Catherine McKeon-O'Malley and Rudolph Tanzi

22

Etiology, Genetics, and Pathogenesis of Alzheimer's Disease

I. Amyloid Hypothesis

Alzheimer's disease (AD) is an age-related neurodegenerative disease that causes a global loss of cognitive function and behavioral deficits. Although cholinesterase inhibitors can improve cognitive function slightly (Knopman and Morris, 1997), truly efficacious drugs for AD treatment and/or prevention are not yet available. However, the etiology of AD is beginning to yield to scientific inquiry. Plausible strategies for treatment and/or prevention have been formulated, but await further research. In the United States, AD afflicts four million individuals and imposes an annual tab of $80–100 billion (Hoyert and Rosenberg, 1999). Thus, one can readily appreciate the importance of recent progress in AD research. This chapter summarizes the current understanding of AD etiology and pathogenesis.

AD cases can be classified as "familial" or "sporadic." Familial AD occurs in both early-onset (before age 65) and late-onset kindreds. In 1990, a mutation in the amyloid precursor protein (APP) was reported to cause hereditary cerebral hemorrhage with amyloidosis Dutch type (Levy *et al.*, 1990). This finding linked amyloid deposition to an APP mutation for the first time and enabled investigators to embrace the idea that plaques were an important cause of neurodegeneration, rather than merely end products of a neurodegenerative process. Later, APP mutations were found to cause AD in a small number of early-onset, autosomal-dominant pedigrees (see Table 22.1).

The neuropathological hallmark of AD is the presence of neuritic plaques in brain parenchyma and cerebral blood vessels. Neuritic plaques consist of a protein core, surrounded by degenerating neurites, astrocytes, and activated macrophages. Alzheimer brain is also characterized by the presence of neurofibrillary tangles (NFT; accumulations of paired helical filaments within neuronal cell bodies), the loss of synapses and neurons, and reduced neurotransmitter concentrations. Cholinergic neurons are especially vulnerable to cell death; these neurons arise in the basal forebrain and terminate in the hippocampus and cerebral cortex. Neurofibrillary tangles consist primarily of ubiquitin and tau, a microtubule-associated protein.

The core protein of plaques is Aβ, a peptide derived from the amyloid precursor protein (APP), which is 39–43 amino acids long (Glenner and Wong, 1984). Plaques also contain numerous other components, including apolipoprotein E (Namba *et al.*, 1991), α_1-antichymotrypsin (Abraham *et al.*, 1988), serum amyloid P (Coria *et al.*, 1988), interleukin-1 (Griffin *et al.*, 1995), basic fibroblast growth factor (Gomez-Pinilla *et al.*, 1990), α_2-macroglobulin (Bauer *et al.*, 1991), low-density lipoprotein-related protein (LRP) (Tooyama *et al.*, 1993), and perlecan (a heparin sulfate proteoglycan). Evidence for an inflammatory contribution to AD is provided by the presence of approximately 40 proteins known to play a role in inflammation (McGeer *et al.*, 1996). Plaques are enriched in the small molecules, zinc, copper, and iron (Lovell *et al.*, 1998).

Within plaques, the primary form of Aβ is Aβ_{42}, a highly-insoluble peptide that readily adopts a β-pleated sheet conformation (Iwatsubo *et al.*, 1994, 1995; Fukumoto *et al.*, 1996). Aβ molecules assemble into fibrils, which then pack into a highly ordered, crystalline-like lattice known as amyloid. The term "amyloid" can be applied to deposits derived from any protein in which a similar arrangement of molecules occurs. In addition to a core protein, all amyloid deposits are marked by the presence of heparan sulfate proteoglycans. "Diffuse" plaques are those in which Aβ molecules have not assembled into fibrils. Generally speaking, diffuse plaques are not surrounded by dystrophic neurites, activated microglia, or astrocytes. They occur in greater numbers than neuritic plaques and may be neuritic plaque precursors (Mackenzie, 1994). Neuritic plaque formation also occurs in Down syndrome and, to a lesser extent, in normal aging (Selkoe, 1991).

The "amyloid hypothesis" refers to the proposition that events leading to the manifestation of AD originate with the deposition of Aβ in amyloid deposits. Although flawed (or incomplete), this hypothesis is now supported by a large body of experimental work. It is worth noting that amyloid deposition occurs in other disorders such as Down syndrome,

TABLE 22.1 Amyloid Precursor Protein Mutations Associated with AD or Stroke

Pathogenic mutation	Reference
K/M 670/671 N/L (Swedish)	Mullan *et al.* (1992)
A682G (Flemish)	Hendricks *et al.* (1992)
E693Q (Dutch)	APP mutation implicated in hereditary cerebral hemorrhage with amyloidosis-Dutch (Levy *et al.*, 1990)
V715M (French)	Ancolio *et al.* (1999)
I716V	Eckman *et al.* (1997)
V717I (London)	Goate *et al.* (1991)
V717F	Murrell *et al.* (1991)
V717G	Chartier-Harlin *et al.* (1991)

Creutzfeldt–Jacob disease, Gertsmann–Sträussler–Scheinker disease, type II diabetes, familial amyloid polyneuropathy, and multiple myeloma. Aberrant protein deposition is a common theme in neurodegenerative disease (Kaytor and Warren, 2000). In Parkinson's disease, two mutations in the α-synuclein gene that cause early onset disease also accelerate α-synuclein aggregation (Narhi *et al.*, 1999).

Clearly, both environmental and genetic factors contribute to the risk of AD. Evidence from twin studies supports a role for environmental variables in AD (Breitner *et al.*, 1995; Nee and Lippa, 1999). Among the factors suggested to modify the risk for AD are head injury (Molgaard *et al.*, 1990; Roberts *et al.*, 1994), educational attainment (Stern *et al.*, 1992; Cobb *et al.*, 1995; Callahan *et al.*, 1996; Geerlings *et al.*, 1999), depression (Kokmen *et al*, 1996; Chen *et al.*, 1999), smoking (Hebert *et al.*, 1992; Lee, 1994; Hillier and Salib, 1997; Merchant *et al.*, 1999), vitamin E consumption (Vatassery *et al.*, 1999), diabetes (Leibson *et al.*, 1997) and hypertension (Skoog *et al.*, 1998; Behl, 1999). A review of the literature cited earlier will reveal that the legitimacy of many so-called environmental risk factors is open to debate. Stronger, but not conclusive, evidence indicates that estrogen replacement (Birge, 1997; Haskell *et al.*, 1997) and the chronic use of nonsteroidal anti-inflammatory drugs may reduce the risk of AD (McGeer *et al.*, 1996). AD and peripheral vascular disease share several risk factors in common; this indicates that impaired cholesterol metabolism may play a role in AD etiology (McKeon-O'Malley *et al.*, 1998). It remains to be determined whether any environmental factor is sufficient to cause AD in the absence of a permissive genotype.

On the genetic front, the identification of several AD-linked genes has yielded important insights into the etiology of AD, and steady progress continues to be made in this area. Female gender is a risk factor for AD, even when the longer life span of females is taken into account (Letenneur *et al.*, 1999). Almost all individuals with Down syndrome (trisomy 21) develop AD in midlife (Katzmann, 1986), presumably due to the overexpression of the APP gene on chromosome 21. The linkage of specific genes to AD risk is discussed in the following section.

II. Genetic Contributions to the Etiology of AD

To date, seven of the genes linked to AD include: the amyloid precursor protein (Chartier-Harlin *et al.*, 1991; Goate *et al.*, 1991; Murrell *et al.*, 1991), presenilin 1 (PS1) (Sherrington *et al.*, 1995), presenilin 2 (PS2) (Levy-Lahad *et al.*, 1995b), apolipoprotein E (Corder *et al.*, 1993), α_2-macroglobulin (Blacker *et al.*, 1998; Liao *et al.*, 1998), LRP (also known as the α2M receptor) (Kang *et al.*, 1997; Wavrant-DeVriéze *et al.*, 1997; Hollenbach *et al.*, 1998), and tau (Lilius *et al.*, 1999; Bullido *et al.*, 2000). Importantly, four of these proteins are related to each other: APP, ApoE, and α_2-macroglobulin are ligands for LRP.

Preliminary evidence has been presented for the involvement of other genes with AD (see Table 22.2). However, data do not permit firm conclusions to be drawn about other genes at this time. Deterministic mutations (i.e., mutations that can cause AD with 100% penetrance) occur in the genes for the amyloid precursor protein, presenilin 1 and presenilin 2, but these mutations account for only a small percentage of total AD cases. Mutations in APP and the presenilins cause AD by increasing the extracellular load of $A\beta_{42}$ (Scheuner *et al.*, 1996). Polymorphisms in the genes for apolipoprotein

TABLE 22.2 Genes in Which Mutations or Polymorphisms May Modify the Risk of AD[a]

Gene	Reference
Angiotensin 1-converting enzyme	Alvarez *et al.* (1999)
α_1-Antichymotrypsin	Haines *et al.* (1996); Talbot *et al.* (1996)
Bleomycin hydrolase	Farrer *et al.* (1998); Montoya *et al.* (1998)
Butyrylcholinesterase K	Brindle *et al.* (1998); Crawford *et al.* (1998); Hiltunen *et al.* (1998); Singleton *et al.* (1998); Tilley *et al.* (1999)
Cathepsin D	Papassotiropoulos *et al.* (1999)
Dihydrolipoyl succinyltransferase (DLST)	Sheu *et al.* (1999)
Estrogen receptor α gene	Brandi *et al.* (1999)
HLA	Ballerini *et al.* (1999)
Lipoprotein lipase	Baum *et al.* (1999)
Mitochondrial genome	Davis *et al.* (1997); Egensperger *et al.* (1997); Chagnon *et al.* (1999)
Myeloperoxidase	Reynolds *et al.* (1999)
Neurotropin-3	Kunugi *et al.* (1998)
Nitric oxide synthase 3	Dahiyat *et al.* (1999)
Serotonin transporter gene	Oliveira *et al.* (1998, 1999); Li *et al.* (1999)
Very low density lipoprotein receptor	Okuizumi *et al.* (1995, 1996); Pritchard *et al.* (1996)

[a]The genes may be associated with AD. Cited references include articles that report the association of a particular gene with AD, or a lack of such an association.

E, α_2-macroglobulin, LRP, and tau are known to increase the risk of AD. A brief description of each of these genes is given.

A. Amyloid Precursor Protein

As indicated earlier, the core protein of neuritic plaques, Aβ, is derived from APP. The APP gene is located at the boundary of 21.q.3 and 21.q22.1 (Kang *et al.*, 1987) and is widely expressed in human tissues (Tanzi *et al.*, 1988). Ten transcripts can be produced by alternative splicing of 19 exons (Wisniewski *et al.*, 1994). However, Aβ cannot be generated by alternative splicing (Lemaire *et al.*, 1989). Three major transcripts contain the Aβ sequence, and these encode proteins with 695, 771, or 770 amino acids (APP_{695}, APP_{751}, APP_{770}). The expression of APP_{695} is confined to the brain (Sola *et al.*, 1993). APP_{751} and APP_{770} contain a region with 50% homology to a Kunitz protease inhibitor domain.

The APP gene lacks a TATA box and has a high GC content, features characteristic of a housekeeping gene (Salbaum *et al.*, 1988). Gene expression produces a protein with the following domains: a signal peptide for transport of APP into the endoplasmic reticulum, a cysteine-rich sequence, a sequence including many negatively charged residues (glutamic acid and aspartic acid), and an uninterrupted stretch of seven threonine residues (Kang *et al.*, 987) (see Fig. 22.1). A zinc II-binding site is located between the cysteine-rich domain and the negatively charged region (Bush *et al.*, 1993). Two consensus sequences for N-linked glycosylation are located at amino acids 467–469 and 496–498. APP also contains a potential heparin-binding site (Small, 1994). APP is modified by post-translational mechanisms including N- and O-glycosylation (Weidemann *et al.*, 1989; Pahlsson *et al.*, 1992), sulfation of tyrosine residues (Weidemann *et al.*, 1989), and phosphorylation (Oltersdorf *et al.*, 1990). APP matures in the endoplasmic reticulum and the Golgi and then becomes inserted into the plasma membrane (Weidemann *et al.*, 1989).

APP is a member of a family of highly conserved proteins. Other members of this family include amyloid precursor protein-like protein (APLP1) and APLP2 (Sprecher *et al.*, 1993; Wasco *et al.*, 1993), but the two latter proteins do not encode Aβ. APP plays a role in many normal functions, including wound healing (Smith *et al.*, 1990; Van Nostrand *et al.*, 1990), proliferation (Saitoh *et al.*, 1989; Ninomiya *et al.*, 1993), adhesion (Schubert *et al.*, 1989; Breen *et al.*, 1991; Chen and Yankner, 1991; Ghiso *et al.*, 1992), neurite extension (Araki *et al.*, 1991; Milward *et al.*, 1992; Small, 1994), survival under stress (Mattson *et al.*, 1993; Yamamoto *et al.*, 1994), and synaptic plasticity (Mattson, 1994). Processes involved in the production of Aβ from APP are discussed under Section III.

B. Apolipoprotein E

Apolipoprotein E is a 34 kDa (299 residues) protein known primarily for its role in lipid transport (Mahley and Huang, 1999). The gene for ApoE has been mapped to chromosome 19 (Das *et al.*, 1985). Three polymorphic alleles, ϵ2, ϵ3, and ϵ4 (corresponding to the proteins ApoE2, ApoE3, and ApoE4), occur at frequencies of 8, 75, and 15%, respectively. ApoE3 has Cys^{112} and Arg^{152}; in ApoE2, Arg^{158} is replaced by cysteine, and in ApoE4 , Cys^{112} is replaced by arginine. In the periphery, ApoE is synthesized primarily in the liver. ApoE cannot penetrate the blood–brain barrier, but is manufactured within the brain by astrocytes (Pitas *et al.*, 1987). Apolipoprotein E is a ligand for three cell surface receptors: the low-density lipoprotein (LDL) receptor, the low-density lipoprotein-related protein, and the very-low-density lipoprotein (VLDL) receptor.

The ϵ4 allele of apolipoprotein E increases the risk of AD in a dose-dependent manner and lowers the age at onset (Corder *et al.*, 1993). This finding has been confirmed repeatedly in a host of ethnic groups around the world. However, it is clear that apolipoprotein E alone does not cause AD because a proportion of elderly homozygotes are unaffected (Roses *et al.*, 1994). Interestingly, the ϵ4 allele has also been shown to increase the risk of cardiovascular disease as well as AD (Lambert *et al.*, 2000). Mutations occurring in noncoding regions of the ApoE gene are also associated with AD (see Table 22.3). In

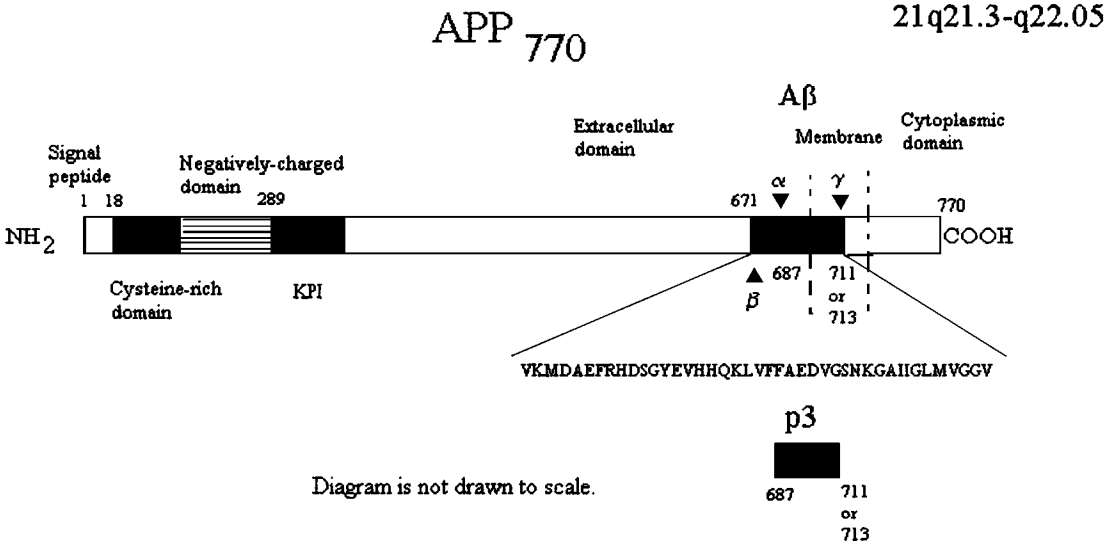

FIG. 22.1. Schematic diagram of APP (not drawn to scale).

TABLE 22.3 Apolipoprotein E Polymorphisms Associated with AD

Polymorphism	Reference
$\epsilon 2/\epsilon 3/\epsilon 4$ polymorphism	The $\epsilon 4$ allele is associated with sporadic and late-onset AD. This finding has been confirmed in more that 100 studies (Corder *et al.*, 1993)
APOE*4 Pittsburgh (APOE*4P) (this is a missense mutation, L28P, caused by a T → C substitution in exon 3)	All APOE*4P carriers identified thus far also carry $\epsilon 4$. However, the risk of AD is five-fold greater in $\epsilon 4$ carriers who also carry APOE*4P than in those who bear the $\epsilon 4$ allele alone (Kamboh *et al.*, 1999)
Th1/E47 cs polymorphism: G to mutation occurs at −186 bp in relation to the TATA box; this polymorphism is located in a consensus sequence for the transcription factor Th1E47	The presence of at least one T allele increases the risk of AD (Lambert *et al.*, 1998)

contrast to the $\epsilon 4$ allele, the $\epsilon 2$ allele decreases the risk of AD (Corder *et al.*, 1994; Talbot *et al.*, 1994).

C. Presenilin 1 and Presenilin 2

Mutations occurring within the homologous, transmembrane proteins PS1 and PS2 cause early-onset familial AD with 100% penetrance. About 70 such mutations have been identified in PS1, whereas only 5 have been observed in PS2 (see Tables 22.4 and 22.5; Table 22.4 includes only a partial list of all PS1 mutations). Remarkably, mutations in PS1 and PS2 increase the $A\beta_{42}/A\beta_{40}$ ratio, or total $A\beta$ both *in vitro* and *in vivo* (Borchelt *et al.*, 1996; Lemere *et al.*, 1996; Mann *et al.*, 1996; Scheuner *et al.*, 1996; Citron *et al.*, 1997, 1998; Tomita *et al.*, 1997). The presenilins may be γ-secretases, the enzymes that generate final cleavage in the release of $A\beta$ from APP (Wolfe *et al.*, 1999).

PS1 and PS2 share a similar pattern of gene expression. Both proteins are expressed primarily within neurons and are localized to the endoplasmic reticulum and Golgi (Kovacs *et al.*, 1996; Blanchard *et al.*, 1997).

The gene for PS1 is located on chromosome 14q24.3 and encodes a 43 to 45 kDa protein, 467 amino acids long (George-Hyslop *et al.*, 1992; Schellenberg *et al.*, 1992). The PS2 gene has been mapped to chromosome 1q42.1 and encodes a 53 to 55 kDa protein, 448 amino acids long (Levy-Lahad *et al.*, 1995a,b; Takano *et al.*, 1997). The presenilins are predicted to contain six (Lehmann *et al.*, 1997), seven (Dewji and Singer, 1997), or eight (Li and Greenwald, 1998) transmembrane domains and a large hydrophilic loop. The N-terminal, C-terminal, and the large hydrophilic loop of PS1 protrude from the endoplasmic reticulum membrane into the cytoplasm (Doan *et al.*, 1996).

The presenilins are cleaved endoproteolytically to produce N-terminal fragments and C-terminal fragments, with approximate molecular masses of 30 and 20 kDa, respectively (Thinakaran *et al.*, 1996). This cleavage occurs at a site within the large hydrophilic loop. N- and C-terminal fragment associate with one another in a 1:1 ratio, forming a stable complex (Capell *et al.*, 1998). Measurable quantities of the full-length proteins are difficult to detect by conventional methods, strongly suggesting that the cleavage products are the physiologically relevant entities.

Potential roles for the presenilins have been suggested in protein processing, Notch signaling and development, and apoptosis (Mattson *et al.*, 1997, 1998; Guo *et al.*, 1998a,b). Mice homozygous for a null mutation in the murine homologue of PS1 (i.e., PS1 knockout mice) die within minutes of birth (Shen *et al.*, 1997). These animals exhibit gross skeletal defects, cerebral hemorrhage, and massive neuronal loss. The lethal phenotype of these animals indicates that PS1 plays an essential role in normal development.

D. α_2-Macroglobulin

α_2-Macroglobulin (α2M) is a "pan-protease" inhibitor involved in the clearance of proteins from the blood via endocytosis (Borth, 1992). α2M is composed of four identical subunits (180 kDa), each of which contains a 25 residue "bait region," a cytokine-binding domain, and a receptor-binding domain. The bait region contains an internal cyclic thiol ester that is cleaved when a protease binds to α2M. This cleavage provokes a conformational change in α2M that permits it to enclose or "capture" the protease and to make its cytokine-binding and receptor-binding domains accessible for ligand binding. Activated α2M binds to its plasma membrane receptor, LRP, to deliver its captured protease. Ligand–LRP complexes are internalized via clathrin-coated pits and are then directed into an endosomal/lysosomal compartment (Kowal *et al.*, 1989). There, ligands are released from LRP and degraded; LRP is recycled to the plasma membrane.

The gene for α2M is located on chromosome 12 (Fukushima *et al.*, 1988). An AD-linked polymorphism is located in the 5′ splice site of exon 18. Two polymorphisms, occurring in this locale, α2M-2 and α2M-1, specify the presence or absence of a pentanucleotide deletion. Inheritance of a single α2M-2 allele increases the risk of AD three- to fourfold, but does not change the age at onset (Blacker *et al.*, 1998). To date, it is not known whether the risk of AD increases with α2M-2 dosage. AD is also associated with a second polymorphic site, Val1000(GTC)/Ile1000(ATC), located near the cyclic thiol ester (Poller *et al.*, 1992). AD risk is increased by the presence of a valine residue at this site (Liao *et al.*, 1998). The risks conferred by the Val1000(GTC) allele of α2M, and the $\epsilon 4$ allele of ApoE, are independent and additive.

E. Lipoprotein-Related Protein

As described previously, LRP is a plasma membrane receptor for APP, ApoE, and α2M. Importantly, an $A\beta$-serine

TABLE 22.4 A Partial List of Presenilin I Mutations Associated with AD

Mutation	Reference	Mutation	Reference
A79V	Cruts *et al.* (1998)	A231V	Cruts *et al.* (1998)
V82L	Campion *et al.* (1995)	M233L	Aldudo *et al.* (1999)
V96F	Kamino *et al.* (1996)	M233T	Kwok *et al.* (1997)
F105L	Finckh *et al.* (2000)	L235P	Campion *et al.* (1996)
Y115H	Campion *et al.* (1995)	A246E	Sherrington *et al.* (1995)
Y115C	Cruts *et al.* (1998)	L250S	Harvey *et al.* (1998)
T116N	Romero *et al.* (1999)	A260V	Rogaev *et al.* (1995)
P117L	Wisniewski *et al.* (1998)	L262F	Forsell *et al.* (1997)
E120D	Poorkaj *et al.* (1998b)	C263R	Wasco *et al.* (1995)
Q120D	Reznik-Wolf *et al.* (1996)	P264L	Campion *et al.* (1995)
E120K	Reznik-Wolf *et al.* (1998)	P267S	Alzheimer's Disease Collaborative Group (1995)
E123K	Yasuda *et al.* (1999)	R269G	Perez-Tur *et al.* (1996)
N135D	Crook *et al.* (1997)	R269H	Gomez-Isla *et al.* (1997)
M139K	Dumanchin *et al.* (1998)	E273A	Kamimura *et al.* (1998)
M139T	Campion *et al.* (1995)	R278T	Kwok *et al.* (1997)
M139I	Boteva *et al.* (1996)	E280A	Alzheimer's Disease Collaborative Group (1995)
I143F	Rossor *et al.* (1996)	E280G	Alzheimer's Disease Collaborative Group (1995)
I143T	Cruts *et al.* (1995)	L282R	Aldudo *et al.* (1998a)
M146I	Jorgensen *et al.* (1996)	A285V	Aoki *et al.* (1997)
M146L	Sherrington *et al.* (1995)	L286V	Sherrington *et al.* (1995)
M146V	Alzheimer's Disease Collaborative Group (1995)	E318G	Reznik-Wolf *et al.* (1998); Aldudo *et al.* (1998b); Mattila *et al.* (1998)
T147I	Campion *et al.* (1999)	G378E	Besançon *et al.* (1998)
H163R	Sherrington *et al.* (1995)	G384A	Cruts *et al.* (1995)
H163Y	Perez-Tur *et al.* (1995); Axelman *et al.* (1998)	S390I	Campion *et al.* (1999)
W165C	Campion *et al.* (1999)	C410Y	Sherrington *et al.* (1995)
S169P	Ezquerra *et al.* (1999)	L424R	Kowalska *et al.* (1999)
S169L	Taddei *et al.* (1998)	A426P	Poorkaj *et al.* (1998b)
L171P	Ramirez-Duenas *et al.* (1998)	P436Q	Taddei *et al.* (1998)
L173W	Campion *et al.* (1999)	To to G at intron 9	Nishiwaki *et al.* (1997)
E184D	Yasuda *et al.* (1997)	Intronic polymorphism located 3' to exon 8	Wragg *et al.* (1996); but see Cai *et al.* (1997), Tysoe *et al.* (1997), Sorbi *et al.* (1997)
G209R	Sugiyama *et al.* (1999)	Deletion Delta9Finn	Crook *et al.* (1998); Prihar *et al.* (1999)
G209V	Poorkaj *et al.* (1998b)	58304G>A Delta9	Sato *et al.* (1998)
I213T	Kamino *et al.* (1996)	58304G>T Delta9	Perez-Tur *et al.* (1995)
A231T	Campion *et al.* (1995)	A deletion of G from the intron four splice donor consensus sequence	Tysoe *et al.* (1998); PS1 truncating mutation

TABLE 22.5 Presenilin 2 Mutations Associated with AD

Pathogenic mutation	Reference
T122P	Finckh *et al.* (2000)
N141I	Levy-Lahad *et al.* (1995a)
M239I	Finckh *et al.* (2000)
M239V	Rogaev *et al.* (1995)
Splice variant: mRNA lacks exon 5	Sato *et al.* (1999)

protease-α2M pathway is one of several mechanisms by which Aβ is cleared (Qiu *et al.*, 1999). LRP belongs to a family of proteins that includes the LDL receptor, megalin (also known as gp330), the VLDL receptor, and the vitellogenin receptor (Krieger and Herz, 1994).

F. Tau

The diagnosis of AD in postmortem brain requires the presence of both NFT and neuritic plaques. Unlike plaques, NFT are not specific to AD; these entities occur in many neurodegenerative disorders, including supranuclear palsy, dementia pugilistica, corticobasal degeneration, and fronto-temporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Goedert *et al.*, 1997). However, NFT density correlates well with dementia severity, whereas plaque density does not (Braak and Braak, 1991).

The primary protein component of NFT is tau (45–60 kDa), a protein that promotes the stabilization of microtubules (Weingarten *et al.*, 1975). In the central nervous system, alternative splicing of the tau gene results in the expression of six isoforms primarily (see Fig. 22.2). A seventh isoform containing a "big tau insert" is expressed prominently in the peripheral nervous system and to a much lesser extent in the central nervous system (Georgieff *et al.*, 1993). Each isoform possesses either three or four microtubule-binding domains (repeat units of 31 amino acids, encoded by exon 10) and 0, 1, or 2 different amino-terminal inserts. Differential expression of tau isoforms occurs during development (Couchie *et al.*, 1988; Kosik *et al.*, 1989). Within NFT, tau is hyperphosphorylated (Grundke-Iqbal *et al.*, 1986), which prevents its binding to microtubules (Bramblett *et al.*, 1993).

The finding made in 1991 that mutations in the amyloid precursor protein gene can cause AD with 100% penetrance focused attention on the role of Aβ in AD. Lately, there has been a resurgence of interest in tau. Tau mutations have been linked to frontotemporal dementia with parkinsonism (FTDP-17; previously known as Pick disease) (Poorkaj *et al.*, 1998a; Hutton *et al.*, 1998; Spillantini *et al.*, 1998; Iijima *et al.*, 1999) and to supranuclear palsy (Chambers *et al.*, 1999). Because plaque deposits are largely absent in FTDP-17 tauopathies, the latter findings indicate that tau aggregation is sufficient for neurodegeneration (Ghetti *et al.*, 1999).

Several groups have searched for AD-linked mutations in the tau gene without success (Crawford *et al.*, 1999; Roks *et al.*, 1999). However, two reports indicate that polymorphisms in the tau gene may increase the risk for AD when found in combination with the ApoE ϵ4 allele (Lilius *et al.*, 1999; Bullido *et al.*, 2000).

III. Pathogenesis

A. Fundamental Questions in AD Research

Most of the current research in AD is designed to address one of the following questions:

What factors regulate the cleavage of Aβ from APP, and Aβ assembly into fibrils?

How does Aβ, alone or in combination with other plaque components, cause neuronal cell death?

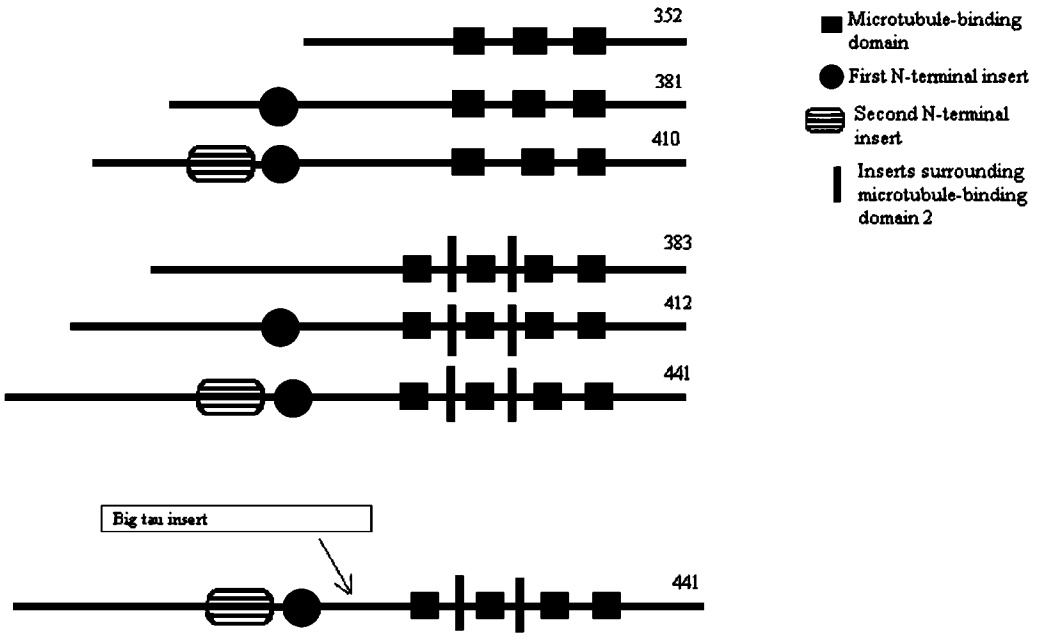

FIG. 22.2. Schematic diagram of tau protein isoforms.

What is the role of inflammation in the maturation of neuritic plaques?

What is the role of tau in AD?

A thorough discussion of last three questions is beyond the scope of this chapter (see Tolnay and Probst, 1999, for a review of the role of tau in AD.) This chapter concentrates, how Aβ is produced from APP, and on Aβ fibrillogenesis.

B. Amyloid, Neuritic Plaques, and Paired Helical Filaments

Within specific brain locale, certain conditions (as yet undefined) permit soluble Aβ molecules to adopt a high degree of β-pleated sheet conformation; this alteration facilitates the rearrangement of Aβ molecules into fibrils. Fibrils pack in cross-β conformation to form amyloid, a highly ordered arrangement of protein molecules that can be detected using Congo Red or Thioflavin S (Kirschner *et al.*, 1987). Neuritic plaques, but not diffuse plaques, are visualized using these stains. Fibril stability is critically dependent on the primary structure of the peptide. This is exemplified by the fact that an APP mutation associated with early-onset FAD, $A\beta_{E22Q}$, increases the stability of Aβ fibrils dramatically (Fraser *et al.*, 1992).

Within plaques, Aβ molecules differ in length, exhibiting heterogeneity at both amino (Tekirian *et al.*, 1998) and carboxyl termini (Jarrett *et al.*, 1993a). Plaques contain a mixture of Aβ molecules; $A\beta_{42}$ and $A\beta_{40}$ are the predominant species in extracellular neuritic plaques, whereas the vascular amyloid consists primarily of $A\beta_{39-40}$ (Dickson, 1997). As indicated earlier, a highly amyloidogenic form of Aβ, $A\beta_{42}$, is the initial Aβ species deposited in neuritic plaques (Iwatsubo *et al.*, 1995). Diffuse plaques contain $A\beta_{42}$, but not $A\beta_{40}$ (Cummings *et al.*, 1996). Plaque enlargement requires a nidus for further protein deposition; $A\beta_{42}$ may serve as a "seed," which permits the deposition of $A\beta_{40}$ (Jarrett *et al.*, 1993b). $A\beta_{40}$ constitutes the bulk of the Aβ produced by normal metabolism (Haass *et al.*, 1992; Seubert *et al.*, 1992).

Neuritic plaques can be surrounded by dystrophic neurites or by neurites containing paired helical filaments (PHF-type neurites). [PHF occurring within neurites are essentially equivalent to those found in neuronal cell bodies. In AD, PHF occur in three locations: within plaques, neurites, or neuronal cell bodies (Braak *et al.*, 1986). PHF-type neurites found in the absence of plaques are known as "neuropil threads" (Dickson, 1997).] Among dystrophic neurites, diverse neuronal types are represented (Struble *et al.*, 1987). In contrast, there is a hierarchy of neuronal types that are susceptible to PHF formation (Price *et al.*, 1991). Both dystrophic neurites and PHF-type neurites are recognized by ubiquitin-specific antibodies, but only the latter are recognized by the Alz-50 antibody.

C. Aβ Formation, Aggregation, and Clearance

1. APP Cleavage Sites

APP is cleaved within Aβ at Lys^{687}-Leu^{688} (APP_{770} numbering), by one or more "α-secretases." This cleavage generates a soluble N-terminal fragment ($sAPP_\alpha$) and a membrane-associated C-terminal fragment. Thus, α-secretase-mediated cleavage of APP precludes the production of Aβ. The amino and carboxy termini of Aβ are produced by the actions of "β-secretase" and "γ-secretase," respectively. As in the case of α-secretase, β-secretase-mediated cleavage of APP produces an amino-terminal fragment ($sAPP_\beta$) and a membrane-associated C-terminal fragment. The "p3" peptide (3 kDa) is created when the C-terminal fragment produced by α-secretase cleavage becomes a substrate for γ-secretase (Haass *et al.*, 1993). The p3 sequence may be amyloidogenic (Lalowski *et al.*, 1996; Dickson, 1997). The cleavage of APP by γ-secretase is unusual in that it occurs within the transmembrane domain of APP. Aβ and p3 are normal constituents of biological fluids (Shoji *et al.*, 1992). Secreted $sAPP_\alpha$ protects cells from toxic insults (Goodman and Mattson, 1994; Furukawa *et al.*, 1996). In this regard, it may be much more effective than $sAPP_\beta$ (Barger and Mattson, 1996).

After its synthesis on ribosomes, APP is directed into the endoplasmic reticulum by its signal peptide (Kang *et al.*, 1987). During its transit through the constitutive secretory pathway (endoplasmic reticulum, Golgi, and *trans*-Golgi network), APP is phosphorylated on its ectodomain. A small percentage of total holoprotein is inserted into the plasma membrane, where it is subject to cleavage by α- or β-secretases (Selkoe, 1998). Uncleaved holoprotein and the C-terminal fragments remaining in the plasma membrane after α-secretase or β-secretase cleavage (C83 and C99, respectively) are reinternalized via clathrin-coated vesicles. The latter molecules can be recycled to the cell surface or enter an endosomal/lysosomal pathway.

2. α-Secretase-Mediated Cleavage of APP

APP has a hydrophobic sequence near its carboxyl terminus (about 23 residues long), which directs its insertion into the plasma membrane and internal membranes of the endoplasmic reticulum, Golgi, and *trans*-Golgi network (Selkoe, 1998). α-Secretase-mediated cleavage of APP occurs both at the cell surface and within the constitutive secretory pathway. This cleavage does not depend on a specific sequence of amino acids; rather, it cuts APP at a specific distance from the plasma membrane (Maruyama *et al.*, 1991).

Total α-secretase activity can be divided into PKC-independent and PKC-dependent components (Buxbaum *et al.*, 1994), which represent basal and stimulated α-secretase activity, respectively. PKC-independent regulation of α- secretase activity involves the elevation of intracellular calcium (Buxbaum *et al.*, 1994). Several metalloproteases belonging to the ADAM family have been shown to possess α-secretase-like activity; among these are TACE (tumor necrosis $factor_\alpha$ converting enyme, also known as ADAM-17), MDC9, and ADAM-10. ADAM metalloproteases are membrane-anchored proteins that contain a catalytic domain, an autoinhibitory domain, a disintegrin-like domain, a cysteine-rich sequence, and epidermal growth factor-like sequence. TACE, MDC9, and ADAM-10 also possess a consensus sequence (HEXXH) for a zinc-binding domain. TACE mediates most cellular PKC-dependent α-secretase activity (Buxbaum *et al.*, 1998; Lammich *et al.*, 1999). MDC9 mediates both basal and PKC-induced cleavage of APP_{695} at the α-secretase site, and inhibition of MDC9 increases β-secretase cleavage (Koike *et al.*, 1999).

Overexpression of ADAM-10 in HEK 293 cells stimulates both basal and PKC-dependent α-secretase activity (Lammich *et al.*, 1999).

3. Cleavage of Aβ from APP

There are several pathways for the production of Aβ_{42} and Aβ_{40}. Aβ_{40} is generated in recycling endosomes following reinternalization from the cell surface (Koo and Squazzo, 1994). Both peptides are produced in the secretory pathway (Chyung *et al.*, 1997; Wild-Bode *et al.*, 1997), but the primary site for Aβ_{42} production is the endoplasmic reticulum, whereas the primary site for Aβ_{40} production is the *trans*-Golgi network (Hartmann *et al.*, 1997). Intracellular production of Aβ may be unique to neurons because nonneuronal cells produce significant amounts of Aβ_{42} and Aβ_{40} only at the cell surface (Hartmann *et al.*, 1997).

Four groups independently identified an unusual membrane-bound aspartyl protease as the elusive β-secretase (Hussain *et al.*, 1999; Sinha *et al.*, 1999; Vassar *et al.*, 1999; Yan *et al.*, 1999). This enzyme has been named BACE (β-site APP-cleaving enzyme) by one group and Asp2 by another group. Although other proteases, such as cathepsin D, can cleave APP at the β-secretase site, BACE meets all the requirements of a true β-secretase. BACE is located within the Golgi and endosomes and has a pH optimum of 5–5.5.

To date, γ-secretase has not been identified. Because inhibition of γ-secretase activity is a prime therapeutic major therapeutic target, the identity of this enzyme may be known by the time this chapter is published. Clearly, presenilin 1 is implicated in γ-secretase cleavage, either as an essential cofactor or as the enzyme itself (Wolfe *et al.*, 1999).

4. Regulation of APP Processing

The regulation of APP processing is extremely complex. It varies across species and also differs between neuronal and nonneuronal cell types. In nonneuronal cell lines or nonhuman cell lines, acetylcholine binding to muscarinic receptor subtypes concurrently increases $sAPP_\alpha$ production and *inhibits* Aβ production (Buxbaum *et al.*, 1992; Hung *et al.*, 1993; Jacobsen *et al.*, 1994). This effect can be mimicked by phorbol ester, indicating the involvement of PKC in signal transduction. However, in cultures of primary human cerebral neurons, PKC activation increases the rate of $sAPP_\alpha$ release and *increases* the production of Aβ (LeBlanc *et al.*, 1998). The latter finding, in conjunction with others, indicates that distinct pathways exist for α- and β-secretase-mediated cleavage of APP (Dyrks *et al.*, 1994). PKC-induced α-secretase cleavage is regulated by protein phosphorylation, but does not depend on the phosphorylation of APP (Jacobsen *et al.*, 1994). The *trans*-Golgi network is the site of regulated, intracellular α-secretase cleavage (Skovronsky *et al.*, 2000).

5. Aβ Aggregation

Aβ aggregation is dependent on concentration, pH, and the length of incubation in aqueous media (Burdick *et al.*, 1992). Aβ exists in a random conformation at low pH, a β-pleated sheet conformation at pH 4–7, and a random conformation at high pH (Barrow and Zagorski, 1991; Fraser *et al.*, 1992). The effect of pH on Aβ conformation indicates that Aβ aggregation is influenced by the ionization state of key residues. Histidine-aspartic acid/glutamic acid salt bridges stabilize β-pleated sheets, facilitating fibril assembly (Fraser *et al.*, 1991). Due to the presence of hydrophobic residues at its carboxyl terminus, Aβ_{42} is very insoluble in water at pH 7.4 (Burdick *et al.*, 1992).

Aqueous solutions of Aβ exhibit kinetic rather than thermodynamic solubility (Jarrett and Lansbury, 1993). In other words, Aβ will precipitate from apparently soluble solutions, given sufficient time. The rate-limiting step in the formation of amyloid is nucleation, i.e., the formation of a certain sized Aβ oligomer, which can serve as a scaffold for further aggregation (Jarrett and Lansbury, 1992). Lag time, i.e., the time until a solution exhibiting kinetic solubility precipitates, is directly proportional to the size of the Aβ oligomer required for nucleation and inversely proportional to peptide concentration.

Biometals can induce Aβ aggregation *in vitro* (Bush *et al.*, 1994). In AD, concentrations of zinc, copper, and iron are likely to be particularly important (for a review, see Atwood *et al.*, 1999). Submicromolar copper induces the aggregation of Aβ_{40} at mildly acidic pH values, similar to those that might be encountered during mild acidosis (Atwood *et al.*, 1998). Under acidic conditions, nanomolar concentrations of Aβ_{40} form aggregates, which can subsequently be dissociated by chelation or alkalinization. Aβ_{42}, but not Aβ_{40}, is precipitated by copper at pH 7.4.

In vitro, low micromolar concentrations of zinc induce the aggregation of Aβ_{40} at pH 7.4 (Huang *et al.*, 1997). This reaction is mediated by dimeric Aβ, potentiated by α-helical-promoting solvents, inhibited by multimeric forms of Aβ, and requires NaCl. At pH 7.4, zinc-induced aggregation of Aβ_{40} is reversible by chelation over the course of several precipitation/solubilization cycles. Aβ aggregation also occurs in acidic solution (pH 5.5), but aggregates formed in this manner cannot be resolubilized by alkalinization (Huang *et al.*, 1997). In canine CSF, half-maximal aggregation of endogenous Aβ is produced by zinc concentrations ranging from 120 to 140 μM (Brown *et al.*, 1997). The ability of zinc to induce Aβ aggregation is dependent on the presence of a histidine residue at position 13 (Liu *et al.*, 1999). Neuronal depolarization can trigger the massive release of zinc in response to pathological events (Howell *et al.*, 1984), causing extracellular zinc concentrations to rise dramatically (Tonder *et al.*, 1990; Koh *et al.*, 1996). Thus, elevated zinc concentrations may facilitate Aβ aggregation *in vivo*.

Aβ aggregates formed in the presence of zinc are more dense and are solubilized less easily than those formed in the presence of copper (Moir *et al.*, 1999). Apolipoprotein E inhibits zinc-induced Aβ aggregation, but enhances copper-induced aggregation. Furthermore, the extent to which metal-induced aggregation of Aβ occurs *in vitro* is altered by specific apolipoprotein E isoforms. Zinc- or copper-induced Aβ aggregation is greater in the presence of apolipoprotein E4 than apolipoprotein E3. This is consistent with the increased risk for AD conferred by the ϵ4 allele.

6. Aβ Clearance

Aβ clearance is not well understood, but may be critically important to AD pathogenesis. Pathways for Aβ clearance include, but are not limited to, the following:

In soluble fractions of human and rat brain, maximal clearance of Aβ occurs at pH 4–5 and is mediated by cathepsin D, an aspartyl protease (McDermott and Gibson, 1996, 1997; Hamazaki, 1996a). Because cathepsin D requires a low pH for catalytic activity, Aβ-degradation by cathepsin D must occur in an acidic intracellular compartment. Cathepsin D cleaves a wild-type Aβ sequence 20 times faster than it does a mutant Aβ sequence (a glycine for alanine substitution at position 21) associated with early-onset AD (Hamazaki, 1996b). This suggests that Aβ clearance by cathepsin D may be relevant to AD.

In vitro, Aβ proteolysis by insulin-degrading enzyme occurs at neutral pH (McDermott and Gibson, 1997).

In vivo, microglia cause Aβ degradation by releasing a protease thought to be a member of the disintegrin family (Mentlein *et al.*, 1998).

Aβ can be cleared by a serine protease–α2M complex (Narita *et al.*, 1997; Qiu *et al.*, 1999). Because polymorphisms in the genes for both α2M and LRP are associated with AD, it is tempting to speculate that Aβ might compete with other molecules, such as cholesterol, for lysosomal clearance (Kowal *et al.*, 1989).

IV. Therapeutic Strategies

Although AD treatment and prevention are still in the future, many potential therapeutic targets exist, each of which could be implemented via several routes. These include the following:

Inhibition of $A\beta_{42}$ or $A\beta_{40}$ secretion, with or without concomitant stimulation of $sAPP_{\alpha}$ secretion
Inhibition of Aβ aggregation or fibril formation
Resolubilization of plaques
Limitation of Aβ-induced toxicity
Stimulation of Aβ clearance
Prevention or limitation of brain inflammation
Administration of neurotrophic agents
Inhibition of neurofibrillary tangle formation
Inhibition of β-secretase or γ-secretase

For a fuller discussion of potential therapeutic strategies, see McKeon-O'Malley *et al.* (1998).

V. Summary

Tremendous progress has been made in the area of AD genetics. Several other genes, yet to be unidentified, may have a major impact on AD risk. However, it seems likely that many more genes will be discovered, each of which increases AD risk slightly. A poor combination of genetic risk factors may be sufficient to cause disease or to permit detrimental environmental factors to operate. The fact that four genes, which alter AD risk (APP, ApoE, α2M, and LRP), are related to each other indicates that cholesterol and other lipids may play a role in AD etiology. We speculate that AD may be caused by risk factors leading to sublethal vascular disease. If this speculation is substantiated by further research, it may be possible to implement lifestyle alterations for AD prevention. Presenilin research is likely to bring about many important findings in area of development, as well as in AD.

References

Abraham, C. R., Selkoe, D. J., and Potter, H. (1988). Immunochemical identification of the serine protease inhibitor, α1-antichymotrypsin in the brain amyloid deposits of Alzheimer's disease. *Cell (Cambridge, Mass.)* **52**, 487–501.

Aldudo, J., Bullido, M. J., Arbizu, T. *et al.* (1998a). Identification of a novel mutation (Leu282Arg) of the human presenilin 1 gene in Alzheimer's disease. *Neurosci. Lett.* **240**, 174–176.

Aldudo, J., Bullido, M. J., Frank, A. *et al.* (1998b). Missense mutation E318G of the presenilin-1 gene appears to be a nonpathogenic polymorphism *Ann. Neurol.* **44**, 985–986.

Aldudo, J., Bullido, M. J., and Valdivieso, F. (1999). DGGE method for the mutational analysis of the coding and proximal promoter regions of the Alzheimer's disease presenilin-1 gene: Two novel mutations. *Hum. Mutation* **14**, 433–439.

Alvarez, R., Alvarez, V., Lahoz, C. H. *et al.* (1999). Angiotensin converting enzyme and endothelial nitric oxide synthase DNA polymorphisms and late onset Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **67**, 733–736.

Alzheimer's Disease Collaborative Group. (1995). The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. *Nat. Genet.* **11**, 219–222.

Ancolio, K., Dumanchin, C., Barelli, H. *et al.* (1999). Unusual phenotypic alteration of beta amyloid precursor protein (betaAPP) maturation by a new Val-715 → Met βAPP-770 mutation responsible for probable early-onset Alzheimer's disease. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4119–4124.

Aoki, M., Abe, K., Oda, N. *et al.* (1997). A presenilin-1 mutation in a Japanese family with Alzheimer's disease and distinctive abnormalities on cranial MRI. *Neurology* **48**, 1118–1120.

Araki, W., Kitaguchi, N., Tokushima, Y. *et al.* (1991). Trophic effect of β-amyloid precursor protein on cerebral cortical neurons in culture. *Biochem. Biophys. Res. Commun.* **181**, 265–271.

Atwood, C. S., Moir, R. D., Huang, X. *et al.* (1998). Dramatic aggregation of Alzheimer abeta by Cu(II) is induced by conditions representing physiological acidosis. *J. Biol. Chem.* **273**, 12817–12826.

Atwood, C. S., Huang, X., Moir, R. D. *et al.* (1999). Role of free radicals and metal ions in the pathogenesis of Alzheimer's disease. *Met. Ions Biol. Syst.* **36**, 309–364.

Axelman, K., Basun, H., and Lannfelt, L. (1998). Wide range of disease onset in a family with Alzheimer disease and a His163Tyr mutation in the presenilin-1 gene. *Arch. Neurol. (Chicago)* **55**, 698–702.

Ballerini, C., Nacmias, B., Rombola, G. *et al.* (1999). HLA A2 allele is associated with age at onset of Alzheimer's disease. *Ann. Neurol.* **45**, 397–400.

Barger, S. W., and Mattson, M. P. (1996). Induction of neuroprotective kappa B-dependent transcription by secreted forms of the Alzheimer's beta-amyloid precursor. *Mol. Brain Res.* **40**, 116–126.

Barrow, C. J., and Zagorski, M. G. (1991). Solution structures of β peptide and its constituent fragments: Relation to amyloid deposition. *Science* **253**, 179–182.

Bauer, J., Strauss, S., Schreiter-Gasser, U. *et al.* (1991). Interleukin-6 and alpha-2-macroglobulin indicate an acute-phase state in Alzheimer's disease cortices. *FEBS Lett.* **285**, 111–114.

Baum, L., Chen, L., Masliah, E. *et al.* (1999). Lipoprotein lipase mutations and Alzheimer's disease. *Am. J. Med. Genet.* **88**, 136–139.

Behl, C. (1999). Alzheimer's disease and oxidative stress: Implications for novel therapeutic approaches. *Prog. Neurobiol.* **57**, 301–323.

Besanon, R., Lorenzi, A., Cruts, M. *et al.* (1988). Missense mutation in exon 11 (Codon 378) of the presenilin-1 gene in a French family with early-onset Alzheimer's disease and transmission study by mismatch enhanced allele specific amplification. *Hum. Mutation* **11**, 481.

Birge, S. J. (1997). The role of estrogen in the treatment of Alzheimer's disease. *Neurology* **48**, S36–S41.

Blacker, D., Wilcox, M. A., Laird, N. M. *et al.* (1998). Alpha-2 macroglobulin is genetically associated with Alzheimer disease. *Nat. Genet.* **19**, 357–360.

Blanchard, V., Czech, C., Bonici, B. *et al.* (1997). Immunohistochemical analysis of presenilin 2 expression in the mouse brain: Distribution pattern and co-localization with presenilin 1 protein. *Brain Res.* **758**, 209–217.

Borchelt, D. R., Thinakaran, G., Eckman, C. *et al.* (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1-42/1-40 ratio in vitro and in vivo. *Neuron* **17**, 1005–1013.

Borth, W. (1992). α2-Macroglobulin, a multifunctional binding protein with targeting characteristics. *FASEB J.* **6**, 3345–3353.

Boteva, K., Vitek, M., Mitsuda, H. *et al.* (1996). Mutation analysis of presenillin 1 gene in Alzheimer's disease *Lancet* **347**, 130–131.

Braak, H., and Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol.* **82**, 239–259.

Braak, H., Braak, E., Grundke-Iqbal, I. *et al.* (1986). Occurrence of neuropil threads in the senile human brain and in Alzheimer's disease: A third location of paired helical filaments outside of neurofibrillary tangles and neuritic plaques. *Neurosci. Lett.* **65**, 351–355.

Bramblett, G. T., Goedert, M., Jakes, R. *et al.* (1993). Abnormal tau phosphorylation at Ser396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. *Neuron* **10**, 1089–1099.

Brandi, M. L., Becherini, L., Gennari, L. *et al.* (1999). Association of the estrogen receptor alpha gene polymorphisms with sporadic Alzheimer's disease. *Biochem. Biophys. Res. Commun.* **265**, 335–338.

Breen, K. C., Bruce, M., and Anderton, B. H. (1991). Beta amyloid precursor protein mediates neuronal cell-cell and cell-surface adhesion. *J. Neurosci. Res.* **28**, 90–100.

Breitner, J. C., Welsh, K. A., Gau, B. A. *et al.* (1995). Alzheimer's disease in the National Academy of Sciences-National Research Council Registry of Aging Twin Veterans. III. Detection of cases, longitudinal results, and observations on twin concordance. *Arch. Neurol. (Chicago)* **52**, 763–771.

Brindle, N., Song, Y., Rogaeva, E. *et al.* (1998). Analysis of the butyrylcholinesterase gene and nearby chromosome 3 markers in Alzheimer disease. *Hum. Mol. Genet.* **7**, 933–935.

Brown, A. M., Tummolo, D. M., Rhodes, K. J. *et al.* (1997). Selective aggregation of endogenous beta-amyloid peptide and soluble amyloid precursor protein in cerebrospinal fluid by zinc. *J. Neurochem.* **69**, 1204–1212.

Bullido, M. J., Aldudo, J., Frank, A. *et al.* (2000). A polymorphism in the tau gene associated with risk for Alzheimer's disease. *Neurosci. Lett.* **278**, 49–52.

Burdick, D., Soreghan, B., Kwon, M. *et al.* (1992). Assembly and aggregation properties of synthetic Alzheimer's A4/beta amyloid peptide analogs. *J. Biol. Chem.* **267**, 546–554.

Bush, A. I., Multhaup, G., Moir, R. D. *et al.* (1993). A novel zinc(II) binding site modulates the function of the βA4 amyloid protein precursor of Alzheimer's disease. *J. Biol. Chem.* **268**, 16109–16112.

Bush, A. I., Pettingell, W. H., Multhaup, G. *et al.* (1994). Rapid induction of Alzheimer Aβ amyloid formation by zinc. *Science* **265**, 1464–1467.

Buxbaum, J. D., Oishi, M., Chen, H. I. *et al.* (1992). Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10075–10078.

Buxbaum, J. D., Ruefli, A. A., Parker, C. A. *et al.* (1994). Calcium regulates processing of the Alzheimer amyloid protein precursor in a protein kinase C-independent manner. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4489–4493.

Buxbaum, J. D., Liu, K.-N., Luo, Y. *et al.* (1998). Evidence that tumor necrosis factor α converting enzyme is involved in regulated α-secretase cleavage of the Alzheimer amyloid protein precursor. *J. Biol. Chem.* **273**, 27765–27767.

Cai, X., Stanton, J., Fallin, D. *et al.* (1997). No association between the intronic presenilin-1 polymorphism and Alzheimer's disease in clinic and population-based samples. *Am. J. Med. Genet.* **74**, 202–203.

Callahan, C. M., Hall, K. S., Hui, S. L. *et al.* (1996). Relationship of age, education, and occupation with dementia among a community-based sample of African Americans. *Arch. Neurol. (Chicago)* **53**, 134–140.

Campion, D., Flaman, J. M., Brice, A. *et al.* (1995). Mutations of the presenilin I gene in families with early-onset Alzheimer's disease. *Hum. Mol. Genet.* **4**, 2373–2377.

Campion, D., Brice, A., Dumanchin, C. *et al.* (1996). A novel presenilin 1 mutation resulting in familial Alzheimer's disease with an onset age of 29 years. *NeuroReport* **7**, 1582–1584.

Campion, D., Dumanchin, C., Hannequin, D. *et al.* (1999). Early-onset autosomal dominant Alzheimer disease: Prevalence, genetic heterogeneity, and mutation spectrum. *Am. J. Hum. Genet.* **65**, 664–670.

Capell, A., Grunberg, J., Pesold, B. *et al.* (1998). The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100-150-kDa molecular mass complex. *J. Biol. Chem.* **273**, 3205–3211.

Chagnon, P., Gee, M., Filion, M. *et al.* (1999). Phylogenetic analysis of the mitochondrial genome indicates significant differences between patients with Alzheimer disease and controls in a French-Canadian founder population. *Am. J. Med. Genet.* **85**, 20–30.

Chambers, C. B., Lee, J. M., Troncoso, J. C. *et al.* (1999). Overexpression of four-repeat tau mRNA isoforms in progressive supranuclear palsy but not in Alzheimer's disease. *Ann. Neurol.* **46**, 325–332.

Chartier-Harlin, M. C., Crawford, F., and Houlden, H. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. *Nature (London)* **353**, 844–846.

Chen, M., and Yankner, B. A. (1991). An antibody to the β amyloid and the amyloid precursor protein inhibits cell-substratum adhesion in many mammalian cell types. *Neurosci. Lett.* **125**, 223–226.

Chen, P., Ganguli, M., Mulsant, B. H. *et al.* (1999). The temporal relationship between depressive symptoms and dementia: A community-based prospective study. *Arch. Gen. Psychiatry* **56**, 261–266.

Chyung, A. S. C., Greenberg, B. D., Cook, D. G. *et al.* (1997). Novel β-secretase cleavage of β-amyloid precursor protein in the endoplasmic reticulum/intermediate compartment of NT2N cells. *J. Cell Biol.* **138**, 671–680.

Citron, M., Westaway, D., Xia, W. *et al.* (1997). Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid β-protein in both transfected cells and transgenic mice. *Nat. Med.* **3**, 67–72.

Citron, M., Eckman, C. B., Diehl, T. S. *et al.* (1998). Additive effects of PS1 and APP mutations on secretion of the 42- residue amyloid beta-protein. *Neurobiol. Dis.* **5**, 107–116.

Cobb, J. L., Wolf, P. A., Au, R. *et al.* (1995). The effect of education on the incidence of dementia and Alzheimer's disease in the Framingham Study. *Neurology* **45**, 1707–1712.

Corder, E. H., Saunders, A. M., Strittmatter, W. J. *et al.* (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921–923.

Corder, E. H., Saunders, A. M., Risch, N. J. *et al.* (1994). Protective effect of apolipoprotein E type 2 for late-onset Alzheimer's disease. *Nat. Genet.* **7**, 184.

Coria, F., Castano, E., Larrondo-Lillo, M. *et al.* (1988). Isolation and characterization of amyloid P component from Alzheimer's disease and other types of cerebral amyloidosis. *Lab. Invest.* **58**, 454–458.

Couchie, D., Charrière-Bertrand, C., and Nuñez, J. (1988). Expression of the mRNA for tau proteins during brain development and in cultured neurons and astroglial cells. *J. Neurochem.* **50**, 1894–1899.

Crawford, F., Fallin, D., Suo, Z. *et al.* (1998). The butyrylcholinesterase gene is neither independently nor synergistically associated with late-onset AD in clinic- and community-based populations. *Neurosci. Lett.* **249**, 115–118.

Crawford, F., Freeman, M., Town, T. *et al.* (1999). No genetic association between polymorphisms in the Tau gene and Alzheimer's disease in clinic or population based samples. *Neurosci. Lett.* **266**, 193–196.

Crook, R., Ellis, R., Shanks, M. *et al.* (1997). Early-onset Alzheimer's disease with a presenilin-1 mutation at the site corresponding to the Volga German presenilin-2 mutation. *Ann. Neurol.* **42**, 124–128.

Crook, R., Verkkoniemi, A., Perez-Tur, J. *et al.* (1998). A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenillin 1. *Nat. Med.* **4**, 452–455.

Cruts, M., Martin, J. J., and van Broeckhoven, C. (1995). Molecular genetic analysis of familial early-onset Alzheimer's Disease linked to chromosome 14q24.3. *Hum. Mol. Genet.* **4**, 2363–2371.

Cruts, M., van Duijn, C. M., Backhovens, H. *et al.* (1998). Estimation of the genetic contribution of presenilin-1 and -2 mutations in a population-based study of presenile Alzheimer disease. *Hum. Mol. Genet.* **7**, 43–51.

Cummings, B. J., Satou, T., Head, E. *et al.* (1996). Diffuse plaques contain C-terminal Aβ42 and not Aβ40: Evidence from cats and dogs. *Neurobiol. Aging* **17**, 653–659.

Dahiyat, M., Cumming, A., Harrington, C. *et al.* (1999). Association between Alzheimer's disease and the NOS3 gene. *Ann. Neurol.* **46**, 664–667.

Das, H. K., McPherson, J., Bruns, G. A. *et al.* (1985). Isolation, characterization, and mapping to chromosome 19 of the human apolipoprotein E gene. *J. Biol. Chem.* **260**, 6240–6247.

Davis, R. E., Miller, S., Herrnstadt, C. *et al.* (1997). Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 4526–4531.

Dewji, N. N., and Singer, S. J. (1997). The seven-transmembrane spanning topography of the Alzheimer disease-related presenilin proteins in the plasma membranes of cultured cells. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 14025–14030.

Dickson, D. W. (1997). The pathogenesis of senile plaques. *J. Neuropathol. Exp. Neurol.* **56**, 321–339.

Doan, A., Thinakaran, G., Borchelt, D. R. *et al.* (1996). Protein topology of presenilin 1. *Neuron* **17**, 1023–1030.

Dumanchin, C., Brice, A., Campion, D. *et al.* (1998). De novo presenilin 1 mutations are rare in clinically sporadic, early onset Alzheimer's disease cases. French Alzheimer's Disease Study Group. *J. Med. Genet.* **35**, 672–673.

Dyrks, T., Monning, U., Beyreuther, K. *et al.* (1994). Amyloid precursor protein secretion and βA4 amyloid generation are not mutually exclusive. *FEBS Lett.* **349**, 210–214.

Eckman, C. B., Mehta, N. D., Crook, R. *et al.* (1997). A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of Aβ42(43). *Hum. Mol. Genet.* **6**, 2087–2089.

Egensperger, R., Kosel, S., Schnopp, N. M. *et al.* (1997). Association of the mitochondrial tRNA(A4336G) mutation with Alzheimer's and Parkinson's diseases. *Neuropathol. Appl. Neurobiol.* **23**, 315–321.

Ezquerra, M., Carnero, C., Blesa, R. *et al.* (1999). A presenilin 1 mutation (Ser169Pro) associated with early-onset AD and myoclonic seizures. *Neurology* **52**, 566–570.

Farrer, L. A., Abraham, C. R., Haines, J. L. *et al.* (1998). Association between bleomycin hydrolase and Alzheimer's disease in caucasians. *Ann. Neurol.* **44**, 808–811.

Finckh, U., ller-Thomsen, T., Mann, U. *et al.* (2000). High prevalence of pathogenic mutations in patients with early-onset dementia detected by sequence analyses of four different genes. *Am. J. Hum. Genet.* **66**, 110–117.

Forsell, C., Froelich, S., Axelman, K. *et al.* (1997). A novel pathogenic mutation (Leu262Phe) found in the presenilin 1 gene in early-onset Alzheimer's disease. *Neurosci. Lett.* **234**, 3–6.

Fraser, P. E., Nguyen, J. T., Surewicz, W. K. *et al.* (1991). pH-dependent structural transitions of Alzheimer amyloid peptides. *Biophys. J.* **60**, 1190–1201.

Fraser, P. E., Nguyen, J. T., Inouye, H. *et al.* (1992). Fibril formation by primate, rodent, and Dutch-hemorrhagic analogues of Alzheimer amyloid β-protein. *Biochemistry* **31**, 10716–10723.

Fukumoto, H., Asami-Odaka, A., Suzuki, N. *et al.* (1996). Amyloid beta protein deposition in normal aging has the same characteristics as that in Alzheimer's disease. Predominance of A beta 42(43) and association of A beta 40 with cored plaques. *Am. J. Pathol.* **148**, 259–265.

Fukushima, Y., Bell, G. I., and Shows, T. B. (1988). The polymorphic human alpha-2-macroglobulin gene (A2M) is located in chromosome region 12p12.3–p13.3. *Cytogenet. Cell Genet.* **48**, 58–59.

Furukawa, K., Sopher, B. L., Rydel, R. E. *et al.* (1996). Increased activity-regulating and neuroprotective efficacy of α-secretase-derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. *J. Neurochem.* **67**, 1882–1896.

Geerlings, M. I., Schmand, B., Jonker, C. *et al.* (1999). Education and incident Alzheimer's disease: A biased association due to selective attrition and use of a two-step diagnostic procedure? *Int. J. Epidemiol.* **28**, 492–497.

George-Hyslop, P., Haines, J., Rogaev, E. *et al.* (1992). Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. *Nat. Genet.* **2**, 330–334.

Georgieff, I. S., Liem, R. K., Couchie, D. *et al.* (1993). Expression of high molecular weight tau in the central and peripheral nervous systems. *J. Cell Sci.* **105**(Pt. 3), 729–737.

Ghetti, B., Murrell, J., and Spillantini, M. G. (1999). Mutations in the Tau gene cause frontotemporal dementia. *Brain Res. Bull.* **50**, 471–472.

Ghiso, J., Rostagno, A., Gardella, J. E. *et al.* (1992). A 109-amino-acid C-terminal fragment of Alzheimer's-disease amyloid precursor protein contains a sequence, - RHDS-, that promotes cell adhesion. *Biochem. J.* **288**, 1053–1059.

Glenner, G. G., and Wong, C. W. (1984). Alzheimer's disease: Initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem. Biophys. Res. Commun.* **120**, 885–890.

Goate, A., Chartier-Harlin, M. C., Mullan, M. *et al.* (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature (London)* **349**, 704–706.

Goedert, M., Trojanowski, J. Q., and Lee, V. M. Y. (1997). *In* "Mechanisms of Dementia" (W. Wasco, and R. E. Tanzi, ed.), p. 199. Humana Press, Totowa, NJ.

Gomez-Isla, T., Wasco, W., Pettingell, W. P. *et al.* (1997). A novel presenilin-1 mutation: Increased beta-amyloid and neurofibrillary changes. *Ann. Neurol.* **41**, 809–813.

Gomez-Pinilla, F., Cummings, B. J., and Cotman, C. W. (1990). Induction of basic fibroblast growth factor in Alzheimer's disease pathology. *NeuroReport* **1**, 211–214.

Goodman, Y., and Mattson, M. P. (1994). Secreted forms of beta-amyloid precursor protein protect hippocampal neurons against amyloid beta-peptide-induced oxidative injury. *Exp. Neurol.* **128**, 1–12.

Griffin, W. S. T., Sheng, J. G., Roberts, G. W. *et al.* (1995). Interleukin-1 expression in different plaques types in Alzheimer's disease: Significance in plaque evolution. *J. Neuropathol. Exp. Neurol.* **54**, 276–281.

Grundke-Iqbal, I., Iqbal, K., Tung, Y. *et al.* (1986). Abnormal phosphorylation of the microtubule-associated protein τ (tau) in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4913–4917.

Guo, Q., Christakos, S., Robinson, N. *et al.* (1998a). Calbindin D28k blocks the proapoptotic actions of mutant presenilin 1: Reduced oxidative stress and preserved mitochondrial function. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 3227–3232.

Guo, Q., Sopher, B., Furukawa, K. *et al.* (1998b). Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: Involvement of calcium and oxyradicals. *J. Neurosci.* **17**, 4212–4222.

Haass, C., Schlossmacher, M. G., Hung, A. Y. *et al.* (1992). Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature (London)* **359**, 322–325.

Haass, C., Hung, A. Y., Schlossmacher, M. G. *et al.* (1993). β-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. *J. Biol. Chem.* **268**, 3021–3024.

Haines, J. L., Pritchard, M. L., Saunders, A. M. *et al.* (1996). No genetic effect of α1-antichymotrypsin in Alzheimer's disease. *Genomics* **33**, 53–56.

Hamazaki, H. (1996a). Cathepsin D is involved in the clearance of Alzheimer's beta-amyloid. *FEBS Lett.* **396**, 139–142.

Hamazaki, H. (1996b). A beta-amyloid peptide variant related with familial Alzheimer's disease and hereditary cerebral hemorrhage with amyloidosis is poorly eliminated by cathepsin D. *FEBS Lett.* **397**, 313–315.

Hartmann, T., Bieger, S. C., Bruhl, B. *et al.* (1997). Distinct sites of intracellular production for Alzheimer's disease Aβ40/42 amyloid peptides. *Nat. Med.* **3**, 1016–1020.

Harvey, R. J., Ellison, D., Hardy, J. *et al.* (1998). Chromosome 14 familial Alzheimer's disease: The clinical and neuropathological characteristics of a family with a leucine→serine (L250S) substitution at codon 250 of the presenilin 1 gene. *J. Neurol., Neurosurg. Psychiatry* **64**, 44–49.

Haskell, S. G., Richardson, E. D., and Horwitz, R. I. (1997). The effect of estrogen replacement therapy on cognitive function in women: A critical review of the literature. *J. Clin. Epidemiol.* **50**, 1249–1264.

Hebert, L. E., Scherr, P. A., Beckett, L. A. *et al.* (1992). Relation of smoking and alcohol consumption to incident Alzheimer's disease. *Am. J. Epidemiol.* **135**, 347–355.

Hendricks, L., van Duijn, C. M., Cras, P. *et al.* (1992). Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. *Nat. Genet.* **1**, 218–221.

Hillier, V., and Salib, E. (1997). A case-control study of smoking and Alzheimer's disease. *Int. J. Geriatr. Psychiatry* **12**, 295–300.

Hiltunen, M., Mannermaa, A., Helisalmi, S. *et al.* (1998). Butyrylcholinesterase K variant and apolipoprotein E4 genes do not act in synergy in Finnish late-onset Alzheimer's disease patients. *Neurosci. Lett.* **250**, 69–71.

Hollenbach, E., Ackermann, S., Hyman, B. T. *et al.* (1998). Confirmation of an association between a polymorphism in exon 3 of the low-density lipoprotein receptor-related protein gene and Alzheimer's disease. *Neurology* **50**, 1905–1907.

Howell, G. A., Welch, M. G., and Frederickson, C. J. (1984). Stimulation-induced uptake and release of zinc in hippocampal slices. *Nature (London)* **308**, 736–738.

Hoyert, D. L., and Rosenberg, H. M. (1999). Mortality from Alzheimer's disease: An update. *Natl. Vital Stat. Rep.* **47**, 1–8.

Huang, X., Atwood, C. S., Moir, R. D. *et al.* (1997). Zinc-induced Alzheimer's Aβ1-40 aggregation is mediated by conformational factors. *J. Biol. Chem.* **272**, 26464–26470.

Hung, A. Y., Haass, C., Nitsch, R. M. *et al.* (1993). Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. *J. Biol. Chem.* **268**, 22959–22962.

Hussain, I., Powell, D., Howlett, D. R. *et al.* (1999). Identification of a novel aspartic protease (Asp 2) as β-secretase. *Mol. Cell. Neurosci.* **14**, 419–427.

Hutton, M., Lendon, C. L., Rizzu, P. *et al.* (1998). Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature (London)* **393**, 702–705.

Iijima, M., Tabira, T., Poorkaj, P. *et al.* (1999). A distinct familial presenile dementia with a novel missense mutation in the tau gene. *NeuroReport* **10**, 497–501.

Iwatsubo, T., Odaka, A., Suzuki, N. *et al.* (1994). Visualization of Aβ42(43) and Aβ40 in senile plaques with end- specific Aβmonoclonals: Evidence that an initially deposited species is Aβ42(43). *Neuron* **13**, 45–53.

Iwatsubo, T., Mann, D. M., Odaka, A. *et al.* (1995). Amyloid beta protein (Aβ) deposition: Aβ42(43) precedes Aβ40 in Down syndrome. *Ann. Neurol.* **37**, 294–299.

Jacobsen, J. S., Spruyt, M. A., Brown, A. M. *et al.* (1994). The release of Alzheimer's disease β-amyloid is reduced by phorbol treatment. *J. Biol. Chem.* **269**, 8376–8382.

Jarrett, J. T., and Lansbury, P. T., Jr. (1992). Amyloid fibril formation requires a chemically discriminating nucleation event: Studies of an amyloidogenic sequence from the bacterial protein OsmB. *Biochemistry* **31**, 12345–12352.

Jarrett, J. T., and Lansbury, P. T., Jr. (1993). Seeding "one-dimensional crystallization" of amyloid: A pathogenic mechanism in Alzheimer's disease and scrapie? *Cell (Cambridge, Mass.)* **73**, 1055–1058.

Jarrett, J. T., Berger, E. P., and Lansbury, P. T. (1993a). The C-terminus of the β protein is critical in amyloidogenesis. *Ann. N. Y. Acad. Sci.* **695**, 144–148.

Jarrett, J. T., Berger, E. P., and Lansbury, P. T., Jr. (1993b). The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: Implications for the pathogenesis of Alzheimer's disease. *Biochemistry* **32**, 4693–4697.

Jorgensen, P., Bus, C., Pallisgaard, N. *et al.* (1996). Familial Alzheimer's disease co-segregates with a Met146Ile substitution in presenilin-1. *Clin. Genet.* **50**, 281–286.

Kamboh, M. I., Aston, C. E., Perez-Tur, J. *et al.* (1999). A novel mutation in the apolipoprotein E gene (APOE*4 Pittsburgh) is associated with the risk of late-onset Alzheimer's disease. *Neurosci. Lett.* **263**, 129–132.

Kamimura, K., Tanahashi, H., Yamanaka, H. *et al.* (1998). Familial Alzheimer's disease genes in Japanese. *J. Neurol. Sci.* **160**, 76–81.

Kamino, K., Sato, S., Sakaki, Y. *et al.* (1996). Three different mutations of presenilin 1 gene in early-onset Alzheimer's disease families. *Neurosci. Lett.* **208**, 195–198.

Kang, D. E., Saitoh, T., Chen, X. *et al.* (1997). Genetic association of the low density lipoprotein receptor-related protein gene (LRP), an apolipoprotein E receptor, with late-onset Alzheimer's disease. *Neurology* **49**, 56–61.

Kang, J., Lemaire, H. G., and Unterbeck, A. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature (London)* **325**, 733–736.

Katzmann, R. (1986). Alzheimer's disease. *N. Engl. J. Med.* **314**, 964–973.

Kaytor, M. D., and Warren, S. T. (2000). Aberrant protein deposition and neurological disease. *J. Biol. Chem.* **274**, 37507–37510.

Kirschner, D. A., Inouye, H., Duffy, L. K. *et al.* (1987). Synthetic peptide homologous to beta protein from Alzheimer disease forms amyloid-like fibrils in vitro. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6953–6957.

Knopman, D. S., and Morris, J. C. (1997). An update on primary drug therapies for Alzheimer disease. *Arch. Neurol. (Chicago)* **54**, 1406–1409.

Koh, J. Y., Suh, S. W., Gwag, B. J. *et al.* (1996). The role of zinc in selective neuronal death after transient global cerebral ischemia. *Science* **272**, 1013–1016.

Koike, H., Tomioka, S., Sorimachi, H. *et al.* (1999). Membrane-anchored metalloprotease MDC9 has an α-secretase activity responsible for processing the amyloid precursor protein. *Biochem. J.* **343**(Pt. 2), 371–375.

Kokmen, E., Beard, C. M., O'Brien, P. C. *et al.* (1996). Epidemiology of dementia in Rochester, Minnesota. *Mayo Clin. Proc.* **71**, 275–282.

Koo, E. H., and Squazzo, S. L. (1994). Evidence that production and release of amyloid β-protein involves the endocytic pathway. *J. Biol. Chem.* **269**, 17386–17389.

Kosik, K. S., Orecchio, L. D., Bakalis, S. *et al.* (1989). Developmentally regulated expression of specific tau sequences. *Neuron* **2**, 1389–1397.

Kovacs, D. M., Fausett, H. J., Page, K. J. *et al.* (1996). Alzheimer-associated presenilins 1 and 2: Neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nat. Med.* **2**, 224–229.

Kowal, R. C., Herz, J., Goldstein, J. L. *et al.* (1989). Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5810–5814.

Kowalska, A., Forsell, C., Florczak, J. *et al.* (1999). A Polish pedigree with Alzheimer's disease determined by a novel mutation in exon 12 of the presenilin 1 gene: Clinical and molecular characterization. *Folia Neuropathol.* **37**, 57–61.

Krieger, M., and Herz, J. (1994). Structures and functions of multi-ligand lipoprotein receptors: Macrophage scavenger receptors and LDL receptor-related protein. *Annu. Rev. Biochem.* **63**, 601–637.

Kunugi, H., Hattori, M., Ueki, A. *et al.* (1998). Possible association of missense mutation (Gly[-63]Glu) of the neurotrophin-3 gene with Alzheimer's disease in Japanese. *Neurosci. Lett.* **241**, 65–67.

Kwok, J. B., Taddei, K., Hallupp, M. *et al.* (1997). Two novel (M233T and R278T) presenilin-1 mutations in early-onset Alzheimer's disease pedigrees and preliminary evidence for association of presenilin-1 mutations with a novel phenotype. *NeuroReport* **8**, 1537–1542.

Lalowski, M., Golabek, A., Lemere, C. A. *et al.* (1996). The "nonamyloidogenic" p3 fragment (amyloid β17–42) is a major constituent of Down's syndrome cerebellar preamyloid. *J. Biol. Chem.* **271**, 33623–33631.

Lambert, J. C., Pasquier, F., Cottel, D. *et al.* (1998). A new polymorphism in the APOE promoter associated with risk of developing Alzheimer's disease. *Hum. Mol. Genet.* **7**, 533–540.

Lambert, J. C., Brousseau, T., Defosse, V. *et al.* (2000). Independent association of an APOE gene promoter polymorphism with increased risk of myocardial infarction and decreased APOE plasma concentrations-the ECTIM Study. *Hum. Mol. Genet.* **9**, 57–61.

Lammich, S., Kojro, E., Postina, R. *et al.* (1999). Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 3922–3927.

LeBlanc, A. C., Koutroumanis, M., and Goodyer, C. G. (1998). Protein kinase C activation increases release of secreted amyloid precursor protein without decreasing Abeta production in human primary neuron cultures. *J. Neurosci.* **18**, 2907–2913.

Lee, P. N. (1994). Smoking and Alzheimer's disease: A review of the epidemiological evidence. *Neuroepidemiology* **13**, 131–144.

Lehmann, S., Chiesa, R., and Harris, D. A. (1997). Evidence for a six-transmembrane domain structure of presenilin 1. *J. Biol. Chem.* **272**, 12047–12051.

Leibson, C. L., Rocca, W. A., Hanson, V. A. *et al.* (1997). The risk of dementia among persons with diabetes mellitus. *Ann. N. Y. Acad. Sci.* **826**, 422–427.

Lemaire, H. G., Salbaum, J. M., Multhaup, G. *et al.* (1989). The PreA4 695 precursor protein of Alzheimer's disease A4 amyloid is encoded by 16 exons. *Nucleic Acids Res.* **17**, 517–522.

Lemere, C. A., Lopera, F., Kosik, K. S. *et al.* (1996). The E280A presenilin 1 Alzheimer mutation produces increased Aβ42 deposition and severe cerebellar pathology. *Nat. Med.* **2**, 1146–1150.

Letenneur, L., Gilleron, V., Commenges, D. *et al.* (1999). Are sex and educational level independent predictors of dementia and Alzheimer's disease? Incidence data from the PAQUID project. *J. Neurol. Neurosurg. Psychiatry* **66**, 177–183.

Levy, E., Carman, M. D., Fernandez-Madrid, I. J. *et al.* (1990). Mutation of the Alzheimer's disease amyloid gene in hereditary cerebral hemorrhage, Dutch-type. *Science* **248**, 1124–1126.

Levy-Lahad, E., Wijsman, E. M., Nemens, E. *et al.* (1995a). A familial Alzheimer's disease locus on chromosome 1. *Science* **269**, 970–973.

Levy-Lahad, E., Wasco, W., Poorkaj, P. *et al.* (1995b). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973–977.

Li, T., Holmes, C., Sham, P. C. *et al.* (1999). Allelic functional variation of serotonin transporter expression is a susceptibility factor for late onset Alzheimer's disease. *NeuroReport* **8**, 683–686.

Li, X., and Greenwald, I. (1998). Additional evidence for an eight-transmembrane-domain topology for *Caenorhabditis elegans* and human presenilins. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7109–7114.

Liao, A., Nitsch, R. M., Greenberg, S. M. *et al.* (1998). Genetic association of an alpha2-macroglobulin (Val1000Ile) polymorphism and Alzheimer's disease. *Hum. Mol. Genet.* **7**, 1953–1956.

Lilius, L., Froelich, Fabre S., Basun, H. *et al.* (1999). Tau gene polymorphisms and apolipoprotein E ϵ4 may interact to increase risk for Alzheimer's disease. *Neurosci. Lett.* **277**, 29–32.

Liu, S. T., Howlett, G., and Barrow, C. J. (1999). Histidine-13 is a crucial residue in the zinc ion-induced aggregation of the A beta peptide of Alzheimer's disease. *Biochemistry* **38**, 9373–9378.

Lovell, M. A., Robertson, J. D., Teesdale, W. J. *et al.* (1998). Copper, iron and zinc in Alzheimer's disease senile plaques. *J. Neurosci. Res.*

Mackenzie, I. R. A. (1994). Senile plaques do not progressively accumulate with normal aging. *Acta Neuropathol.* **87**, 520–525.

Mahley, R. W., and Huang, Y. (1999). Apolipoprotein E: From atherosclerosis to Alzheimer's disease and beyond. *Curr. Opin. Lipidol.* **10**, 207–217.

Mann, D. M., Iwatsubo, T., Cairns, N. J. *et al.* (1996). Amyloid beta protein (Abeta) deposition in chromosome 14-linked Alzheimer's disease: Predominance of Aβ42(43). *Ann. Neurol.* **40**, 149–156.

Maruyama, K., Kametani, F., Usami, M. *et al.* (1991). "Secretase," Alzheimer amyloid protein precursor secreting enzyme is not sequence-specific. *Biochem. Biophys. Res. Commun.* **179**, 1670–1676.

Mattila, K. M., Forsell, C., Pirttila, T. *et al.* (1998). The Glu318Gly mutation of the presenilin-1 gene does not necessarily cause Alzheimer's disease. *Ann. Neurol.* **44**, 965–967.

Mattson, M. P. (1994). Secreted forms of β-amyloid precursor protein modulate dendrite outgrowth and calcium responses to glutamate in cultured embryonic hippocampal neurons. *J. Neurobiol.* **25**, 439–450.

Mattson, M. P., Cheng, B., Culwell, A. R. *et al.* (1993). Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the β-amyloid precursor protein. *Neuron* **10**, 243–254.

Mattson, M. P., Robinson, N., and Guo, Q. (1997). Estrogens stabilize mitochondrial function and protect neural cells against the pro-apoptotic action of mutant presenilin-1. *NeuroReport* **8**, 3817–3821.

Mattson, M. P., Guo, Q., Furukawa, K. *et al.* (1998). Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J. Neurochem.* **70**, 1–14.

McDermott, J. R., and Gibson, A. M. (1996). Degradation of Alzheimer's beta-amyloid protein by human cathepsin D. *NeuroReport* **7**, 2163–2166.

McDermott, J. R., and Gibson, A. M. (1997). Degradation of Alzheimer's beta-amyloid protein by human and rat brain peptidases: Involvement of insulin-degrading enzyme. *Neurochem. Res.* **22**, 49–56.

McGeer, P. L., Schulzer, M., and McGeer, E. G. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: A review of 17 epidemiologic studies. *Neurology* **47**, 425–432.

McKeon-O'Malley, C., Saunders, A. J., Bush, A. I. *et al.* (1998). Potential therapeutic targets for Alzheimer's disease. *Emerg. Ther. Targets* **2**, 157–179.

Mentlein, R., Ludwig, R., and Martensen, I. (1998). Proteolytic degradation of Alzheimer's disease amyloid β-peptide by a metalloproteinase from microglia cells. *J. Neurochem.* **70**, 721–726.

Merchant, C., Tang, M. X., Albert, S. *et al.* (1999). The influence of smoking on the risk of Alzheimer's disease. *Neurology* **52**, 1408–1412.

Milward, E. A., Papadopoulos, R., Fuller, S. J. *et al.* (1992). The amyloid protein precursor of Alzheimer's disease is a mediator of the effects of nerve growth factor on neurite outgrowth. *Neuron* **9**, 129–137.

Moir, R. D., Atwood, C. S., Romano, D. M. *et al.* (1999). Differential effects of apolipoprotein E isoforms on metal-induced aggregation of A beta using physiological concentrations. *Biochemistry* **38**, 4595–4603.

Molgaard, C. A., Stanford, E. P., Morton, D. J. *et al.* (1990). Epidemiology of head trauma and neurocognitive impairment in a multi-ethnic population. *Neuroepidemiology* **9**, 233–242.

Montoya, S. E., Aston, C. E., DeKosky, S. T. *et al.* (1998). Bleomycin hydrolase is associated with risk of sporadic Alzheimer's disease. *Nat. Genet.* **19**, 404.

Mullan, M., Crawford, F., Axelman, K. *et al.* (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of β-amyloid. *Nat. Genet.* **1**, 345–347.

Murrell, J., Farlow, M., and Ghetti, B. (1991). A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. *Science* **254**, 97–98.

Namba, Y., Tomonaga, M., Kawasaki, H. *et al.* (1991). Apolipoprotein E immunoreactivity in cerebral deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. *Brain Res.* **541**, 163–166.

Narhi, L., Wood, S. J., Steavenson, S. *et al.* (1999). Both familial Parkinson's disease mutations accelerate α-synuclein aggregation. *J. Biol. Chem.* **274**, 9843–9846.

Narita, M., Holtzman, D. M., Schwartz, A. L. *et al.* (1997). α2-macroglobulin complexes with and mediates the endocytosis of β-amyloid via cell surface low-density lipoprotein receptor-related protein. *J. Neurochem.* **69**, 1904–1911.

Nee, L. E., and Lippa, C. F. (1999). Alzheimer's disease in 22 twin pairs—13-year follow-up: Hormonal, infectious and traumatic factors. *Dementia Geriatr. Cogn. Disord.* **10**, 148–151.

Ninomiya, H., Roch, J., Sundsmo, M. P. *et al.* (1993). Amino acid sequence RERMS represents the active domain of amyloid β/A4 protein precursor that promotes fibroblast growth. *J. Cell Biol.* **121**(4), 879–886.

Nishiwaki, Y., Kamino, K., Yoshiiwa, A. *et al.* (1997). T/G polymorphism at intron 9 of presenilin 1 gene is associated with, but not responsible for sporadic late-onset Alzheimer's disease in Japanese population. *Neurosci. Lett.* **227**, 123–126.

Okuizumi, K., Onodera, O., Namba, Y. *et al.* (1995). Genetic association of the very low density lipoprotein (VLDL) receptor gene with sporadic Alzheimer's disease. *Nat. Genet.* **11**, 207–209.

Okuizumi, K., Onodera, O., Seki, K. *et al.* (1996). Lack of association of very low density lipoprotein receptor gene polymorphism with Caucasian Alzheimer's disease. *Ann. Neurol.* **40**, 251–254.

Oliveira, J. R., Gallindo, R. M., Maia, L. G. *et al.* (1998). The short variant of the polymorphism within the promoter region of the serotonin transporter gene is a risk factor for late onset Alzheimer's disease. *Mol. Psychiatry* **3**, 438–441.

Oliveira, J. R., Shimokomaki, C. M., Brito-Marques, P. R. *et al.* (1999). The association of the short variant of the 5-HTTPLR polymorphism and the ApoE epsilon4 allele does not increase the risk for late onset Alzheimer's disease. *Mol. Psychiatry* **4**, 19–20.

Oltersdorf, T., Ward, P. J., and Henriksson, T. (1990). The Alzheimer amyloid precursor protein. Identification of a stable intermediate in the biosynthetic/degradative pathway. *J. Biol. Chem.* **265**, 4492–4497.

Pahlsson, P., Shakin-Eshleman, S. H., and Spitalnik, S. L. (1992). N-linked glycosylation of β-amyloid precursor protein. *Biochem. Biophys. Res. Commun.* **189**, 1667–1673.

Papassotiropoulos, A., Bagli, M., Feder, O. *et al.* (1999). Genetic polymorphism of cathepsin D is strongly associated with the risk for developing sporadic Alzheimer's disease. *Neurosci. Lett.* **262**, 171–174.

Perez-Tur, J., Froelich, S., Prihar, G. *et al.* (1995). A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. *NeuroReport* **7**, 297–301.

Perez-Tur, J., Croxton, R., Wright, K. *et al.* (1996). A further presenilin 1 mutation in the exon 8 cluster in familial Alzheimer's disease. *NeuroDegeneration* **5**, 207–212.

Pitas, R. E., Boyles, J. K., Lee, S. H. *et al.* (1987). Astrocytes synthesize apolipoprotein E-containing lipoproteins. *Biochim. Biophys. Acta* **917**, 148–161.

Poller, W., Faber, J. P., Klobeck, G. *et al.* (1992). Cloning of the human alpha 2-macroglobulin gene and detection of mutations in two functional domains: The bait region and the thiolester site. *Hum. Genet.* **88**, 313–319.

Poorkaj, P., Bird, T. D., Wijsman, E. *et al.* (1998a). Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol.* **43**, 815–825.

Poorkaj, P., Sharma, V., Anderson, L. *et al.* (1998b). Missense mutations in the chromosome 14 familial Alzheimer's disease presenilin 1 gene. *Hum. Mutation* **11**, 216–221.

Price, J. L., Davis, P. B., Morris, J. C. *et al.* (1991). The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* **12**, 295–312.

Prihar, G., Verkkoniem, A., Perez-Tur, J. *et al.* (1999). Alzheimer disease PS-1 exon 9 deletion defined. *Nat. Med.* **5**, 1090.

Pritchard, M. L., Saunders, A. M., Gaskell, P. C. *et al.* (1996). No association between very low density lipoprotein receptor (VLDL-R) and Alzheimer disease in American Caucasians. *Neurosci. Lett.* **209**, 105–108.

Qiu, Z., Strickland, D. K., Hyman, B. T. *et al.* (1999). Alpha2-macroglobulin enhances the clearance of endogenous soluble beta-amyloid peptide via low-density lipoprotein receptor-related protein in cortical neurons. *J. Neurochem.* **73**, 1393–1398.

Ramirez-Duenas, M. G., Rogaeva, E. A., Leal, C. A. *et al.* (1998). A novel Leu171Pro mutation in presenilin-1 gene in a Mexican family with early onset Alzheimer disease. *Ann. Genet.* **41**, 149–153.

Reynolds, W. F., Rhees, J., Maciejewski, D. *et al.* (1999). Myeloperoxidase polymorphism is associated with gender specific risk for Alzheimer's disease. *Exp. Neurol.* **155**, 31–41.

Reznik-Wolf, H., Treves, T. A., Davidson, M. *et al.* (1996). A novel mutation of presenilin 1 in familial Alzheimer's disease in Israel detected by denaturing gradient gel electrophoresis. *Hum. Genet.* **98**, 700–702.

Reznik-Wolf, H., Treves, T. A., Shabtai, H. *et al.* (1998). Germline mutational analysis of presenilin 1 and APP genes in Jewish-Israeli individuals with familial or early-onset Alzheimer disease using denaturing gradient gel electrophoresis (DGGE). *Eur. J. Hum. Genet.* **6**, 176–180.

Roberts, G. W., Gentleman, S. M., Lynch, A. *et al.* (1994). β amyloid protein deposition in the brain after severe head injury: Implications for the pathogenesis of Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* **57**, 419–425.

Rogaev, E. I., Sherrington, R., Rogaeva, E. A. *et al.* (1995). Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature (London)* **376**, 775–778.

Roks, G., Dermaut, B., Heutink, P. *et al.* (1999). Mutation screening of the tau gene in patients with early-onset Alzheimer's disease. *Neurosci. Lett.* **277**, 137–139.

Romero, I., Jorgensen, P., Bolwig, G. *et al.* (1999). A presenilin-1 Thr116Asn substitution in a family with early-onset Alzheimer's disease. *NeuroReport* **10**, 2255–2260.

Roses, A. D., Strittmatter, W. J., Pericak-Vance, M. A. *et al.* (1994). Clinical application of apolipoprotein E genotyping to Alzheimer's disease. *Lancet* **343**, 1564–1565.

Rossor, M. N., Fox, N. C., Beck, J. *et al.* (1996). Incomplete penetrance of familial Alzheimer's disease in a pedigree with a novel presenilin-1 gene mutation. *Lancet* **347**, 1560.

Saitoh, T., Sundsmo, M. P., Roch, J. *et al.* (1989). Secreted form of amyloid β protein precursor is involved in the growth regulation of fibroblasts. *Cell (Cambridge, Mass.)* **58**, 615–622.

Salbaum, J. M., Weidemann, A., Lemaire, H. G. *et al.* (1988). The promoter of Alzheimer's disease amyloid A4 precursor gene. *EMBO J.* **7**, 2807–2813.

Sato, N., Hori, O., Yamaguchi, A. *et al.* (1999). A novel presenilin-2 splice variant in human Alzheimer's disease brain tissue. *J. Neurochem.* **72**, 2498–2505.

Sato, S., Kamino, K., Miki, T. *et al.* (1998). Splicing mutation of presenilin-1 gene for early-onset familial Alzheimer's disease. *Hum. Mutation Suppl.* **1**, S91–S94.

Schellenberg, G. D., Bird, T. D., Wijsman, E. M. *et al.* (1992). Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. *Science* **258**, 668–671.

Scheuner, D., Eckman, C., Jensen, M. *et al.* (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat. Med.* **2**, 864–870.

Schubert, D., Jin, L-W., Saitoh, T. *et al.* (1989). The regulation of amyloid β protein precursor secretion and its modulatory role in cell adhesion. *Neuron* **3**, 689–694.

Selkoe, D. J. (1991). The molecular pathology of Alzheimer's disease. *Neuron* **6**, 487–498.

Selkoe, D. J. (1998). The cell biology of beta-amyloid precursor protein and presenilin in Alzheimer's disease. *Trends Cell Biol.* **8**, 447–453.

Seubert, P., Vigo-Pelfrey, C., and Esch, F. S. (1992). Isolation and quantitation of soluble Alzheimer's β-peptide from biological fluids. *Nature (London)* **359**, 325–327.

Shen, J., Bronson, R. T., Chen, D. F. *et al.* (1997). Skeletal and CNS defects in presesilin-1-deficient mice. *Cell (Cambridge, Mass.)* **89**, 629–639.

Sherrington, R., Rogaev, E. I., Liang, Y. *et al.* (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature (London)* **375**, 754–760.

Sheu, K. F., Brown, A. M., Haroutunian, V. *et al.* (1999). Modulation by DLST of the genetic risk of Alzheimer's disease in a very elderly population. *Ann. Neurol.* **45**, 48–53.

Shoji, M., Golde, T., Ghiso, J. *et al.* (1992). Production of the Alzheimer amyloid β protein by normal proteolytic processing. *Science* **258**, 126–129.

Singleton, A. B., Smith, G., Gibson, A. M. *et al.* (1998). No association between the K variant of the butyrylcholinesterase gene and pathologically confirmed Alzheimer's disease. *Hum. Mol. Genet.* **7**, 937–939.

Sinha, S., Anderson, J. P., Barbour, R. *et al.* (1999). Purification and cloning of amyloid precursor protein beta-secretase from human brain. *Nature (London)* **402**, 537–540.

Skoog, I., Wallin, A., Fredman, P. *et al.* (1998). A population study on blood-brain barrier function in 85-year-olds: Relation to Alzheimer's disease and vascular dementia. *Neurology* **50**, 966–971.

Skovronsky, D. M., Moore, D. B., Milla, M. E. *et al.* (2000). Protein kinase C-dependent α-secretase competes with β-secretase for cleavage of amyloid-beta precursor protein in the trans-Golgi network. *J. Biol. Chem.* **275**, 2568–2575.

Small, D. H. (1994). A heparin binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. *J. Neurosci.* **14**, 2117–2127.

Smith, R. P., Higuchi, D. A., and Broze, G. J. (1990). Platelet coagulation factor XIa-inhibitor, a form of Alzheimer amyloid precursor protein. *Science* **248**, 1126–1128.

Sola, C., Mengod, G., Probst, A. *et al.* (1993). Differential regional and cellular distribution of β-amyloid precursor protein messenger RNAs containing and lacking the Kunitz protease inhibitor domain in the brain of the human, rat and mouse. *Neuroscience* **53**, 267–295.

Sorbi, S., Nacmias, B., Tedde, A. *et al.* (1997). Presenilin-1 gene intronic polymorphism in sporadic and familial Alzheimer's disease. *Neurosci. Lett.* **222**, 132–134.

Spillantini, M. G., Murrell, J. R., Goedert, M. *et al.* (1998). Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7737–7741.

Sprecher, C. A., Grant, F. J., Grimm, G. *et al.* (1993). Molecular cloning of the cDNA for a human amyloid precursor protein homolog: Evidence for a multigene family. *Biochemistry* **32**, 4481–4486.

Stern, Y., Alexander, G. E., Prohovnik, I. *et al.* (1992). Inverse relationship between education and parietotemporal perfusion deficit in Alzheimer's disease. *Ann. Neurol.* **32**, 371–375.

Struble, R. G., Powers, R. E., Casanova, M. F. *et al.* (1987). Neuropeptidergic systems in plaques of Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **46**, 567–584.

Sugiyama, N., SUZUKI, K., Matsumura, T. *et al.* (1999). A novel missense mutation (G209R) in exon 8 of the presenilin 1 gene in a Japanese family with presenile familial Alzheimer's disease. *Hum. Mutation* **14**, 90.

Taddei, K., Kwok, J. B., Kril, J. J. *et al.* (1998). Two novel presenilin-1 mutations (Ser169Leu and Pro436Gln) associated with very early onset Alzheimer's disease. *NeuroReport* **9**, 3335–3339.

Takano, T., Sahara, N., Yamanouchi, Y. *et al.* (1997). Assignment of Alzheimer's presenilin-2 (PS-2) gene to 1q42.1 by fluorescence in situ hybridization. *Neurosci. Lett.* **221**, 205–207.

Talbot, C. J., Lendon, C., Craddock, N. *et al.* (1994). Protection against Alzheimer's disease with APOE $\epsilon 2$. *Lancet* **343**, 1432–1433.

Talbot, C. J., Houlden, H., Craddock, N. *et al.* (1996). Polymorphism in AACT gene may lower age of onset of Alzheimer's disease. *NeuroReport* **7**, 534–536.

Tanzi, R. E., McClatchey, A. I., Lamperti, E. D. *et al.* (1988). Protease inhibitor domain encoded by an amyloid protein precursor mRNA associtated with Alzheimer disease. *Nature (London)* **331**, 528–530.

Tekirian, T. L., Saido, T. C., Markesbery, W. R. *et al.* (1998). N-terminal heterogeneity of parenchymal and cerebrovascular Abeta deposits. *J. Neuropathol. Exp. Neurol.* **57**, 76–94.

Thinakaran, G., Borchelt, D. R., Lee, M. K. *et al.* (1996). Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron* **17**, 181–190.

Tilley, L., Morgan, K., Grainger, J. *et al.* (1999). Evaluation of polymorphisms in the presenilin-1 gene and the butyrylcholinesterase gene as risk factors in sporadic Alzheimer's disease. *Eur. J. Hum. Genet.* **7**, 659–663.

Tolnay, M., and Probst, A. (1999). Tau protein pathology in Alzheimer's disease and related disorders. *Neuropathol. Appl. Neurobiol.* **25**, 171–187.

Tomita, T., Maruyama, K., Saido, T. C. *et al.* (1997). 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. U.S.A.* **94**, 2025–2030.

Tonder, N., Johansen, F. F., Frederickson, C. J. *et al.* (1990). Possible role of zinc in the selective degeneration of dentate hilar neurons after cerebral ischemia in the adult rat. *Neurosci. Lett.* **109**, 247–252.

Tooyama, I., Kawamata, T., Akiyama, H. *et al.* (1993). Immunohistochemical study of a $\alpha 2$-macroglobulin receptor in Alzheimer and control postmortem human brain. *Mol. Chem. Neuropathol.* **18**, 153–160.

Tysoe, C., Whittaker, J., Cairns, N. J. *et al.* (1997). Presenilin-1 intron 8 polymorphism is not associated with autopsy-confirmed late-onset Alzheimer's disease. *Neurosci. Lett.* **222**, 68–69.

Tysoe, C., Whittaker, J., Xuereb, J. *et al.* (1998). A presenilin-1 truncating mutation is present in two cases with autopsy-confirmed early-onset Alzheimer disease. *Am. J. Hum. Genet.* **62**, 70–76.

Van Nostrand, W. E., Schmaier, A. H., Farrow, J. S. *et al.* (1990). Protease Nexin-II (Amyloid β-Protein Precursor): A platelet α-granule protein. *Science* **248**, 745–748.

Vassar, R., Bennett, B. D., Babu-Khan, S. *et al.* (1999). β-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **286**, 735–741.

Vatassery, G. T., Bauer, T., and Dysken, M. (1999). High doses of vitamin E in the treatment of disorders of the central nervous system in the aged. *Am. J. Clin. Nutr.* **70**, 793–801.

Wasco, W., Gurubhagavatula, S., Paradis, M. D. *et al.* (1993). Isolation and characterization of the human APLP2 gene, encoding a homologue of the Alzheimer's associated β protein precursor. *Nat. Genet.* **5**, 95–100.

Wasco, W., Pettingell, W. P., Jondro, P. D. *et al.* (1995). Familial Alzheimer's chromosome 14 mutations. *Nat. Med.* **1**, 848.

Wavrant-DeVrièze, F., Perez-Tur, J., Lambert, J. C. *et al.* (1997). Association between the low density lipoprotein receptor-

related protein (LRP) and Alzheimer's disease. *Neurosci. Lett.* **227**, 68–70.

Weidemann, A., Konig, G., Bunke, D. *et al.* (1989). Identification, biogenesis, and localization of precursors of Alzheimer's disease A4 protein. *Cell (Cambridge, Mass.)* **57**, 115–126.

Weingarten, M. D., Lockwood, A. H., Hwo, S. Y. *et al.* (1975). A protein factor essential for microtubule assembly. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1858–1862.

Wild-Bode, C., Yamazaki, T., Capell, A. *et al.* (1997). Intracellular generation and accumulation of amyloid beta-peptide terminating at amino acid 42. *J. Biol. Chem.* **272**, 16085–16088.

Wisniewski, T., Ghiso, J., and Frangione, B. (1994). Alzheimer's disease and soluble Aβ. *Neurobiol. Aging* **15**, 143–152.

Wisniewski, T., Dowjat, W. K., Buxbaum, J. D. *et al.* (1998). A novel Polish presenilin-1 mutation (P117L) is associated with familial Alzheimer's disease and leads to death as early as the age of 28 years. *NeuroReport* **9**, 217–221.

Wolfe, M. S., Xia, W., Ostaszewski, B. L. *et al.* (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature (London)* **398**, 513–517.

Wragg, M., Hutton, M., and Talbot, C. J. (1996). Genetic association between intronic polymorphism in presenilin-1 gene and late-onset Alzheimer's disease. Alzheimer's Disease Collaborative Group. *Lancet* **347**, 509–512.

Yamamoto, K., Miyoshi, T., Yae, T. *et al.* (1994). The survival of rat cerebral cortical neurons in the presence of trophic APP peptides. *J. Neurobiol.* **25**, 585–594.

Yan, R., Bienkowski, M. J., Shuck, M. E. *et al.* (1999). Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature (London)* **402**, 533–537.

Yasuda, M., Maeda, K., Ikejiri, Y. *et al.* (1997). A novel missense mutation in the presenilin-1 gene in a familial Alzheimer's disease pedigree with abundant amyloid angiopathy. *Neurosci. Lett.* **232**, 29–32.

Yasuda, M., Maeda, K., Hashimoto, M. *et al.* (1999). A pedigree with a novel presenilin 1 mutation at a residue that is not conserved in presenilin 2. *Arch. Neurol. (Chicago)* **56**, 65–69.