MOLECULAR GENETICS OF ALZHEIMER DISEASE

S. PARVATHY
JOSEPH D. BUXBAUM

MOLECULAR PATHOLOGY OF ALZHEIMER DISEASE

Pathologic Changes

Alzheimer disease (AD) is characterized histopathologically by the intraneuronal accumulation of paired helical filaments (PHFs) composed of abnormal tau proteins and extracellular deposits of an amyloid peptide (Aβ) in plaques (1). AD plaques are round, spheric structures, 15 to 20 μM in diameter, consisting of a peripheral rim of abnormal neuronal processes and glial cells surrounding a core of deposited material. Several associated proteins have been identified in the plaques including heparan sulfate proteoglycans (2), apolipoprotein E (Apo E), and α-antichymotrypsin (3), as well as metal ions (4).

Alzheimer Amyloid Precursor Protein

Aβ is proteolytically derived from a larger integral membrane protein, the amyloid precursor protein (APP). Because there are mutations in APP (discussed in detail later), that lead to AD, the molecular and cell biology of APP will be discussed here at some length. APP is a type I integral membrane glycoprotein containing the Aβ region, which includes 28 amino acids of the ectodomain and 12 to 14 amino acids of the adjacent transmembrane domain (1,5). APP is a member of a family that also includes the amyloid precursor–like proteins 1 and 2 (APLP1 and APLP2). The APP gene is localized on chromosome 21 at 21q21.2 (6,7), and it is encoded by 18 exons, of which exons 16 and 17 encode the Aβ peptide domain (8). Three major splice variants of APP have been identified containing the Aβ sequence, that is, the APP695, APP751, and the APP770 isoform, of which APP695 is the major isoform found in neurons (6,7,9). The two longer forms (APP751 and APP770) contain a 56-amino-acids domain with homology to Kunitz family of serine protease inhibitors (KPI) (10).

APP Processing

APP can be processed by at least three secretases: α-, β-, and γ-secretases. The site of cleavage of each of these enzymes is shown in Fig. 83.1. In the nonamyloidogenic pathway, α-secretase cleaves the amyloid precursor protein within the Aβ domain. The cleavage within the Aβ domain prevents deposition of the intact amyloidogenic peptide. α-Secretase activity generates a soluble N-terminal fragment of APP known as sAPPα, and its C-terminal counterpart of approximately 10 to 11 kd remains embedded in the membrane. The site of cleavage targeted by α-secretase has been identified at the Lys16-Leu17 bond of the Aβ sequence corresponding to Lys687-Leu688 peptidyl bond of APP770 (11). The 10- to 11-kd C-terminal product may undergo an additional cleavage by a protease γ-secretase activity. This process leads to the formation of p3 and its complementary product p7 (Fig. 83.1).

The protease termed β-secretase initiates Aβ generation by cleaving APP after methionine 671 (using APP770 numbering), thus creating an approximately 12-kd membrane-retained C-terminal fragment having residue 1 (aspartate) of Aβ at its N-terminus (12). This can result in the secretion of a truncated soluble APP molecule, called sAPPβ, into the medium (13). The 12-kd fragment may then undergo γ-secretase cleavage within the hydrophobic transmembrane domain at either valine 710, alanine 712, or threonine 713 to release the 40, 42, or 43 residue Aβ peptides (13). The varying C-terminal of Aβ may be a feature of crucial importance because Aβ peptides display distinct physical properties and, in particular, exhibit aggregation behavior that can vary according to their length (14).

Buxbaum et al. showed by using fibroblasts with a disrupted TACE (tumor necrosis factor α-converting enzyme)
gene that two classes of α-secretase exist (16), one involved in the basal secretion and the other involved in regulated secretion (16). These investigators demonstrated that TACE, a member of the ADAM family (a disintegrin and metalloprotease family) of proteases, plays a central role in regulated α-secretase cleavage of APP. The existence of two classes of α-secretases is supported by the finding that the potency of inhibition by different hydroxamic acid–based inhibitors is different between basal α-secretase activity and TACE (17). The mammalian kuzbanian orthologue mKuz (ADAM 10) has α-secretase activity and is involved in the basal release of sAPPα (18). Four groups have now identified a candidate for β-secretase (BACE), also known as Asp-2 (19–22). BACE has been shown to be an aspartyl protease that is activated from the proenzyme form in cell lines. Finally, presenilin 1 (PS1) appears to facilitate a proteolytic activity that cleaves the integral membrane domain of APP by γ-secretase (23). It is possible that presenilins are γ-secretase or they facilitate γ-secretase activity through some other mechanism (discussed in detail later).

**FUNCTIONS OF APP**

The functions of APP and related members of the APP superfamily remain to be fully clarified (32), although APP has been shown to have a wide range of biological properties. The extracellular domain of APP is capable of binding to a range of proteoglycan molecules, and this allows it to function as a regulator of cell–cell or cell–matrix interactions, cell growth, and synaptic plasticity. APP itself may function as a cell-surface receptor. Secreted forms of APP liberated from the cells by the action of secretases may regulate Ca²⁺, thereby having a neuroprotective effect (33). In addition, sAPPα has been shown to stimulate a protein kinase–mediated signal transduction cascade in cultured cells (34). APP also contains heparin-binding sites and metal ion-binding sites. Finally, APP isoforms containing the KPI domain can act as potent inhibitor of serine proteases (9). The physiologic consequences of the enhanced secretion of either sAPPα or Aβ have not been defined.

The intracellular C-terminal domain of APP has been shown to bind to several intracellular proteins, including X11 (35), Fe65 (36), disabled protein (Dab) (37), G protein Go (38), and BP1 (39). The phosphotyrosine interaction domains of X11, Fe65, and Dab bind to the YENPTY motif found in the C-terminus of APP. These proteins can function as adapter proteins enabling APP to bind to other proteins.

**REGULATION OF APP PROCESSING**

Several lines of evidence suggest that protein phosphorylation can modulate APP processing in various cell lines. This was shown by a series of studies that consistently reported on the effect of several agents known to activate protein kinase C (PKC) directly. Thus, cell treatment with phorbol esters such as phorbol 12-myristate 13-acetate (PMA) or phorbol 12,13-dibutyrate (PDBu) leads to significant increases in the production of sAPPα (40,41). Concomitant
with increased sAPPα secretion, PKC activation lowers the production of Aβ (42–44).

The hypothesis that APP processing could be regulated by phosphorylation-dependent processes was reinforced by a study showing that okadaic acid, an inhibitor of phosphatases 1 and 2A, potentiated the PDBu-induced increase of sAPPα (42). The same studies showed that Aβ formation was also under the control of phosphatase activity (42). It was demonstrated that drugs that increase cytoplasmic Ca²⁺ levels stimulated sAPPα release in the extracellular medium and increased Aβ formation (45,46). Therefore it can be said APP processing can be regulated by PKC and by intracellular Ca²⁺ concentrations. The exact mechanism by which PKC and Ca²⁺ modulate APP processing is not clear.

The density of cholinergic receptors is affected in patients with AD, an observation that prompted some authors to examine whether the stimulation of muscarinic receptors could, through activation of the phospholipase C/PKC cascade, ultimately modulate APP processing. Release of sAPPα was found to be stimulated after treatment with various agonists of muscarinic receptors in cells overexpressing M1 and M3 receptor subtypes (47,48). Several other agents have been known to activate APP processing. These include nerve growth factor, epidermal growth factor, tacrine (anticholinesterase drug), estrogen, and electrical depolarization (49). All the foregoing observations emphasize the complexity of the regulatory mechanisms of APP processing.

**Localization of APP Processing**

By using a mixture of protease inhibitors and by approaches such as metabolic labeling, pulse chase experiments, and cell treatments with various agents known to affect a characteristic step of the intracellular trafficking, De Strooper et al. established that α-secretase activity occurred in a late compartment of the constitutional secretory pathway (50,51). These data were supported by Sambamurti and co-workers (52), who demonstrated using [³⁵S] labeling and temperature block that α-secretase activity occurs in the trans-Golgi network or in a late trans-Golgi compartment just after sulfate incorporation (53).

APP can escape intracellular cleavage by α-secretase and reach the cell surface as a full-length mature product, as evidenced by labeling of APP after biotinylation (54) or radioiodination (55) of cell-surface membranes. Such an approach allows recovering secreted biotinylated or iodinated sAPPα in the cell medium (55,56). This finding indicates that α-secretase activity can also occur at the plasma membrane level in several cell systems (see also ref. 57).

Full-length APP that arrives to the plasma membrane can be endocytosed and recycled (58), and Aβ appears to be generated both in the endocytic and the exocytic pathway. The cellular sites of Aβ production have been thoroughly investigated in cell culture systems. Initial studies indicated that endosomal/lysosomal processing of APP leads to the production of fragments that contain the APP C-terminus and entire Aβ region and hence are potentially amyloidogenic (56,59). Despite the initial excitement generated by the discovery of these Aβ-containing fragments, several lines of evidence suggest that the lysosomal degradation of APP is unlikely to contribute to the production of Aβ. However, agents that interfere with pH gradients (i.e., ammonium chloride and chloroquine) inhibit the production of Aβ (25), a finding suggesting that Aβ may be generated in acidic compartments (i.e., endosomes or late Golgi).

Furthermore, cells that express APP with various deletions in the cytoplasmic tail release lower levels of soluble Aβ (60), a finding suggesting that the internalization of APP from the cell surface and subsequent recycling to the plasma membrane may be responsible for the generation of Aβ. Two lines of evidence are consistent with this idea: secreted Aβ can be generated from [¹²⁵I]surface-labeled APP (55), and the surface APP "tagged" with either monoclonal antibodies or biotin can be recycled to the plasma membrane after endocytosis (58). These studies offered a model wherein β-secretase cleavage occurs within endocytic compartments, and γ-secretase cleavage of the residual approximately 100 amino acid membrane-bound fragment within the APP transmembrane domain occurs virtually simultaneously with the formation and release of Aβ.

Although the majority of Aβ appears to be generated in the endosomal recycling pathway, a few secreted Aβ species are also generated in a secretory pathway (27,61). Aβ secretion could be totally prevented by brefeldin A (24,62), and by monensin (24), two agents that potently block intracellular trafficking. This finding suggests that Aβ may be generated during the transport of APP through the Golgi apparatus en route to the plasma membrane.

Taking together all the available data, it is possible to formulate a model for APP processing. APP polypeptide undergoes complex post-translational modifications, including sulfation, phosphorylation, and both N- and O-linked glycosylation (44,63). These modifications occur during the trafficking of the protein through the secretory pathway. Thus, APP is cotranslationally translocated into the endoplasmic reticulum by its signal peptide and then matured during passage through the Golgi by acquiring sulfate, phosphate, and sugar groups. At this stage, some of the APP may have already been processed by β- and γ-secretases. Then, a percentage of mature molecules is transported to the plasma membrane by secretory vesicles (64). At or near the cell surface, some APP molecules undergo proteolysis by the protease designated α-secretase. Alternatively, uncleaved surface APP molecules can undergo endocytosis through clathrin-coated vesicles, apparently mediated by a YENPTY signal sequence in the distal cytoplasmic tail (65), after which the full-length precursor is trafficked to late endosomes and lysosomes for apparent
degradation (56,59), or it is rapidly recycled within early endosomes to the cell surface (66). The latter pathway has been shown to be a principal site for the two proteolytic cleavages that generate the Aβ peptide (55). It appears that, at least in cultured cells, only a few of all biosynthesized APP molecules undergo either the α-secretase or the β-secretase fate; many full-length precursor molecules remain inserted into internal membranes, particularly in the Golgi.

Tangles

Neurofibrillary tangles are another important histologic feature of AD. They consist of PHFs in a double helix (diameter, 20 nm) found in the cytoplasm of neurons, particularly of the pyramidal cells of the cerebral cortex and hippocampus (67). PHFs are composed principally of phosphorylated tau, a low-molecular-weight microtubule-associated protein (68).

Tau is a family of six proteins derived by alternative mRNA splicing from a single gene located on chromosome 17. These molecular isoforms of tau differ in whether they contain three or four tubulin-binding domains of 31 or 32 amino acids each near the C-terminal end and no, one, or two inserts of 29 amino acids each at N-terminal end of the molecule.

Tau in AD brain, especially in PHFs, is abnormally hyperphosphorylated and glycosylated. At the later stages of tangle formation, the tau is increasingly ubiquitinated. In a normal neuron, biological function depends on an intact microtubule network through which much of the axoplasmic transport is supported. The AD abnormally phosphorylated tau (AD P-tau) competes with tubulin in binding to normal tau, MAP1, and MAP2 and inhibits their microtubule assembly-promoting activities. The disruption of the microtubule network probably compromises the axonal transport and starts retrograde degeneration of the affected neurons. The neuronal cytoskeleton in AD is progressively disrupted and is replaced by bundles of PHFs, leading to the formation of neurofibrillary tangles (67).

To date, 21 phosphorylation sites in the AD abnormally phosphorylated tau have been identified (69). Among the several protein kinases that have been implicated in the phosphorylation are glycogen synthase kinase-3 (70), neuronal Cdc-like protein kinase (71), mitogen-activated protein kinase (72), Ca2+/calmodulin-dependent protein kinase II (73), casein kinase I (74), and cyclic adenosine monophosphate–dependent protein kinase (75). AD P-tau can be dephosphorylated by protein phosphatases PP-2B, PP-2A, and PP-1, but not by PP-2C. Tau phosphatase activity is decreased by approximately 30% in AD brain (76); thus, the decrease in phosphatase activities may contribute to the abnormal hyperphosphorylation of tau in AD resulting from the inhibition of dephosphorylation reaction. To date, no mutations in tau have been implicated in AD; however, mutations in tau are associated with frontotemporal dementia (77).

MUTATIONS IN APP IN EARLY-ONSET AD

Genes may be related to disease in two ways: through mutations that by themselves are sufficient to cause the disease (i.e., deterministic mutations), or alternatively, through gene variations (polymorphisms) that may increase disease risk without being sufficient (or necessary) by themselves to cause the disorder. This latter group is referred to as susceptibility genes. Although it is currently thought that most cases of AD occur sporadically, autosomal dominant transmission has been identified in families with early-onset AD, defined as beginning before the age of 65 years. These cases are relatively rare; worldwide, only several hundred families are currently known to carry deterministic mutations (78). Extensive research carried out since the early 1980s has isolated certain genes that, when mutated, cause AD, notably APP on chromosome 21 (79,80), the PS1 gene on chromosome 14 (81), and the PS2 gene on chromosome 1 (81). Mutations in these genes lead to early-onset AD and explain only a small proportion of total AD cases. Furthermore, trisomy 21 (Down syndrome or DS) increases the risk of AD, perhaps because of the tripled genetic dosage of APP. In addition, some susceptibility genes are currently being studied, of which polymorphisms of the APOE gene have received the most attention. The presence of the APOE-4 allele has been identified as a genetic risk factor for sporadic AD and familial AD (FAD) of late onset. All these genetic causes of AD are discussed in detail later.

AD in Down Syndrome

In considering the molecular pathology of DS, a matter of critical interest is that virtually all patients with DS who survive beyond 35 years of age develop neuropathologic changes that closely resemble AD (82). Thus, there is the abnormal accumulation of Aβ in the brains of both patients with AD and those with DS, followed by cognitive decline. Patients with DS are thought to express high levels of APP because of an extra copy of chromosome 21. The most straightforward explanation for dementia in DS is the presence of three instead of two copies of APP in patients with DS (83). Other genes that are potentially overexpressed in DS are located within a segment of chromosome 21, termed the Down locus. Genes that are contained within the Down locus include APP, superoxide dismutase 1, S100-B (a calcium-binding protein), and BACE-2 (a homologue of BACE).

The occurrence of +1 frameshifted proteins, such as APP +1 and ubiquitin-B +1 (UBB +1) has been linked to the onset of AD in patients with DS. Frameshifts are caused by dinucleotide deletions in GAGAG motifs in mRNA and are now thought to be the result of unfaithful transcription
of normal DNA by a novel process termed molecular misreading. The aberrant mRNAs are translated in the +1 reading frame as “+1 proteins,” that is, proteins with a wild-type N-terminus and frameshifted and often truncated C-terminus. It has been shown that expression of APP +1 protein (84,85) and UBB +1 protein is found in all patients with DS (85). In patients with DS and AD, the mRNA decay pathway may be impaired, and therefore +1 mRNA is translated into +1 protein. Conversely, UBB +1 may have a role in directly interfering with the ubiquitin/proteasome system and may lead to an inefficient protein breakdown through the proteasomal pathway.

APP Mutations and Their Effect on Aβ Formation

Several different pathogenic mutations have been found in exons 16 and 17 of the APP gene to date (Table 83.1). These mutations are missense mutations. The early-onset AD mutations (according to APP770 numbering), APP715, 716, 717, and APP670/671, are located outside the Aβ amyloid sequence, with APP715-717 (Val715Met, Ile716Val, and Val717Ile/Gly/Phe) close to the C-terminal γ-secretase cleavage site (79) within the transmembrane domain and APP670/671 (Lys670Asn/Met671Leu) at the N-terminal β-secretase cleavage site within the extracellular part of APP (90). In contrast, the APP692 (Ala692Gly) mutation is located inside the Aβ amyloid sequence next to the α-secretase cleavage site (89). Other sequence variations in the Aβ region are thought not to be pathogenic.

The localization of mutations led to the hypothesis that these mutations could influence the activity of the respective secretases, resulting in the aberrant processing of APP (95). Indeed, mutations at codons 716 and 717 lead to a selective increase in the production of Aβ peptides ending at residue 42/43 (91,96–99). The Lys670Asn/Met671Leu mutation, conversely, appears to augment the production of both Aβ40 and Aβ42/43 (100), whereas the Ala692Gly mutation has a more complicated effect on APP processing by causing impaired α-secretase cleavage, increased heterogeneity of secreted Aβ species, and increased hydrophobicity of the Aβ (98). The Ala692Gly mutation also has clinical features in some cases similar to those of cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D) (93), and in other cases more similar to AD. Recently, another mutation in APP (E693G) has been identified as the Arctic APP mutation that enhances Aβ protofibril formation (94).

### MUTATIONS IN THE PRESENILINS IN EARLY-ONSET AD

The homologous membrane proteins presenilin 1 (PS1) and presenilin 2 (PS2) were identified in 1995 as the genes responsible for a substantial fraction of early onset, autosomal dominant AD (81,101). The most common causes of autosomal dominant FAD are mutations in the PS1 gene on chromosome 14 (81). These account for 30% to 50% of all early-onset cases (102), and they are the primary cause of AD with onset before the age of 55 years. To date, more than 50 PS1 mutations and two PS2 mutations have been reported (Tables 83.2 and 83.3) in FAD. All mutations in the presenilins (PS) are missense mutations, except for the mutation of a splice acceptor site resulting in the deletion of exon 9. The lack of mutations leading to loss of gene expression or frameshifts suggests that the disease phenotype results from a gain of function. The hydrophobic regions of PS1 and PS2 are almost completely identical and are highly conserved among species.

### Structure, Localization, and Post-Translational Modification of Presenilins

#### Structure

Hydropathy analysis of PS1 and PS2, using the indices of Kyte and Doolittle, indicates the presence of a hydrophilic N-terminal followed by ten hydrophobic regions (HR) of at least 15 amino acids in length, which could potentially span the membrane (81,101,120). Most of these segments are connected by small hydrophilic loops, except one longer stretch of mostly hydrophilic residues between HR7 and HR8 called the “large loop.”

Multiple studies have been aimed to determine the number of transmembrane domains as well as the orientation of the N- and C-terminus of PS. These approaches included staining with antibodies after selective permeabilization of cellular membranes, construction of chimeric proteins with protease cleavage sites, and the use of β-galactosidase or glycosylation tags (121–127). All the proposed models agree that the first six hydrophobic regions represent transmembrane domains, whereas the predictions of the total number of regions varies between six and eight. In all these models except one (126), the N-terminal and the C-termi-

<table>
<thead>
<tr>
<th><strong>TABLE 83.1. APP MUTATIONS THAT CAUSE AD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutation</strong></td>
</tr>
<tr>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>V717F</td>
</tr>
<tr>
<td>V717L</td>
</tr>
<tr>
<td>A692G (Flemish)</td>
</tr>
<tr>
<td>K/M670/1N/L (Swedish)</td>
</tr>
<tr>
<td>I716V (Florida)</td>
</tr>
<tr>
<td>V715M (French)</td>
</tr>
</tbody>
</table>
TABLE 83.2. MISSENSE MUTATIONS IN THE PRESENILIN 1 GENE

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age of Onset (y)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>V82L</td>
<td>55</td>
<td>Campion et al., 1995 (103)</td>
</tr>
<tr>
<td>V96F</td>
<td></td>
<td>Kamino et al., 1996 (104)</td>
</tr>
<tr>
<td>Y115H</td>
<td>37</td>
<td>Campion et al., 1996 (105)</td>
</tr>
<tr>
<td>Y115C</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>P117L</td>
<td></td>
<td>Wisniewski et al., 1998 (107)</td>
</tr>
<tr>
<td>E120D</td>
<td>48</td>
<td>St. George-Hyslop, 1998 (78)</td>
</tr>
<tr>
<td>E120K</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>K123E</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>M139T</td>
<td>49</td>
<td>Campion et al., 1996 (105)</td>
</tr>
<tr>
<td>M139V</td>
<td></td>
<td>40 Alzheimer’s Disease Collaborative Group, 1995 (109)</td>
</tr>
<tr>
<td>M139K</td>
<td>40</td>
<td>Dumanchin et al., 1998 (110)</td>
</tr>
<tr>
<td>I143F</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>I143T</td>
<td>35</td>
<td>Cruts et al., 1995 (111)</td>
</tr>
<tr>
<td>M146L</td>
<td>45</td>
<td>Sherrington et al., 1995 (81)</td>
</tr>
<tr>
<td>M146V</td>
<td>38</td>
<td>Alzheimer’s Disease Collaborative Group, 1995 (109)</td>
</tr>
<tr>
<td>M146I</td>
<td>40</td>
<td>St. George-Hyslop, 1998 (78)</td>
</tr>
<tr>
<td>H163R</td>
<td>50</td>
<td>Sherrington et al., 1995 (81)</td>
</tr>
<tr>
<td>K123E</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>M139V</td>
<td>40</td>
<td>Alzheimer’s Disease Collaborative Group, 1995 (109)</td>
</tr>
<tr>
<td>L171P</td>
<td>40</td>
<td>Ramirez-Duenas et al., 1998 (112)</td>
</tr>
<tr>
<td>G209V</td>
<td></td>
<td>Kamino et al., 1996 (104)</td>
</tr>
<tr>
<td>I213T</td>
<td>52</td>
<td>Campion et al., 1996 (105)</td>
</tr>
<tr>
<td>A231V</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>M233T</td>
<td>35</td>
<td>Kwok et al., 1997 (113)</td>
</tr>
<tr>
<td>L235P</td>
<td>32</td>
<td>Campion et al., 1996 (105)</td>
</tr>
<tr>
<td>A246E</td>
<td>55</td>
<td>Sherrington et al., 1995 (81)</td>
</tr>
<tr>
<td>L250S</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>A260V</td>
<td>40</td>
<td>Ikeda et al., 1996 (114)</td>
</tr>
<tr>
<td>L262F</td>
<td>50</td>
<td>Forsell et al., 1997 (115)</td>
</tr>
<tr>
<td>C263R</td>
<td>47</td>
<td>St. George-Hyslop, 1998 (78)</td>
</tr>
<tr>
<td>P264L</td>
<td>45</td>
<td>Campion et al., 1995 (103)</td>
</tr>
<tr>
<td>P267S</td>
<td></td>
<td>Alzheimer’s Disease Collaborative Group, 1995 (109)</td>
</tr>
<tr>
<td>R269G</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>R269H</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>E273A</td>
<td></td>
<td>Kamimura et al., 1998 (116)</td>
</tr>
<tr>
<td>E278T</td>
<td></td>
<td>Kwok et al., 1997 (113)</td>
</tr>
<tr>
<td>E280A</td>
<td>47</td>
<td>Alzheimer’s Disease Collaborative Group, 1995 (109)</td>
</tr>
<tr>
<td>E280G</td>
<td>42</td>
<td>Alzheimer’s Disease Collaborative Group, 1995 (109)</td>
</tr>
<tr>
<td>L286V</td>
<td></td>
<td>50 Alzheimer’s Disease Collaborative Group, 1995 (109)</td>
</tr>
<tr>
<td>291-319</td>
<td></td>
<td>deletion</td>
</tr>
<tr>
<td>E317G</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>G384A</td>
<td>35</td>
<td>Cruts et al., 1995 (111)</td>
</tr>
<tr>
<td>L392V</td>
<td>25–40</td>
<td>Campion et al., 1995 (103)</td>
</tr>
<tr>
<td>A231V</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
<tr>
<td>P436S</td>
<td></td>
<td>Hardy, 1997 (106)</td>
</tr>
</tbody>
</table>

TABLE 83.3. MISSENSE MUTATIONS IN THE PRESENILIN 2 GENE

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Age of Onset (y)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1411</td>
<td>50-65</td>
<td>Rogaev et al., 1995 (120)</td>
</tr>
<tr>
<td>M239V</td>
<td></td>
<td>Florence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Onset variable Rogaev et al., 1995 (120)</td>
</tr>
</tbody>
</table>

Two studies using β-galactosidase fusion proteins support the model of eight transmembrane domains (TM) with N-terminal and C-terminal in the cytosol (124,125). In this model, HR7 and HR10 do not pass through but are associated with the membrane. Alternative topologies have also been suggested.

Post-Translational Modification

Presenilins are neither glycosylated nor modified by sulfation, acylation, or the addition of glycosaminoglycans (121), but both proteins are phosphorylated on serine residues (128,129). The most prominent post-translational modification of both PS1 and PS2 is proteolytic cleavage (130,131). PS1 is rapidly cleaved into a 27- to 28-kd N-terminal fragment (NTF) and an 18- to 20-kd C-terminal fragment (CTF), and PS2 is cleaved into two polypeptides of 34 and 20 kd, respectively.

Epitope mapping studies (131) and radiosequencing analysis (130) revealed that PS1 endoproteolysis occurs in the cytoplasmic loop domain, within a domain in which several of the identified FAD-linked PS1 mutations occur. The N-terminal of the CTF is heterogeneous, with the two predominant species beginning at amino acids 293 and 299 (130), encoded by exon 9. These findings are consistent with the demonstration that the FAD-linked PS1/E9 variant, which lacks exon 9 encoded sequences (amino acids 290 to 319), fails to be cleaved (131). Several of the PS mutations are clustered around this region.

The PS holoproteins are unstable, with half-lives about 1.5 hours (130,132), and their degradation is apparently mediated in part through the proteosome (133,134). In contrast, PS fragments produced through normal endoproteolysis of wild type and FAD-mutant presenilins are quite stable (half-life of approximately 24 hours) (130,132), a finding consistent with the hypothesis that the heterodimeric complexes represent the biologically active form of the protein. Presenilin molecules that are not incorporated into the complex are rapidly degraded by several proteases, including the caspases and calpain-like enzymes (134,135). It appears that endoproteolysis of the presenilins is not needed for activation of their putative activities but may be required to convert unstable presenilins into stable complexes (134).
Various C-terminally truncated and chimeric PS poly-peptides were used to characterize the interaction between NTF and CTF. It was observed that transgene-derived human PS1 NTF expressed in mouse N2a cells neither assembled with the endogenous CTF nor inhibited the cleavage or accumulation of the endogenous mouse PS1. Furthermore, in cells coexpressing PS1 and PS2, PS1- and PS2-derived fragments did not form mixed assemblies. In contrast, cells expressing a chimeric PS1/PS2 polypeptide formed PS1 NTF, PS2 CTF assemblies. These studies provide strong evidence that intramolecular associations between PS domains precede endoproteolytic processing (136).

In addition to the regulated endoproteolytic processing cleavage by the yet hypothetical presenilinase, presenilins also undergo additional cleavage, termed alternative cleavage, within the hydrophilic loop domain (133). Full-length PS1, as well as PS1- or PS2 derived CTFs, can be cleaved by caspases in transfected cells and cells induced to undergo apoptosis. Several members of the caspase family of proteases, including caspases 1, 3, 6, 7, 8, and 11, are capable of cleaving PS1 and PS2 in vitro (138).

**Localization**

Endogenous presenilins have a relatively limited subcellular distribution; they are found in the early compartments of biosynthetic pathway. Presenilin proteins have been localized to the endoplasmic reticulum (ER) and the Golgi subcellular compartments (137). Confocal and electron microscopy, combined with subcellular fractionation experiments, show that presenilins in neurons reside in the smooth and rough ER, the ER Golgi intermediate compartments, and, to a limited extent, in the cis-Golgi, but not beyond (139). The finding of overexpressed presenilin proteins within Golgi compartments should, however, be interpreted with caution, because evidence indicates that membrane proteins with many transmembrane domains can accumulate in structures called aggresomes, structures that reflect cell stress (140). Studies provide convincing evidence that some mammalian PS1 can be found at the cell surface, where it can be biotinylated (141).

**Biological Functions of Presenilins**

**Interaction with APP**

There is strong evidence that presenilins are able to interact directly with APP. Complex formation between APP and presenilins has been demonstrated by communoprecipitation of both proteins in cells either transfected or with endogenous proteins as well as with the yeast two-hybrid system (142,143). Thinakaran and colleagues, in contrast to these other researchers, did not observe physiologic complexes between PS1 and PS2 derivatives with APP (144).

Studies with progressive deletion of presenilin showed that the hydrophilic N-terminal of PS2 (1-87) is sufficient for the interaction with APP (127). Two different domains of APP appear to be involved in the APP-PS interaction. The last 100 C-terminal residues of APP (C100) encompassing Aβ and the TM region are able to interact with PS1 and PS2 (142,143). However, deletion of the cytoplasmic C-terminus domain does not abrogate PS1 binding (143). In addition, two APP constructs representing physiologically secreted forms of APP (sAPPα and sAPPβ) were shown to coprecipitate with PS2 in transfected COS cells (127). Taken together, these result suggest presenilin binds to the Aβ/transmembrane region and at least one additional interaction domain N-terminal of Aβ. Full maturation of APP does not seem to be required for the interaction, because the APP form detected in precipitated complexes is mostly immature.

**Role in APP Processing**

Pathogenic mutations in PS modify APP processing, thereby leading to an augmentation of Aβ42 secretion. Patients with AD who carry PS1 or PS2 mutations have significant increase of plasma Aβ42 levels (145) together with deposition of Aβ42 in the brain (146,147). In fibroblasts from such patients, the APP metabolism is shifted toward an increase of Aβ42 production. Similarly, the presence of mutated PS1 increases Aβ42 in transfected cells (148–151), as well as in transgenic mice (148–150). How the mutant PS influences the production of Aβ42 peptides remains uncertain, but these PS mutations appear to cause aberrant gain, rather than loss, of function. In neurons of PS1-knock-out mice, secretion of Aβ is drastically reduced, leading to the accumulation of α- and β-cleaved C-terminus stubs of APP (23,152). This gives evidence that PS1 is obligatory for proteolysis of APP at the γ-secretase cleavage site.

Wolf et al. mutated the two aspartates located in analogous positions near the middle of TM6 and TM7 to alanines (153). Either mutation, when expressed in various mammalian cell types, prevented both the normal endoproteolysis of PS1 within the hydrophobic region of the TM6-TM7 loop and markedly inhibited the γ-secretase cleavage of the 99-residue C-terminal fragment of APP (C99), thereby lowering levels of Aβ40 and Aβ42. Conservative substitution of aspartate by glutamate still abrogated the γ-secretase cleavage of APP, a finding indicating a specific requirement for the two TM aspartates. These results are consistent with one of two mechanisms: a role for presenilin as a unique cofactor for γ-secretase that could play a role in protein trafficking or a role as a functional γ-secretase, making it an unprecedented intramembranous aspartyl protease. There is evidence for and against both possibilities.

A major concern with the hypothesis that presenilins are proteases is their subcellular localization. Presenilin proteins...
have been localized to early transport compartments, whereas abundant γ-secretase activity is restricted to late transport compartments and the endosomal pathway (55, 61). The same holds true for the release of the Notch intracellular domain (see later), which occurs after ligand binding by Notch at the cell surface (154,155). In contrast, the cellular localization of presenilin proteins in ER and early Golgi overlaps to some degree with the intracellular site of generation of the highly amyloidogenic Aβ42. An additional concern is that the presenilin sequences have no homology to any of the proteases identified so far. Confirmation of the protease involved in cleavage is PS1 not only prevented APP processing by γ-secretase, but also prevented the cleavage of the Notch C-terminus in the membrane. This result immediately suggested that either presenilins are directly involved in cleaving both Notch and APP or mediate both cleavages in a more indirect way. Processing of Notch resembles in some aspects the processing of APP. Notch is processed by a furin-mediated cleavage during its passage through the Golgi system. The resultant two fragments remain in the same protein complex and localize in the cellular membrane to form the functional receptor. The binding of the ligand to the receptor stimulates the cleavage of one of the subunits at a specific extracellular site close to the membrane. A subsequent intramembranous cleavage liberates an intracellular fragment that translocates into the nucleus. This peptide forms an active transcription complex, which activates transcription of Notch target genes. The last of these proteolytic cleavage steps of Notch resembles γ-secretase cleavage of APP.

Complex formation between Notch and PS has been observed (141). There are also data showing that presenilins are functionally implicated in the Notch signaling pathway. The phenotype observed in PS1 knockout animal models (159) consists of a severe impairment of the development of the axial skeleton. The origin of these skeleton abnormalities lies in the impairment of the segmentation of the somites. Interestingly, Notch-1 (160) knockout animals suffered from similar abnormal skeleton deformations, a finding consistent with interaction of presenilins with Notch signaling pathway. De Strooper et al. used a Semiliki forest virus system to express truncated fragments of Notch-1 in primary neuronal cultures of PS1 knockout animals to identify the steps in Notch signaling depending on PS1(161). The authors provide evidence strongly suggesting that PS1 is crucial for the final processing of Notch-1 that generates the intracellular fragments, which subsequently will translocate to the nucleus and activate the expression of Notch-1 target genes.

The genetic studies in C. elegans and Drosophila offer a powerful approach to study presenilin function. Sel-12, a nematode homologue of presenilin, was identified by screening for suppressors of lin-12 (C. elegans homologue of Notch) gain of function mutation (162). Sel-12 is able to facilitate the signaling of transmembrane receptors of the lin-12/Notch family, and human presenilins have been shown to complement for Sel-12 function effectively (163). The egg-laying deficiency in C. elegans caused by the null mutations of Sel-12, the worm’s homologue of mammalian presenilin, can be rescued by wild-type, but not mutated, human presenilin (163,164). However, presenilin cleavage does not seem to be essential for functional activity, because the PS1 with the Δ-exon 9 mutation is still able at least partially to rescue the egg-laying defective phenotype of C. elegans Sel-12 mutants (164).
Proteins Interacting with PS

Presenilins have been found to interact directly with a variety of proteins. Proteins interacting with presenilins include members of the catenin family (165–167). Catenins have at least two different functions in the cell. First, they are components of cell–cell adhesive junctions interacting with the cytoskeletal anchors of cadherin adhesion molecules. Second, there is compelling evidence that β-catenin is a key effector in the Wingless/Wnt signaling cascade. Wingless and its vertebrate counterpart Wnt signaling direct many crucial developmental decisions in Drosophila and vertebrates. β-Catenin has been shown to become destabilized in case of PS1 mutations and PS1 deficiency (168). β- and β-Catenin interact with the large loop of PS1 (165,166).

The yeast two-hybrid system was used for the identification of a neuronal calcium-binding protein termed calsenilin (15), which binds to the C-terminus of PS2. Calsenilin was shown to interact with both PS1 and PS2 in cultured cells and could link presenilin function to pathways regulating intracellular calcium levels.

Several other proteins have been identified that interact with presenilins including the cytoskeletal proteins filamin and filamin homologue (168), µ-calpain (169), Rab11, a small guanosine triphosphatase belonging to the p21 ras-related superfamily (170), G-protein Go (171), and glyco-gen synthase kinase-3b (172).

Apoptosis and Cell Death

There is increasing evidence of causal involvement of presenilins in apoptosis. ALG3, a 103-residue C-terminal fragment of PS2, was isolated in death trap assay as rescuing a T-cell hybridoma from T-cell receptor and Fas-induced apoptosis (173). In PC12 cells, the down-regulation of PS2 by antisense RNA protects the cells from glutamate toxicity. Similar effects of ALG3 overexpression and PS2 down-regulation suggest that this C-terminal fragment of PS2 acts as a dominant negative form of PS2. Expression of mutant PS1 (L286V) in PC12 cells enhanced apoptosis on trophic factor withdrawal or Aβ toxicity (174). The alternative caspase cleavage in the C-terminal fragment of PS1 has been shown to abrogate the binding of PS1 to β-catenin (167) and could therefore modulate the apoptotic outcome.

Finally, the FAD mutation M146V was inserted by homologous recombination in the mouse genome (knock-in) to drive the expression at normal levels of a mutated mouse PS1 protein (175). The knock-in mutation was shown to increase ER calcium mobilization and superoxide and mitochondrial reactive oxygen species production leading to caspase activation (175).

Evidence shows that mutant PS1 also renders cells less efficient to respond to stress conditions in ER. Mutations in PS1 may increase vulnerability to ER stress by altering the unfolded protein response (UPR) signaling pathway that is responsible for ensuring the proper folding of newly synthesized proteins (176,177).

ROLE OF APOLIPOPROTEIN E ISOFORMS IN LATE-ONSET AD

In addition to the deterministic genetic mutations found in APP and presenilins, genetic factors modify the risk of developing AD. The APOE gene on chromosome 19 is considered as an important risk factor for the development of late-onset AD. Apo E is a 34-kd component of various lipoproteins, including chylomicrons, very-low-density lipoproteins (VLDLs), and a subset of high-density lipoproteins (HDLs) (178). These lipoproteins regulate plasma lipid transport and clearance by acting as ligand for lipoprotein receptors such as LDL-R and low-density receptor-related protein (LRP) (179,180). Apo E has been implicated in the transport of cholesterol and phospholipids for the repair, growth, and maintenance of membranes that occur during development or after injury (178).

Apo E is polymorphic and is encoded by three alleles (APOE2,3,4) that differ in two amino acid positions. The most common isoform, E3, has a Cys residue at position 112 and an Arg at position 158. The two variants contain either two Cys residues (E2) or two Arg residues (E4) at these positions. In general, it seems that E4 allele increases the risk of developing AD by approximately threefold, and that the E2 allele decreases the risk (181). The presence of one or two E4 alleles is associated with earlier onset of disease and an enhanced amyloid burden in brain, but it has little effect on the rate of progression of dementia (182). Thus, homozygous E4/E4 subjects have an earlier onset (mean age less than 70 years) than heterozygous E4 subjects (mean age of onset for E2/E3 is more than 90 years) (183).

The most obvious hypothesis is that APOE polymorphisms may influence the production, distribution, or clearance of Aβ. This hypothesis is supported by observations that the subjects with one or more APOE4 alleles have a higher amyloid burden than do subjects with no APOE4 alleles (184). Second, there is evidence that both Apo E and Aβ may be cleared through the LRP receptor, and Apo E4 and Aβ peptide may compete for clearance through the LRP receptor (179). Third, transgenic mice that overexpress APP develop a significantly lower number of Aβ deposits when they are bred to an APOE knockout background (185). These findings strongly support a role of Apo E in the aggregation or clearance of Aβ in the brain. The APOE genotype influences the onset of AD in patients with DS and in those with APP mutations but not in families with presenilin mutations (185–187).

OTHER GENETIC RISK FACTORS IN AD

In addition to the APOE gene, which has been confirmed as a strong risk factor in various studies, polymorphisms in
several other genes have been described to increase susceptibility for AD. Most of these genetic polymorphisms are still subject to discussion because they either need to be confirmed in larger studies or show only weak influence toward the risk of developing AD.

A family-based study revealed an association between late-onset AD and the presence of an exon 2 splice acceptor deletion in the A2M gene on the short arm of chromosome 12 (188). Significantly, A2M binds to a variety of proteins, including proteases (189,190) and Aβ peptide (191). In addition, A2M is also present in senile plaques and can attenuate Aβ fibrillogenesis and neurotoxicity (191). Moreover, A2M may, through LRP-mediated endocytosis, allow the internalization and subsequent lysosomal degradation of Aβ (192).

Other candidate AD genes that have been reported include α₁-antichymotrypsin (193), bleomycin hydrolase (194), and a gene on chromosome 3q25-26 (195). The candidacy for these genes as AD loci awaits further testing and confirmatory studies in greater numbers of AD samples.

Among the several risk factors, age is one of the most important elements that has to be considered with respect to sporadic Alzheimer-type dementia. Various cellular and molecular changes take place in the brain during normal aging, among which changes in glucose and energy metabolism are of pivotal significance (196). Reduced acetylcholine synthesis, formation of advanced glycation end products, membrane instability, and reduced energy availability are some of the other changes that occur with age. The decrease in the pool of available energy may lower or even arrest the transport of newly synthesized membrane proteins such as APP, thereby facilitating Aβ synthesis intracellularly. Therefore, it can be hypothesized that these changes somehow contribute to the formation of Aβ peptide and hyperphosphorylated tau. In a general context, age may be considered as a risk factor for neuronal damage and thus for age-related brain disorders such as sporadic dementia of the Alzheimer type.

AD has been reported to be in higher prevalence among old women compared with men. There is evidence suggesting that estrogen may have a protective role against AD, perhaps through its action as a trophic factor for cholinergic neurons (197), a modulator for the expression of Apo E in the brain (198,199), an antioxidant compound decreasing the neuronal damage caused by oxidative stress (200,201), or a promoter of physiologic nonamyloidogenic processing of the APP decreasing the production of Aβ (202). Therefore, in older postmenopausal women, the lack of circulation of estrogen may contribute to developing AD.

**CONCLUSIONS**

There are clearly very significant genetic contributions to AD. Practically, Apo E is the most significant genetic factor in AD because of the very high prevalence of the APOE4 allele, even though APOE is only a risk factor for AD.

The mutations in APP and PS account for only a small percentage of AD cases, but when present, these are deterministic mutations leading to an aggressive early-onset form of the disease. These mutations in APP and PS, rare though they are, give crucial insight into the molecular process underlying all forms of AD. Thus, the APP mutations clearly underscore critical role of APP in disease initiation. The PS mutations implicate Aβ, and particularly Aβ42, in disease. A similar, crucial role for Aβ in sporadic AD is supported by postmortem and tau studies. Polymorphism in APOE and A2M may also have their effects by interacting with APP and Aβ. It therefore seems likely that treatments that modulate Aβ formation or Aβ clearance may be of benefit in AD.

If Aβ changes initiate disease, it is likely that changes in tau are the actual cause of neuronal dysfunction and cognitive decline. Therefore, abrogating deleterious effects of Aβ on tau phosphorylation may represent another viable therapeutic approach to AD.

**REFERENCES**


130. Podlisny MB, Citron M, Amarante P, et al. Presenilin proteins undergo heterogeneous endoproteolysis between Thr291 and
151. Tomita T, Maruyama K, Saida TC, et al. The presenilin 2 mutation (N141I) linked to familial Alzheimer disease (Volga German families) increases the secretion of amyloid beta protein ending at the 42nd (or 43rd) residue. Proc Natl Acad Sci USA 1997;94:2025–2030.


