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15. MOLECULAR MECHANISMS IN ALZHEIMER'S DISEASE

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15.1 Introduction

Alzheimer's disease (AD), a progressive neurovegetative disorder of the central nervous system and a leading cause of dementia, is partially caused by genetic changes.

The molecular mechanisms and hypothesis of AD is very complex. The key event leading to AD appears to be the formation of a peptide known as amyloid beta (A β) which clusters into amyloid plaques or senile plaque on blood vessels and on the outside surface of neurons of the brain, which ultimately leads to the killing of neuron. Amyloid cascade of events in AD would be:

A β formation \rightarrow amyloid plaques \rightarrow neuron death \rightarrow dementia

The amyloid β -peptide is created by enzyme clipping of the neuron membrane protein known as amyloid precursor protein (APP). Enzymes can clip APP in the ways that do not result in amyloid β formation. Moreover, there are two forms of amyloid β -peptide, one with 40 and other with 42 aminoacids. The 42 amino acid peptide is more hydrophobic than that with 40. Following amyloid plaque formation, two processes play an important role in causing the death of neurons: inflammation and neurofibrillary tangles. Two major types of brain cells that participate in immune and inflammatory process are astrocytes and microglia. The number of astrocytes seems to be enlarged in AD and activated to produce prostaglandin leading to arachidonic acid mediated inflammation. Furthermore, activated microglial cells produce free radicals and this mechanism leads to the death of neurons.

Neurons can be very large and nutrient substances as well as cell regulation components are transported along microtubules. Structural integrity of microtubules is maintained by tau protein. In AD, tau protein loses its capacity to bind to microtubules but binds to each other forming paired helical filaments forming knots known as neurofibrillary tangles (NFTs) which further cause microtubule death.

With these facts in mind, the second part of the amyloid cascade would be:

APP \rightarrow A β 42 \rightarrow amyloid plaque \rightarrow inflammation NFTs \rightarrow neuron death.

The formation of A β 42 from APP is regulated by secretases - two enzymes that compete to cleave APP and consequently cause formation of insoluble amyloid plaques. α -secretase cleaves APP in a way in which A β 42 is not formed. On the contrary, β -secretase and, in the next step, γ -secretase cleaves APP forming either 40 aminoacid amyloid protein which is soluble, or 42 aminoacid amyloid peptide which clumps together forming insoluble amyloid plaques.

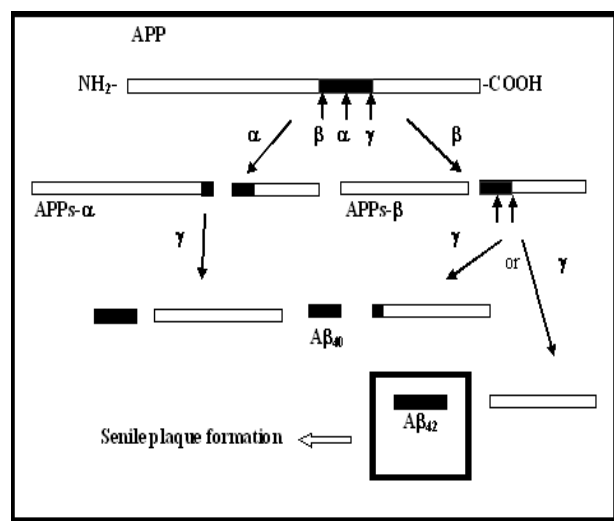


Figure 1. Action of secretases

15.2 Genetics of AD

Familial AD is genetically heterogeneous and appears in two forms: early-onset FAD and late-onset FAD. FAD accounts for only 10% of all AD occurring between 30 and 60 years of age. Mutations in three genes, i.e. coding amyloid β -precursor protein (APP), presenilin-1 (PS-1) and presenilin-2 (PS-2) have been found in FAD.

GENE	CHROMOSOME	PROTEIN
Presenilin-1	14	S182
Presenilin-2	1	STM2
Amyloid Precursor Protein	21	APP
Apo-lipoprotein E	19	APOE

Furthermore, it has been shown that mutations in those genes leads to elevated levels of amyloid β (A β 4), a proteolytic fragment of APP found in a deposited form in the brain of FAD. Not all cases of early-onset FAD are accounted for by APP, PS-1 and PS-2 mutations, so it is likely that other genes remain to be identified.

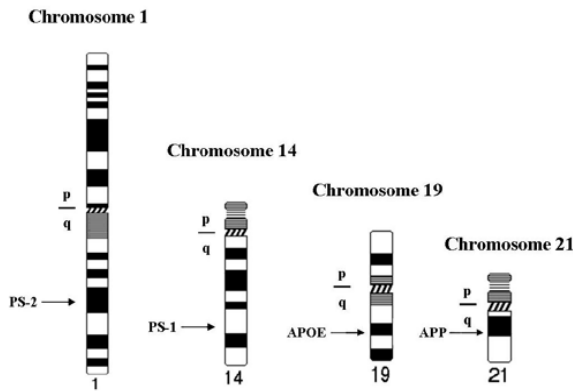


Figure 2. Ideograms of human chromosomes 1, 14, 19 and 21 showing cytogenetic locations of genes presenilin, presenilin 1, apolipoprotein E and amyloid β -precursor protein h

Apolipoprotein (ApoE) gene polymorphism was studied as a potential major susceptibility factor for late-onset AD. ApoE is a glycoprotein involved in cholesterol transport and the metabolism of lipoprotein particles. The polymorphic form of ApoE allele e4 has been studied in multi-national study ApoE Europe in which a Croatian group had taken a significant part. The results of our study support the role of ApoE 4 in AD patients. ApoE binds avidly to synthetic and soluble bA4 peptide in CSF. ApoE4 does not bind to tau protein in vitro unlike E2 and E3. It seems that interaction between ApoE3 and tau protein serves as protection against tau phosphorylation and tangle formation. This protein was supposed to have an additional role in other neurovegetative disorders regulating to some extent cholinergic metabolism in human brain that may have an impact on neuronal plasticity and regenerative capacity in brain.

15.3 Apo E allele frequency in Croatian AD patients

	$\epsilon 2$	$\epsilon 3$	$\epsilon 4$	OR (CI)a	p
Case (87)	2.9	52.3	44.8	13.96	<0.001
Control (88)	2.8	90.0	7.4	6.48-30.08	

In some other studies, it has been found that a-1-antichimotripsin (ACT) and PS-1 (intron 8 polymorphism) modify the risk associated with the ApoE 4 allele to induce AD. To establish the specific role of ApoE in AD, it is essential to determine the extent of association of e4 allele with types of dementia other than AD.

The USA National Institute on Aging and the Alzheimer Association regard ApoE genotyping as an adjunctive test for AD, since patients with AD are more likely to have an ApoA allele than patients with other forms of dementia. However, it is not clear from other studies whether the ApoE4 allele is more strongly associated with AD than with other types of dementia.

There is increasing evidence for the role of ApoE in the pathogenesis of AD. ApoE is detectable by immunochemistry in senile plaques, neurofibrillary tangles and cerebrovascular amyloid in AD brain. The distinctive binding properties of ApoE isoforms to bA4 peptide and tau protein have suggested ways in which ApoE might mediate its action.

The APP gene is located on chromosome 21 and a total of six mis-sense mutations have been described so far. All of them were

shown to be connected with AD, but about 2% of all published cases of FAD and 5-20% of early-onset FAD have one of these mutations. At least 41 different mutations have been found in PS gene that is located on chromosome 14. Almost all mutations were found in AD patients and PS-1 mutations account for 30-50% of AD families. PS-2 gene mutations (chromosome 1) account only for 2% of all early-onset FAD.

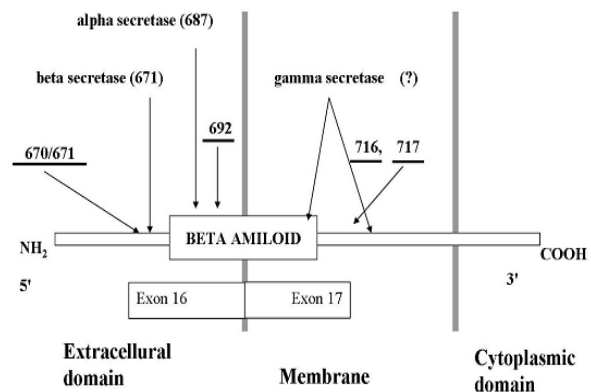


Figure 3. Domain structure of APP showing the position of mutations causing Alzheimer's disease within amyloid β (bA4) and secretase cleavage sites.

Table 2. APP mutations associated with AD

Number of families	Mean age of onset	Mutation	
1		Gly 692	Hendriks et al., 1992
		Ala	
		Lys 670	Mullan et al., 1992
2	55	Asn	
		Met 671	
		Leu	
		Ile 716 Val	Hutton and Hardy 1997
2		Val 717 Ile	Naruse et al., 1991
1		Val 717 Ile	Yoshioka et al., 1991
1	43	Val 717	Murrell et al., 1991
		Phe	
1		Val 717	Chartier-Harlin., et al 1991b
		Gly	
2	54	Val 717 Ile	Goate et al., 1991
1		Val 717 Ile	Fidani et al., 1992
1	48	Val 717 Ile	Karlinsky et al., 1992
1		Val 717 Ile	Yoshizawa et al., 1993
2	54	Val 717 Ile	Sorbi et al., 1993
1		Val 717 Ile	Sorbi et al., 1995
1	49	Val 717 Ile	Brooks et al., 1995
1	55	Val 717 Ile	Matsumura et al., 1996
3		Val 717 Ile	Campion et al., 1996

15.4 How do these mutations impact APP function?

The APP mutation at codon 693 may alter the secondary structure of bA4 peptide in cell membrane resulting in premature deposition. Mutations at codons 670/671 and 717 do not lie within bA4 but flank it, lying very close to sites of secretase cleavage. Soluble form of bA4 (1-40) peptide is rapidly cleared preventing plaque formation in the brain. Longer peptides of 42-44 aminoacids aggregate more rapidly into fibrils. The APP 717 mutations produced up to two times longer and a more insoluble form of APP

that aggregates rapidly and promotes peptide deposition, and APP 692 mutation leads to formation of an APP molecule containing truncated β A4 peptide, which is at the same time overproduced.

Since most early-onset AD families do not have mutations in the APP gene, it was expected that other AD loci might exist. In 1992, many investigators have shown that a second locus :S182, PS-1, AD3 on chromosome 14 may be responsible for the early-onset FAD.

PS-1 gene contains 10 protein-coding exons and 2 or 3 additional exons encoding the 5*-untranslated protein. PS-1 gene undergoes alternate splicing as well as inclusion or exclusion of codons 26-29 at 3* end . At least 41 different FAD mutations in PS gene have been identified in more than 50 unrelated families, most of them in exon 5 and 8. The known FAD mutations are distributed through PS-1 gene, most of them in predicted TM domains. Furthermore, all FAD missense mutations occur in aminoacids that are conserved in PS-1 and PS-2.

Presenilin-2 gene (PS-2) was found and isolated based on its homology to the PS-1 gene. It contains 10 protein-coding exons and two additional exons encoding the 5*-untranslated region. The PS-2 isoform missing exon 8 has been described. Evidence for specificity of function for both presenilins derives from the presence of non-homologous regions in molecules. The mean age of onset in FAD families with PS-1 gene mutation is earlier (44 years) than in families with PS-2 gene mutations (mean: 50 years).

15.5 Other factors

Additional genetic factors may be involved in the manifestation of the AD disease. α 1-antichymotrypsin (ACT) protein levels in plasma and CSF have been observed to be elevated in AD patients, and therefore ACT has been proposed as a biochemical marker for AD. ACT is intimately associated functionally within the β A4 peptide in AD brains and ACT appears to promote fibril formation of the β A4 peptide, and thus the deposition of amyloid ACT protein belongs to the class of serine proteinase inhibitors (serpins) and is encoded by a gene on chromosome 14.

A polymorphism has been described in the signal peptide sequence of ACT with nearly equal frequencies in the general population. This bi-allelic polymorphism of the ACT gene has been described to cause an amino acid exchange Ala (A) to Thr (T) at codon 15 in the signal peptide region and has been stated to confer a significant risk for AD.

Patients with the combination of ACT/AA phenotype and ApoE ϵ 4 allele were reported to have a two- to three-fold increased risk for A.

Some investigators suggest that the degenerative changes in AD are preceded by vascular or atherogenic changes that reduce blood flow and thereby induce ischemic and oxidative stresses leading to AD

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