segregates in a manner consistent with a fully penetrant autosomal dominant trait resulting from a single gene defect. To date, three genes are identified that when mutated cause presenile AD: β-amyloid Precursor Protein gene (APP), Presenilin-1 gene (PSEN1) and Presenilin-2 gene (PSEN2). Mutations in these genes are by themselves sufficient to cause EOAD. No mutations in these genes have
been identified in LOAD patients with onset of the disease after 65 years of age. Mutations in PSEN1 are the most frequently seen in the EOAD families with a frequency of 18-50 per cent (Campion et al., 1995; Hutton et al., 1996; Cruts et al., 1998), followed by APP with five per cent (Van Broeckhoven, 1995) and PSEN2 with less than one per cent (Hutton et al., 1996; Sherrington et al., 1996). Together, these three genes account for 30-50 per cent of the families with autosomal dominant presenile AD, which represents about 1 per cent of the whole AD population.

In contrast to EOAD, in most LOAD families the inheritance pattern is less clear, suggesting that a complex interaction of genetic and environmental factors underlie the etiology of LOAD. Wide range of onset ages creates difficulty to distinguish between individuals who are at risk for the disease and who have escaped it. Another piece of genetic evidence shows the probable role of the ε4 allele of Apolipoprotein E gene (APOE) as a risk factor for late-onset sporadic or familial LOAD. To date, the presence of the ε4 allele is the single most common genetic determinant of susceptibility to AD. However, the presence of the ε4 allele alone is not sufficient to cause AD. Not everyone having the ε4 allele will develop the illness, and many who lack the allele will also develop AD.

The β-amylloid precursor protein gene (APP)

The APP gene (OMIM # 104760) maps to 21q21.2 (Tanzi et al., 1987). APP is a member of a family of evolutionarily conserved APP-like proteins. The promoter region shows typical features of a housekeeping gene that lacks the typical TATA and CAAT boxes and that has multiple transcription start sites (Salbaum et al., 1988). The β-Amloid Precursor Protein (app) is a single transmembrane protein; it has an approximately 23 residue hydrophobic stretch, located near its carboxyl-terminal, that serves to anchor app in the phospholipid bilayer of internal (e.g. Golgi, endosome) and external (plasma-lemma) membranes. With its small cytoplasmic C-terminal domain and a relatively large N-terminal domain, it appears to be a classic type-1 integral membrane glycoprotein.

Alternative transcription of three of the 18 exons results in the production of a variety of mRNA species that have been named according to the number of amino acids they encode (Salbaum et al., 1988; Robakis et al., 1987; Tanzi et al., 1988).

The app undergoes an extensive post-translational processing, including the addition of sugar, phosphate and sulfate groups to the protein. After its synthesis on ribosomes, it is cotranslationally translocated into the endoplasmic reticulum (ER) via its signal peptide and then it matures through the central secretory pathway. After its maturation app is endoproteolytically cleaved by α-, β- and γ-secretases between residues 16 and 17 of the Aβ region, which is located just a few amino acids before the beginning of the single transmembrane sequence. The α-secretase (TACE/ADAM17 and ADAM10) and β-secretase (BACE) enzymes are identified and presenilins are thought to play a role in γ-secretase activity (Vassar et al., 1999; Brou et al., 2000; Asai et al., 2003). The alpha-secretory cleavage done by the α-secretase enzymes results in a large soluble N-terminal fragment of app that is secreted into the lumen of vesicles and from the cell surface and a carboxy terminal fragment (CTF) of 83 amino acids (sometimes referred to as the 10 kDa CTF), which is retained in the membrane (Selkoe et al., 1988; Weidemann et al., 1989; Esch et al., 1990). The further cleavage of 10 kDa CTF by γ-secretase results in the generation of a 3 kDa fragment which is known as p3 and a 6kDa fragment p7. The app can also be cleaved by β-secretase just before aspartic acid_{1} (asp_{1}) of the Aβ region that results in a 12 kDa CTF. The further cleavage of the 12 kDa CTF by γ-secretase generates Aβ. Therefore Aβ is generated when app is sequentially cleaved by β- and γ-secretases. Both β- and γ-secretases do not cleave only at a single amino acid position. Aβ peptides found in the amyloid deposits of AD brains show both N- and C-terminal heterogeneity. In addition to asp_{1}, the N-terminus may begin at residue pyroglutamate_{3}, arginine_{5}, or glutamate_{11}. These N-terminal species are assumed to form by the modification of the Aβ N-terminus after an initial β-secretase cleavage at asp_{1}, but of course they can also form by direct endoproteolytic cleavages at those respective residues. C-terminal heterogeneity is more important than that of the N-terminal. Immunocytochemical studies have shown that the diffuse plaques, which are the earliest form of Aβ deposits to be detectable contain Aβ peptides with alanine_{42} C-terminal only. Aβ_{42} peptides can
form diffuse plaques as early as age 12 years, whereas \( A\beta_{40} \) peptides are first observed almost two decades later (Iwatsubo et al., 1994; Iwatsubo et al., 1995). Only a minority of total cellular \( app \) undergoes \( \alpha \)-secretase cleavage, but even a smaller proportion of total cellular \( app \) undergoes \( \beta \)-secretase cleavage. Not all of the 10 and 12 kDa CTFs are processed to form \( p3 \) and \( A\beta \), respectively; other nonspecific proteolytic pathways in lysosomes can degrade these CTFs to small peptides and individual amino acids (Golde et al., 1992; Haass et al., 1992).

\( app \) is highly conserved in evolution. Rat and human APP mRNAs are 97 per cent homologous (Kang and Müller-Hill, 1990). All 695 residues of \( app \) are identical in monkey and human (Podlisny et al., 1991), and close homologs have been found in \( Drosophila \) and \( C. elegans \) (Rosen et al., 1989; Daigle and Li, 1993). This high degree of conservation throughout evolution suggests that the protein may play an important physiological role.

The function of \( app \) is poorly understood. The secreted form of APP has protease inhibitor motifs, suggesting that longer isoforms can have the ability to inhibit certain serine proteases (Kitaguchi et al., 1988; Ponte et al., 1988). Secreted \( app \) has trophic effects on fibroblasts, PC12 cells, cortical and neuronal cells (Saitoh et al., 1989; Bhasin et al., 1991). Nerve growth factor (NGF) is found to modulate the expression and secretion of \( app \) in PC12 cells (Villa et al., 2001). It is suggested that secreted \( app \) can protect from the excitotoxic effects of glutamate by regulating intracellular calcium levels (Mattson et al., 1993). \( app \) also interacts with the extracellular matrix, where it takes a role in the guidance of neurites in the developing nervous system and during regeneration of neurites after injury. It has been shown that \( app \) promotes cell adhesion to the substrate (Bhasin et al., 1991). It interacts with heparan sulfate proteoglycan, collagen, and laminin, which are major protein components of the extracellular matrix that have been localized in amyloid plaques (Small et al., 1992; Narindrasorak et al., 1992; Buee et al., 1993). These findings suggest that \( app \) can function as an autocrine factor by stimulating cell proliferation and cell adhesion and by supporting NGF-induced neurite outgrowth of PC12 cells.

\( app \) interacts with a number of other proteins, including \( \alpha' \)-antichymotrypsin (Fraser et al., 1993), \( \text{serum amyloid-P} \) (Kalaria et al., 1991), \( \text{apolipoprotein E} \) (Richey et al., 1995) and \( \tau \) (Goate et al., 1991). This suggests that alterations in the interaction between \( \tau \) and \( app \) can be important in the development of AD (Smith and Perry, 1997). It was demonstrated that the cytoplasmic tail of \( app \) forms a multimeric complex with the nuclear adaptor protein \( Fe65 \) and the histone acetyltransferase \( TIP60 \). This complex stimulates transcription via Gal4 or LexA DNA binding domains, suggesting that the release of the cytoplasmic tail of \( app \) by \( \gamma \)-cleavage may function in gene expression (Cao and Sudhof, 2001; Baek et al., 2002).

To date, 28 different mutations of \( app \) have been identified in families with presenile AD or related disorders (Cruts, 2007). All mutations are located at or near the proteolytic cleavage sites involved in the generation of \( A\beta \). A mutation at codon 693 of exon 16, causing a glutamic acid to glutamine substitution results in hereditary cerebral hemorrhage with amyloidosis of the Dutch type, a rare autosomal dominant disorder found in few families in the Netherlands (Bakker et al., 1991). Another mutation that results in an Ala to Gly substitution at codon 692 in exon 17 was found in a family with a mixed phenotype, in which patients have cerebral hemorrhages or EOAD (Hendriks et al., 1992).

The mutations at codon 717 may act by affecting the activity of the factors that interact with the \( A\beta \) regions of \( app \), perhaps by increasing the amounts or type of \( A\beta \) produced. Assays designed to discriminate \( A\beta_{40} \) from \( A\beta_{42} \) indicate that the derivative in conditioned media of cells, transfected with wild type \( app \) is primarily \( A\beta_{40} \), while the introduction of the codon 717 mutation consistently results in a 1.5 to 1.9 fold increase in the percentage of \( A\beta_{42} \) generated (Suziki et al., 1994).

### The presenilin genes

The presenilin genes, Presenilin-1 (PSEN1, OMIM # 104311) and Presenilin-2 (PSEN2, OMIM # 600759) are members of a novel gene family with yet unclear function. They code for integral membrane proteins (\textit{psen1} and \textit{psen2}) of 467 and 448 amino acids, respectively, with 8 transmembrane domains (Doan et al., 1996); they are localized predominantly in the membranes of the rough endoplasmic reticulum (RER), and to a lesser extent...
in the early Golgi complex (Walter et al., 1996; Kovacs et al., 1996).

The PSEN1 is found to be located at 14q 24.3 (Van Broeckhoven et al., 1992). It spans about 70 kb, having 10 protein-coding exons. Several alternatively spliced PSEN1 messengers have been reported resulting from the use of an out-frame splice site in intron 9 or splicing at sites different from defined exon/intron boundaries (Cruts et al., 1996; Sahara et al., 1996; Anwar et al., 1996). Exon 3 of PSEN1 has two different splice donor sites 12 bp apart, and when alternatively spliced, this results in the presence or absence of a Val-Arg-Ser-Gln (VRSQ) motif in the N-terminal domain of psen1 (codons 26-29). This motif, when present, constitutes part for two putative phosphorylation sites for protein kinase C and casein kinase II, respectively. Both isoforms are present in brain tissues, fibroblasts and lymphoblasts, but the shorter isoform is 1.5 times more frequent than the longer transcript (Cruts et al., 1995; Alzheimer's Disease Collaborative Group, 1995).

The PSEN2 is located at 1q42.1. It has 10 coding and three non-coding exons identified in 5' UTR (Levy-Lahad et al., 1996). Although both presenilin genes are homologous, PSEN2 is much smaller than PSEN1 and spans 24 kb. The intron-exon boundaries of both PSEN genes are very similar, and in conserved regions of the genes the boundaries are identical, but intronic sequences immediately flanking the exons are different, excluding the possibility that PSEN1 and PSEN2 are the result of a recent gene duplication event.

In PSEN2, alternative splice forms have also been described that result from the alternative use of in-frame splice acceptor site in introns 9 and 10 (Sherrington et al., 1996; Prihar et al., 1996). The two splice events occur within a region of HL-VI that is not conserved between psen1 and psen2. Thus, the splice products may have functions that are specific only for psen2. In brain the longer isoforms of psen2 are predominant, but the shorter isoform can also be detected.

Both presenilins are integral membrane proteins with eight transmembrane domains. The homology between psen1 and psen2 is 67 per cent. Within the transmembrane domains (TM), the homology can reach 95 per cent (Cruts et al., 1995). The main differences between psen1 and psen2 are in their N-terminal and central regions of hairpin loop-VI domain (HL-VI) (183). Both PSEN1 and PSEN2 are expressed in various human brain regions, heart, placenta, lung, liver, skeletal muscle, kidney and pancreas. In brain psen1 is found in the cell body and dendrites of neurons (Kovacs et al., 1996; Cook et al., 1996).

The presenilins are highly conserved in evolution, and have been identified in nematodes (C. elegans), fruit flies (Drosophila), clawed frogs (Xenopus), zebrafish and mammals (Baumeister and Haass, 1998). The 468 amino acid rat presenilin-1 protein has 88 per cent and 93 per cent amino acid similarity with those of human psen1 and psen2, respectively (Takahashi et al., 1996).

Only a minor amount of the presenilin proteins can be isolated as full-length proteins. The most abundant fragments identified are an approximately 27-28 kDa amino terminal fragment (NTF) and an approximately 18-20 kDa carboxy-terminal fragment (CTF) (Haass, 1997). psen1 is endoproteolytically cleaved in HL-VI with a predominant cleavage site between amino acids 291 and 292. psen2 is proteolytically cleaved into two stable cellular polypeptides of about 20 kDa and 34 kDa (Kim et al., 1997a).

The cleavage of psen1 in the large cytoplasmic loop is obviously tightly regulated and saturable, since only a fraction of overexpressed psen1is converted to stable NTF and CTF, whereas the majority of nascent psen1 polypeptide is rapidly degraded (Thinakaran et al., 1996; Thinakaran et al., 1996).

The presenilins are also proteolytically processed by proteases of the caspase superfamily proteins that take part in programmed cell death, apoptosis (Kim et al., 1997b; Loetscher et al., 1997). This process generates a longer NTF and a smaller CTF if full-length presenilin is the substrate, or only a smaller CTF if the conventional CTF is the substrate. It has been reported that the PSEN2 mutation N141I enhances the intracellular concentration of the alternative CTF derived by caspase-cleavage (Kim et al., 1997b). Besides, Bel-Xl, an anti-apoptotic member of the Bcl-2 family interacts with the C-terminus of psen1 and psen2, suggesting a mechanism by which presenilins may modulate mitochondria-dependent apoptotic cell death (Passer et al., 1999). However, inhibition of caspase cleavage does not affect Aβ generation. Moreover, mutation of the caspase cleavage site prevented apoptotic proteolysis, but did not affect the biological activity of human PSEN1 and PSEN2 in C. elegans (Brockhau et al., 1998).

It was demonstrated that the NTF/CTF assembly is a part of the active γ-secretase enzyme com-
plex. Inhibitors of aspartyl proteases specifically bound to NTF/CTF, and not to psen1 holoprotein, supporting the idea that the endoproteolytic derivatives are biologically active forms of psen1 (Li et al., 2000). Recent studies showed that nicastrin, Aph-1 and Pen-2 are also required for psen1-mediated intramembranous γ-secretase of select type I membrane proteins. Endoproteolysis of psen1 and the accumulation of fragments are regulated by the availability of nicastrin, Aph-1 and Pen-2 (Kimberly et al., 2003).

Presenilin-derived NTF and CTF are components of high molecular weight complexes. Many proteins are found to interact with psen, including β-catenin, E-cadherin, an actin binding protein; filamin, GSk-3β, tau, calcium binding proteins, such as calsenilin, calmyrin, sorcin, mu-calpin and CALP/KChIP4 (Van Gassen et al., 2000). Presenilins have been shown to regulate the rapid turnover of β-catenin which is a multifunctional protein in Wnt-signalling. PSEN1 deficiency results in increased β-catenin stability and AD-linked PSEN1 variants cause defective intracellular trafficking of β-catenin (Nishimura et al., 1999). Mutant Drosophila lacking PSEN1 expression exhibit defective apical localization of β-catenin and accumulation in ubiquitin-positive cytoplasmic inclusions (Noll et al., 2000). But pharmacological inhibition of γ-secretase activity does not affect psen1/β-catenin interaction, β-catenin trafficking, turnover or its transcriptional activity, suggesting that psen1-mediated regulation of β-catenin function is independent of γ-secretase activity. Moreover, deletion of β-catenin binding site in psen1 or psen2 does not affect psen endoproteolysis, stability or Aβ production (Xia et al., 2002).

Presenilin-derived fragments are also found in small synaptic vesicles, synaptic plasma membranes, synaptic adhesion sites and neurite growth cone membranes, indicating that PSEN1 may regulate neuronal differentiation, development or synaptic function (Georgakopoulou-los et al., 1999). Cadherins are Ca^{2+}-dependent cell-surface adhesion molecules with extracellular domains that span cellular junctions. Disruption of cadherin function alters dendritic spine morphology, impairs synaptic vesicle accumulation and recycling, and affects distribution of postsynaptic proteins (Togashi et al., 2002). Induction of apoptosis or Ca^{2+} influx stimulates psen1/γ-secretase cleavage of E-cadherins, releasing the cytosolic tail of E-cadherin from the cytoskeleton into the cytosol along with β- and α-catenin, thus enhancing the disassembly of the E-cadherin-catenin adhesion complex (Marambaud et al., 2002). PSEN1 also interacts with telencephalin, another cell adhesion molecule belonging to the immunoglobulin superfamily (Annaert et al., 2001). Thus, it is suggested that psen1 may participate in regulation of intercellular adhesion in epithelial cells and neurons.

Cell lines expressing PSEN1 mutants show high intracellular Ca^{2+} concentrations (Guo et al., 1996). It is also found that presenilins modulate the capacitative calcium entry (CCE), a refilling mechanism that regulates the coupled process of IP3-mediated release of ER Ca^{2+} and replenishing intracellular Ca^{2+} through plasma membrane channels (Yoo et al., 2000). It was shown that AD-linked PSEN variants attenuate CCE and loss of PSEN1 or expression of dominant negative mutant potentiates CCE (Herms et al., 2003), suggesting that PSEN1 has a role in cellular Ca^{2+} homeostasis.

To date, 164 different AD-related PSEN1 mutations have been identified (Cruts, 2007). Most of the PSEN1 mutations are missense mutations. One exception is a splice-site mutation, which results in an in-frame skipping of exon 9 (Δ9), deleting 29 amino acids in HL-VI and changing the amino acid at codon 290, containing the junction of exons 8 and 10. Besides presenile AD, the Δ9 deletion patients also have additional clinical features of spastic paraplegia (Kwok et al., 1997). Nearly 70 per cent of the PSEN1 mutations are in exons 5, 6, 7 and 8.

All AD patients who have PSEN1 mutations were found to have a positive autosomal dominant family history for AD. There is only one de novo case reported, with a His163Arg mutation that is absent in both unaffected aged parents (Tanahashi et al., 1996). The onset age of PSEN1 mutations vary between 34 and 64 years. But within a given family, range of the onset age gets narrower and correlates well with the nature and location of the amino acid substitution. PSEN1 mutations are fully penetrant. But there is one exception described of a carrier of the Ile143Phe mutation still not demented at age 68 years (Rossor et al., 1996). An association between the intron 8 polymorphism of PSEN1 and LOAD was reported (Wragg et al., 1996), but subsequent studies did not confirm this association (Cruts et al., 1996b). It has been suggested that this polymorphism might be in linkage disequilibrium
with a functional variant elsewhere in the gene (Dermaut et al., 2001).

In contrast to PSEN1, only 10 different mutations have been reported in PSEN2, which showed variable onset ages and incomplete penetrance (Sherrington et al., 1996). It is suggested that with PSEN2 mutations, AD is modified by yet unidentified genetic and/or environmental factors. Several silent mutations and intronic polymorphisms were identified in PSEN2 (Cruts et al., 2007), however there is not enough evidence for their involvement in AD pathology (Cruts et al., 1998; Sherrington et al., 1996).

The molecular mechanisms by which the mutant PS exerts its pathogenic effect are not yet clearly identified. However it was shown that PSEN1 mutations enhance the production of $\beta_42$, without changing the overall $\beta$ production. The splice-site mutation in intron 8 of PSEN1 was associated, in four families, to a form of AD with specific neuropathological findings such as diffuse senile plaques morphologically different from those usually observed in AD brain (Colacicco et al., 2002). These plaques do not show amyloid fibril depositions in the core and are not associated with surrounding dystrophic neurites and inflammatory reactions (Crook et al., 1998). Such features suggest that $\beta$-amyloid deposition is not the key event in the pathogenesis of AD, but that the neurotoxic effect of $\beta_42$ occurs before its cellular aggregation, probably by affecting calcium homeostasis, or by increasing the production of free radicals, or by disturbing the intracellular signalling pathways.

The apolipoprotein E gene (APOE)

The human APOE gene (OMIM # 107741) is located in a gene family cluster with APOC-I, APOC-I' and APOC-II on the proximal arm of chromosome 19, at 19q13.2 (Lusis et al., 1986). The gene contains four exons and spans 3.7 kb (Paik et al., 1989). The transcription start site lies in the second exon therefore the first exon is not translated. To date, 30 APOE variants have been characterized and 14 of them have been found to be associated with familial dysbetalipoproteinemia (Strittmatter and Roses, 1995). The most common allelic variants are APOE-e2, APOE -e3 and APOE -e4 (E2, E3 and E4). The E4 isoform is associated with increased levels of total cholesterol and betalipoprotein (Boerwinkle et al., 2001), that results in an increased susceptibility to heart disease (Davignon et al., 1988). Because of its reduced binding affinity to cellular receptors, the E2 isoform is associated with decreased levels of cholesterol and betalipoprotein (Boerwinkle et al., 1989). Most of the type II hyperlipidemia patients are homozygous for E2 (Breslow et al., 1982).

Apolipoprotein E protein (ApoE) is a major serum lipoprotein of 34.2 kDa, and it is secreted by many organs such as macrophages (Basu et al., 1981), adrenals of kidneys (Reue et al., 1984), but mainly synthesized by liver (Mahley, 1988). After its synthesis ApoE is incorporated into lipoproteins and directs their catabolism via binding receptors (Takahashi et al., 1992). ApoE cannot cross the blood-brain barrier, but is also found in the cerebrospinal fluid as a component of lipoproteins and lipid complexes (Pitas et al., 1987). It is the main apolipoprotein found in the brain and synthesized by astrocytes (Elshourbay et al., 1985). ApoE is thought to be involved in the metabolization and redistribution of cholesterol and phospholipid during membrane modelling, by its ability to bind and transport cholesterol-rich lipids into cells via interaction with its receptors (Nathan et al., 1994; Holtzman et al., 1995). The increase in the synthesis of ApoE after neuronal injury, in both peripheral and central nervous systems, indicates its implication in neuronal regeneration (Fagan et al., 1996). There is also an isoform specific effect of ApoE on the growth and sprouting of cultured dorsal root ganglia neurons (Nathan et al., 1994) and a murine neuroblastoma cell line (Mahley, 1988).

The E4 allele is associated with sporadic and familial AD of early or late onset (St Clair et al., 1995), Lewy body dementia (Betard et al., 1994), severe cerebral amyloid angiopathy (Greenberg et al., 1995), and Creutzfeldt-Jakob Disease (Amou-yel et al., 1994). However, family studies showed that E4 is neither necessary nor sufficient to cause AD. It may accelerate the preclinical progression, and therefore the age of onset (Bennett et al., 1995). The observation of increased frequency of E4 allele was initially made in late onset familial AD (Strittmatter et al., 1993), but further studies demonstrated that E4 allele was also over-represented in sporadic late-onset AD cases as well as young-onset cases with an approximately three-fold higher frequency (Saunders et al., 1993, Rebeck et al., 1993). The decrease in the frequency of the E2 allele in AD suggests that E2 has a negative or protective effect on AD (Corder et al., 1993).
The major neuropathological phenotype of the E4 genotype is increased Aβ deposition. There are two hypotheses how ApoE isoforms can affect amyloid levels in the brains of AD patients: First, ApoE can be involved in the clearance of Aβ; second, ApoE can be involved in the deposition of Aβ.

The first hypothesis includes two steps: association of ApoE with Aβ and interaction between ApoE-Aβ complexes and ApoE receptors. It was shown that ApoE isoforms on lipoproteins interact with Aβ peptide in vitro, with E3 displaying more avid interaction with Aβ than E4 (La Du et al., 1995). It was also found that physiological lipoproteins do interact with Aβ (Koudinov et al., 1996). The low-density lipoprotein receptor-related protein (LRP), one of the ApoE receptors, is also found on plaques and amyloid-containing vessels (Wisniewski et al., 1992). The LRP is a multifunctional receptor that binds a variety of ligands and internalizes them for endosomal and lysosomal degradation (Herz, 1993). Dysfunction of Aβ clearance by such receptors can cause the accumulation of Aβ.

The second hypothesis is the possibility that ApoE affects Aβ deposition. Several studies suggested that E4 causes greater aggregation of Aβ than does E3. It was shown that purified ApoE isoforms interact with Aβ in vitro and that E4 displayed formation of more stable complexes with Aβ than did E3 (Strittmatter et al., 1993a, Strittmatter et al., 1993b). When E4 and Aβ peptide are incubated together, a dense network of very long monofibrils are formed, and the spontaneous fibrillogenesis of Aβ peptides is enhanced, whereas in the presence of E3 the monofibril complexes are less dense and the network is simpler (Sanan et al., 1994). There are also data suggesting that ApoE inhibits amyloid deposition (Evans et al., 1995). It was shown that at low concentrations, only the E2 and E3 isoforms but not E4 are potent inhibitors of the Aβ aggregation in vitro (Pillot et al., 1997). This suggests that ApoE inhibits Aβ aggregation by the formation of stable ApoE-Aβ complexes, with specific interactions between E2 and E3, but not E4, and amyloid C-terminal fragments. It was reported that E2 and E3 bind Aβ more quickly and form more efficient ApoE-Aβ complexes than the E4 isoform (Yang et al., 1997).

The interaction between ApoE and tau remains controversial. It was initially reported that tau interacts with E3 in vitro but not with E4 (Strittmatter et al., 1994). In the light of this finding a model was proposed in which the interaction of E3 with tau could prevent its hyperphosphorylation, allowing its normal function in stabilization of microtubules (MTs). In the presence of E4, tau could become hyperphosphorylated and thus inactive, promoting the formation of PHFs. However, in numerous independent studies it was observed that E4 carriers do not accumulate a greater number of NFTs than duration- and aged-matched E3 individuals (Sparks et al., 1996). It was shown that ApoE regulates tau phosphorylation by increasing cytoplasmic Ca²⁺ via two different signaling pathways; an influx of extracellular Ca²⁺ resulting from the activation of a cell surface Ca²⁺ channel; and release of Ca²⁺ from internal Ca²⁺ stores via G-protein coupled pathway. This study suggests that ApoE may affect several Ca²⁺-associated signal transduction pathways that increase the activity of protein phosphatases 2A and 2B, which in turn dephosphorylate tau (Wang and Gruenstein, 1997; Wang et al., 1998). But still, the crucial link between Aβ and ApoE4 and neuronal alterations present in the AD brain is yet unknown.

Analysis of the ApoE genotypes in EOAD families revealed that families segregating APP mutations other than APP Ala692Gly, the ApoE4 allele acts as a dose-dependent age of onset modifier, and its presence leads to an earlier onset age of disease. Mutation carriers with an E4 allele have onset ages approximately five years earlier, and those with an E2 allele five years later than the mean onset age of AD in the family (Houlden et al., 1998). In contrast, there is no clear evidence that the onset age in PS mutation families is modulated by the ApoE genotype (Van Broeckhoven et al., 1994).

Other possible genetic risk factors

To date more than 100 genes other than APOE have been considered to contribute to sporadic AD pathology, such as the α1-antichymotrypsin gene (Kamboh et al., 1995), the angiotensin converting enzyme (Kehoe et al., 1999), the very low-density lipoprotein receptor gene (Okaizumi et al., 1995), the butyrylcholinesterase gene (Lehmann et al., 1997), the bleomycin hydroxylase gene (Montoya et al., 1998), the LBP-1/CP2/LSF gene (Lambert et al., 2000), the very low-density lipoprotein receptor related protein
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A gene (Wavrant-DeVrieze et al., 1999), the alpha-2 macroglobulin gene (Chen et al., 1999). However, their contribution to AD is not clear yet and needs to be further investigated, since the majority of these studies showed contradictory results. In certain AD patients, synergistic effects of more than one gene locus may have been responsible for increased risk of AD. This may be the reason why the risk factors mentioned above are not found to be associated in every population analysed.

**Promoter polymorphisms**

**APP promoter**

Besides the exonic mutations, duplication of APP gene cause autosomal dominant AD (Rovelet-Lecrux et al., 2006), indicating that overexpression of APP gene is sufficient to cause AD. Trisomy 21 in Down's syndrome (DS) patients leads to a 4-5 fold overexpression of APP, resulting in a 50 years decrease in onset age of AD in DS patients compared with the normal population (Rumble et al., 1989). These results imply that a fundamental component of the molecular etiology of AD may lie in the expression of APP, its biogenesis and turnover. Screening for mutations in the -802/+268 APP promoter region in sporadic and familial EOAD and LOAD cases did not reveal any AD specific mutation (Rooke et al., 1992; Rogaev et al., 1993). However, in a recent study, the proximal promoter (-766/+204) and two functional distal regions (-2634/-2159 and -2096/-1563) of APP were systematically sequenced in two independent AD series with onset ages ≤70 years (Belgian sample, n= 180; Dutch sample, n= 111) and identified eight novel sequence variants (Theuns et al., 2006). Three of them (-118C→A, -369C→G, and -534G→A) were identified only in patients with AD showed, in vitro, a nearly two-fold neuron-specific increase in APP transcriptional activity. Recent sib-pair analyses suggested that genetic variability at the APP locus may contribute to the risk of LOAD (Wavrant-DeVrieze et al., 1999). It is obvious that a systematic screening of the APP regulatory sequences in extended AD populations is necessary.

**The PSEN gene promoters**

Since PSEN genes are assigned a pivotal role in APP processing, altered PSEN expression due to variations in regulatory regions is considered to be a risk factor for AD. Genetic association studies in a population-based EOAD case-control sample showed association of the single nucleotide polymorphism (SNP) -48C→T with EOAD (Van Duijn ey et al., 1999). Systematic screening of 3kb of the PSEN1 upstream region in the same population revealed four novel polymorphism (-1789G/A, -2154G/A, -2319T, and -2823I/D), two of which (2154G/A, and -2823I/D) were also shown to be associated with increased risk for EOAD, and the risk haplotype -48C/-2154G/-2823D was found to be in linkage disequilibrium (Theuns et al., 2000). Additionally, two potentially AD-related mutations (-280C→G and -2818 A→G) were identified on PSEN1 promoter. Luciferase reporter gene assay revealed that the -48C risk allele resulted in a 50% decrease in promoter activity in neurons, which in homozygous individuals can lead to a critical decrease in PSEN1 expression. It was also shown that the -280G mutation results in neuron-specific 30% decrease in promoter activity.

PSEN2 has also been shown to be involved in APP processing, and changes in its expression levels might be important in AD pathology. Specific down-regulation of PSEN2 expression has been suggested in the early stages of sporadic LOAD (McMillan et al., 2000). A weak association has been shown between -1495D and AD in individuals homozygous for this deletion (Riazanskaia et al., 2002), but this relation was not confirmed in an Italian population (Di Natale et al., 2003).

**The APOE promoter**

It was shown that relative ApoE E4 mRNA level is increased in AD compared with controls, and it was suggested that genetic variability in the neuronal expression of APOE contributes to disease risk (Lambert et al., 1997). The C→G transversion at position +113 in the intron 1 enhancer element showed no statistically significant association independent of APOE E4, but in a population based study, three SNPs (-491A→T, -427T→C, and -219T→G) and two heterozygous mutations (-557C→T and -456C→T) were identified. Reporter gene analysis revealed that the three SNPs alter the transcriptional activity of the APOE promoter and show genetic association with AD, independent of APOE E4 (Artiga et al., 1998; Bullido et al., 1998). Although several studies attempted to confirm this association, most reported either absence of associ-
ation or association due to linkage disequilibrium with APOE E4 (Town et al., 1998; Ahmed et al., 1999). However, population-based differences of APOE E4 frequencies, giving rise to differences in relative risk for AD, have been documented (Lucotte et al., 1997). In vivo studies demonstrated that the -219T and -491A risk alleles correlated with an increased expression of the E4 allele in brain (Lambert et al., 1998). Later it was shown that the -491AA risk genotype is associated with increased levels of ApoE in plasma, independently of APOE E4 or AD status, though more pronounced in AD patients (Laws et al., 1999). These data provide evidence that, in addition to the qualitative effect of the ApoE E2/E3/E4 isoforms on risk for AD, the quantitative variation of expression of these isoforms due to functional APOE promoter variations is a determinant in AD development. It is therefore that there is a wide variation in relative risk for AD associated with APOE promoter polymorphisms.

**Full genome screens**

Many researchers have attempted to find chromosomal locations either by linkage or by association studies. Full genome screens revealed many different loci on different chromosomes, but only some of them yielded positive results in at least three independent studies (Table 1).

**Chromosome 6p21**

Three potential AD candidate genes have been previously reported for this location; HLA-A, HFE and TNFA, but full-genome screens gave signals ~10Mb further distal to the associated genes (Renovize, 1984; Culpan et al., 2003; Pulliam et al., 2003). There is a growing evidence of a putative AD locus on 6p21, but it is suggested that the actual disease gene has not been identified yet.

**Chromosome 10q24**

The only locus found to be associated by more than one group of researchers is located between 90 and 94 Mb and encompasses the genes TNFRSF6 (90 Mb) and IDE/KIFF11/HHEX (Eldred et al., 2003; Feuk et al., 2003). Most probably IDE is the best candidate on biological grounds, since it has been shown to degrade monomeric Aβ before it can aggregate in oligomeric forms, and finally into Aβ plaques. In addition to IDE locus, four other genes on the long arm of chromosome 10 were found to be associated with AD. The CDC2 and VR22 map ~30 Mb proximal, while the other two, GSTO1/2 and PRSS11 map 10-30 Mb distal. While none of these genes have been found to be associated with AD, it is noteworthy that two of these genes were also found to be associated with other neurodegenerative illnesses, frontotemporal dementia (CDC2) and Parkinson’s Disease (GSTO1/2) (Nowotny et al., 2001; Li et al., 2003). CDC2 encodes for the cell division cycle 2 protein which is involved in the phosphorylation of both tau and app, and is found inn neurons bearing NFTs. GSTO1 and 2 encode for glutathione S-transferase omega-1 and -2, which are involved in physiological response to oxidative stress, and might regulate the inflammatory cytokines. These findings suggest a more common pathway leading to neuronal cell death across these syndromes.

**Chromosome 11q23**

The region near the tip of the long arm of chromosome 11 has been found to associate with risk of AD in only one of the full genome screens. But, independent studies showed significant association between AD and BACE, which was mapped to this chromosomal region (Kirschling et al., 2003; Clarmon et al., 2003). This gene is a very good AD candidate on biochemical grounds as it is responsible of β-secretase cleavage of app. All positive studies observe over-representation of G-allele located at codon 262 on exon 5 in AD cases compared to controls. In all these studies the effect was more pronounced in carriers of APOE E4 allele. But in contrast to the positive results, there is an equal number of studies showing no relation of BACE polymorphism with risk of AD (Cruts et al., 2001).

**Chromosome 12p13 and 12q13**

Thus far association of five biological and positional candidate genes on chromosome 12 have been reported. Three of the genes, complement 1 r subcomplement (C1R) at 7.1 Mb, α-2 macroglobulin gene (α2M) at 9.1 Mb and OLR1 at 10.2 Mb, are located in 12p13. The other two candidates LRP1 at 55.8 Mb and transcription factor LBP1c/CP2/LSF (TFCP2) at 49.8 Mb, are mapped to 12q13.
Table 1. Summary of genome-wide linkage and linkage disequilibrium studies for chromosomal regions with maximum Lod Score ≥1.0 in LOAD.

<table>
<thead>
<tr>
<th>Linkage Studies</th>
<th>Linkage Disequilibrium</th>
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<tbody>
<tr>
<td>Pericak-Vance et al., 2000; Haines et al., 2001</td>
<td>Zubenko et al., 1998</td>
</tr>
<tr>
<td>Myers et al., 2002</td>
<td>Hiltunen et al., 2001</td>
</tr>
<tr>
<td>1st Stage 292 2nd Stage 451</td>
<td>Farrer et al., 2003</td>
</tr>
<tr>
<td>466 families</td>
<td>100 cases</td>
</tr>
<tr>
<td>ASPs</td>
<td>57 cases</td>
</tr>
<tr>
<td>1p13, 1p36</td>
<td>100 cases</td>
</tr>
<tr>
<td>1p13</td>
<td>100 controls</td>
</tr>
<tr>
<td>2p21</td>
<td>51 controls</td>
</tr>
<tr>
<td>3p14</td>
<td>110 controls</td>
</tr>
<tr>
<td>3p26</td>
<td>2p22</td>
</tr>
<tr>
<td>2p12, 2q22, 2q24, 2q34</td>
<td>2p23</td>
</tr>
</tbody>
</table>

| 4q32 | 4q35 | 4p16, 4p14, 4q35 |
| 5p15 | 5p14 | 5p15 |
| 6q21 | 6q27 | 6p22, 6p12 |
| 7q31 | 8q12 | 6p12, 6p21 |
| 9q22 | 9p21 | 8q12 |
| 9q34 | 9q21 | 9q22 |
| 10p11 | 10q22 | 10p13 |
| 10q21, 10q22 | 10q24 | 10q23 |
| 12q11 | 12p11 | 12q24 |
| 12q32 | 12q14, 12q21 |
| 14q11 | 14q22 | 14q32, 14q22 |
| 15q26 | 14q32, 14q22 |
| 16q23 | 15q26 |
| 18q22 | 16p12 |
| 18q12 | 17q11 |
| 19q13 | 19q13 |
| 21q22 | 19q13 |
| Xp22 | 18q22 |
| Xq21 | 19q13 |
| Xp21 | 19q13 |
The role of $\alpha_2$M in relation to AD risk has been studied extensively. $\alpha_2$M has been immunolocalised in senile plaques (Rebeck et al., 1995). The human $\alpha_2$-macroglobulin gene (OMIM # 103950) is a single copy gene, located on 12p13.3. It is found in a gene cluster, together with a closely related gene; the Pregnancy Zone Protein gene and the $\alpha_2$M-pseudogene (Oesriendt et al., 1998). The $\alpha_2$M gene spans 48 kb and consists of 36 exons (Matthijs et al., 1992).

The $\alpha_2$M gene is expressed by fibroblasts and macrophages, but mainly by hepatocytes. To date, two point mutations have been described, which cause chronic lung disese (Matthijs and Marynen, 1991). There are also several intronic and exonic polymorphisms identified. No complete $\alpha_2$M deficiencies are known in man, suggesting a fundamental function for this protein.

$\alpha_2$M is a very large tetrameric glycoprotein of 720kDa. It is the major human plasma proteinase inhibitor which inhibits proteinases of all classes by steric trapping mechanism. It is suggested that $\alpha_2$M has several pathophysiological processes in AD. $\alpha_2$M immunostains senile plaques and binds $A_\beta$ with high affinity (Bauer et al., 1991; Du et al., 1997). It is suggested that a serine proteinase-$\alpha_2$M complex can degrade $A_\beta$ and trypsin-activated $\alpha_2$M efficiently degrades $A_\beta$ in vitro and prevents in vitro formation of $A_\beta$ fibrils as well as $A_\beta$-induced toxicity of cultured human cortical neuronal cells (Zhang et al., 1996).

After being activated $\alpha_2$M is cleared by the $\alpha_2$M-r/low density lipoprotein receptor-related protein (Lrp/LRP), a multifunctional protein which is also the primary neuronal receptor for ApoE (Sinckland et al., 1990). LRP also binds and clears the isoforms of app. Thus $\alpha_2$M potentially directs the ApoE and app metabolism in the brain (Kounnas et al., 1995).

The I1000V polymorphism and a 5bp deletion polymorphism in the 5' splice site of exon 18 are found to be overrepresented in LOAD cases (Liao et al., 1998; Pirskanen et al., 2001). Although these findings are confirmed by several studies, there are also publications that failed to show any association (Prince et al., 2001; Ki et al., 2001).

LRP1 binds to ApoE, $\alpha_2$M and app thus can affect the risk of AD (Rebeck et al., 1995; Kounnas et al., 1995). Three polymorphisms in the LRP1 gene, a tetranucleotide repeat in the 5' region, same sense mutation in exon 3 and Ala216Val, have been examined but the results are conflicting (Wang et al., 2001).

CR1 gene is located close to $\alpha_2$M. One large case-control study examined a CR1 Ser135Leu polymorphism in relation to AD risk, but no significant association was found (Luedeking-Zimmer et al., 2003).

The TFPCP2 gene is located about 6Mb proximal to the LRP1 gene and is a potential candidate gene, as it affects the expression of $\alpha_2$M and interacts with Fe65 protein that regulates the expression app (Zambrano et al., 1998; Bing et al., 1999). A G/A polymorphism in the 3’UTR of TFPCP2 was shown to confer protection against the risk of AD (Lambert et al., 2000). Additional studies confirmed this association (Taylor et al., 2001, Luedeking-Zimmer et al., 2003). The collective data on six different case-control studies suggest that the TFPCP2 gene has a modest protective effect against the risk of AD.

The fifth gene located near the $\alpha_2$M (~1Mb) is the OLR1 or LOX-1 gene. OLR1 is a cell receptor and is abundantly expressed in the brain (Yamanka et al., 1998). Polymorphisms in the 3’UTR of OLR1 were reported to be in association with risk of AD, but this is not confirmed in other studies (Lambert et al., 2003; Bertram et al., 2004).

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