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REVIEW

Alzheimer Disease: Mechanistic Understanding Predicts Novel Therapies

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Clinical Principles

Alzheimer disease usually begins with gradual failure of recent memory with preserved alertness and motor function.

The syndrome of minimal cognitive impairment (mild cognitive impairment)—a subtle decrease in short-term declarative memory with otherwise normal cognition—is often a harbinger of Alzheimer disease.

Alzheimer disease progresses slowly to involve many cognitive spheres and shortens life expectancy, with most patients ultimately dying of secondary respiratory complications (for example, aspiration and pneumonia).

Treatment with acetylcholinesterase inhibitors can temporarily alleviate some symptoms but does not modify disease progression.

A noncompetitive *N*-methyl-D-aspartate–receptor antagonist (memantine) has recently been approved as a noncholinergic symptomatic treatment.

Epidemiologic data suggest that long-term use of certain cyclooxygenase-1 or -2 inhibitors (for example, ibuprofen) or statin drugs may be associated with a decreased risk for Alzheimer disease.

Methods to image cerebral amyloid deposits are in early clinical development.

Pathophysiologic Principles

A clinical diagnosis of Alzheimer disease is confirmed by observing numerous neuritic (amyloid) plaques and neurofibrillary tangles in the hippocampus, amygdala, and association neocortex.

The plaques (extracellular) are composed of the 42- and 40-residue β-amyloid proteins, whereas the tangles (intraneuronal) are composed of modified forms of the microtubule-associated protein, tau.

β-Amyloid protein is generated normally throughout life from a large, receptor-like precursor (β-amyloid precursor protein) through proteolytic cleavages by the β- and γ-secretases.

Mutations in 3 genes (β-amyloid precursor protein, presenilin 1, and presenilin 2) that cause Alzheimer disease increase cerebral β-amyloid production, whereas inheritance of the apolipoprotein E4 polymorphism enhances its stability, leading to β-amyloid accumulation.

Prospective mechanism-based treatments include inhibitors of the β- and γ-secretases and immunotherapeutics (anti–β-amyloid protein monoclonal antibodies and β-amyloid protein vaccines).

Few diagnoses in medicine are more dispiriting for patients and their families than Alzheimer disease. This insidious dissolution of one's most human qualities—reasoning, abstraction, language, and memory—now affects upwards of 30 million individuals worldwide. Until recently, the study of Alzheimer disease was fraught with mechanistic ignorance and therapeutic nihilism. But rapid scientific progress in 3 areas, biochemical pathology, genetics, and animal modeling has led to an increasingly accepted model of pathogenesis and the emergence of clinical trials of potentially disease-modifying agents.

Alzheimer disease research has been controversial. Not surprisingly, agreement on the temporal sequence of the molecular and cellular events that lead to dementia and which steps are most amenable to intervention has been difficult to achieve. But in the last several years, substantial consensus has developed that certain biochemical changes in the hippocampus and association cortices occur many years or decades before clinical symptoms, and a rough outline of the disease cascade has emerged.

Biochemistry of the Brain Lesions Provides Major Clues to Geneticists

In contrast to research for disorders such as Parkinson and Huntington diseases, in which the cloning of novel

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For author affiliations, see end of text.
For definition of terms used, see Glossary.

Glossary

Amyloid: Extracellular tissue deposits of insoluble proteinaceous fibrils that are enriched in a particular protein conformation called β-pleated sheet.

Cytoplasmic domain: The part of a transmembrane protein (for example, a receptor) that projects into the inside of the cell.

Ectodomain: The part of a transmembrane protein (for example, a receptor) that projects out of the cell into the extracellular space.

Gliosis: A neuropathologic reaction in which the number of glial cells (for example, astrocytes and microglia) increases and their structure is altered. This occurs in many neurologic diseases and is thus a nonspecific response to different brain injuries.

Missense mutation: A single-base mutation in the DNA sequence of a gene that leads to the change of a single amino acid in the corresponding protein.

Neuritic dystrophy: The distortion of the shapes of neurites (that is, axons and dendrites) that can be visualized microscopically in the brains of patients with various neurodegenerative diseases.

Receptors: Specialized proteins that traverse the outer membrane of the cell (usually) and receive a signal by binding to either another protein or a small molecule, such as a neurotransmitter (for example, dopamine).

Secretases: Proteases (that is, protein-cutting enzymes) that mediate the secretion from a cell of a fragment of another protein. In the case of the β-amyloid precursor protein, the 3 secretases (α, β, and γ) cut at different sites within the protein to shed different fragments.

Transgene: An artificially created segment of DNA that matches the coding region of a natural gene (plus certain regulatory elements) and can be microinjected into fertilized mouse ova to produce transgenic mice overexpressing this gene.

causative genes led to the development of biochemical hypotheses, Alzheimer disease research has largely developed in the reverse direction. The identification of the proteins that make up the classic amyloid (see Glossary) plaques and neurofibrillary tangles in Alzheimer disease cortex suggested their respective genes as sites to search for pathogenic mutations, and such mutations were subsequently found. While rare, they have been enormously instructive in attempts to develop a dynamic model of how Alzheimer disease unfolds. Thus, an understanding of the biochemical pathology preceded genetic discoveries in Alzheimer disease.

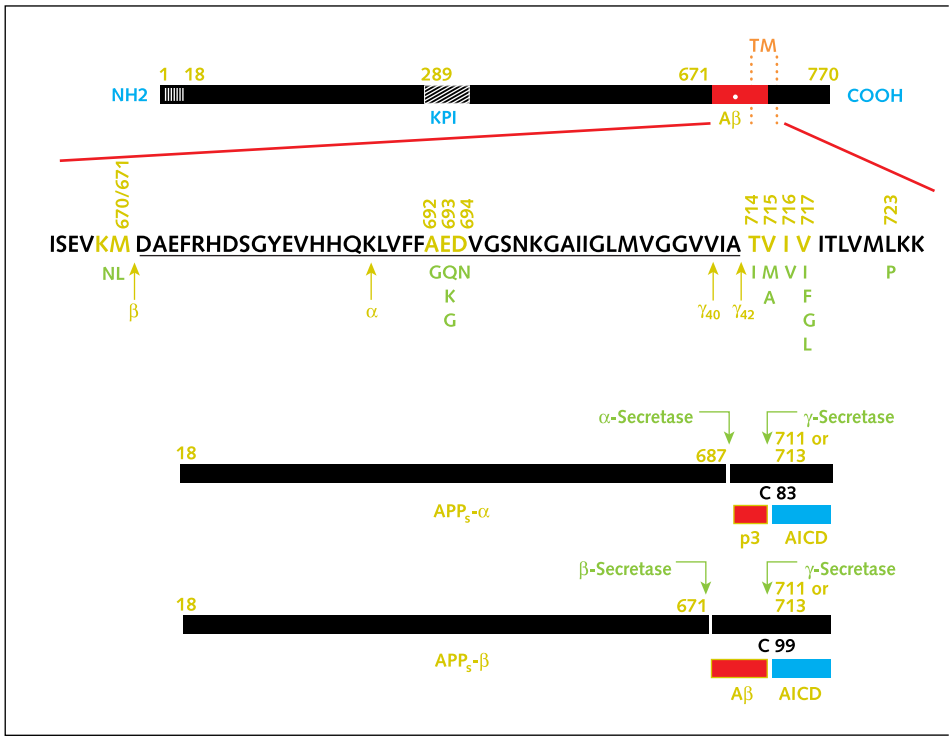
Neuritic (senile) plaques contain extracellular deposits of the 40– and 42–amino acid β-amyloid proteins surrounded by dystrophic neurites (axons and dendrites), activated microglia (monocyte- or macrophage-derived cells that reside in the brain), and reactive astrocytes. A large portion of the β-amyloid protein in these neuritic plaques is in the form of insoluble amyloid fibrils, but these are intermixed with a poorly defined array of nonfibrillar forms of the protein. Once protein sequencing has established that β-amyloid protein was the subunit of fibrillar amyloid (1, 2), β-amyloid protein immunohistochemistry revealed several deposits in brains of patients with Alzheimer disease that lacked the dystrophic neurites and altered glia that characterize the neuritic plaques. Such plain β-amyloid protein deposits, referred to as "diffuse" plaques, exist mostly in a nonfibrillar (that is, "preamyloid") form (3, 4). The diffuse deposits are composed of the 42-residue form of the peptide (β-amyloid protein 42), which is far more prone to aggregation than the slightly shorter and less hydrophobic 40-residue form (β-amyloid protein 40) (5). In healthy individuals, β-amyloid protein 40 and β-amyloid protein 42 make up 90% and about 10%, respectively, of the β-amyloid peptides that are normally produced by brain cells throughout life (discussed later). Note that β-amyloid protein plaques do not occur simply in these 2 extreme forms (diffuse and neuritic) but rather as a continuum in which mixtures of nonfibrillar and fibrillar forms of the peptide can be associated with varying degrees of surrounding neuritic and glial alteration.

Perhaps the most frequent criticism of the "amyloid hypothesis" of Alzheimer disease is that β-amyloid protein deposits can be found in moderate or sometimes high density in the cortex of mentally normal elderly patients. But these deposits are almost exclusively diffuse plaques that seem to represent relatively "benign" precursor lesions associated with little surrounding cytopathology. In this context, drawing a rough analogy to the early fatty streaks observed in the arteries of individuals who have not yet experienced clinically noticeable cardiovascular events is not unreasonable. Statistically significant relationships between β-amyloid protein plaque burden (measured postmortem) and degree of cognitive impairment (measured just premortem) have been established (6), and such data have been complemented by even stronger correlations with cortical β-amyloid protein 42 levels measured biochemically (7).

β-Amyloid protein can also accumulate in the basement membranes of some cortical and meningeal microvessels (8). The extent of this vascular amyloidosis often does not correlate closely with the number of β-amyloid protein plaques in a brain, and its importance in contributing to the dementia is not well defined. Some older humans develop many β-amyloid protein–bearing cortical vessels, often without the abundant plaques and tangles of Alzheimer disease. They can experience sudden vessel rupture and lobar cerebral hemorrhages, an increasingly recognized stroke syndrome referred to as congophilic amyloid angiopathy (8).

The other classic lesion observed in the Alzheimer index case of 1906 was the neurofibrillary tangle. Tangles are intraneuronal masses of paired, helically wound filaments (paired helical filaments) (9). Neurofibrillary tangles usually occur in large numbers in the brain of a patient with Alzheimer disease, particularly in the entorhinal cortex; hippocampus; amygdala; association cortices of the frontal, temporal, and parietal lobes; and certain subcortical nuclei that project to these regions. The subunit protein of the paired helical filaments is the microtubule-associated protein, tau (10, 11). Paired helical filaments are not limited to the tangles found in the neuronal cell bodies, but also occur in smaller bundles in many of the dystrophic neu-

Figure 1. **Schematic diagrams of the β-amyloid precursor protein (*APP*) and its principal metabolic derivatives.**



The first line depicts the largest of the known β-amyloid precursor protein alternate splice forms, comprising 770 amino acids. Regions of interest are indicated at their correct relative positions. A 17-residue signal peptide occurs at the *N*-terminus (*box with vertical lines*). Two alternatively spliced exons of 56 and 19 amino acids are inserted at residue 289; the first contains a serine protease inhibitor domain of the Kunitz type (*KPI*). A single membrane-spanning domain (transmembrane [*TM*]) at amino acids 700 through 723 is indicated (*dotted lines*). The β-amyloid protein (*Aβ*) fragment includes 28 residues just outside the membrane plus the first 12 to 14 residues of the TM domain. In the second line, the sequence within β-amyloid precursor protein that contains the β-amyloid protein and TM regions is expanded. The underlined residues represent the β-amyloid proteins 1 to 42 peptide. The green letters below the wild-type sequence indicate the currently known missense mutations identified in certain families with Alzheimer disease or hereditary cerebral hemorrhage with amyloidosis. The 3-digit numbers are codon numbers (β-amyloid precursor protein 770 isoform). In the third line, the first arrow indicates the site (after residue 687) of a cleavage by α-secretase that enables secretion of the large, soluble ectodomain of β-amyloid precursor protein (APP_s-α) into the medium and retention of the 83-residue C-terminal fragment (*C83*) in the membrane. The C83 fragment can undergo cleavage by the protease called γ-secretase at residue 711 or residue 713 to release the p3 peptides. The fourth line depicts the alternative proteolytic cleavage after residue 671 by β-secretase that causes the secretion of the slightly truncated APP_s-β molecule and the retention of a 99 residue C-terminal fragment (*C99*). The C99 fragment can also undergo cleavage by γ-secretase to release the β-amyloid peptides. Cleavage of both C83 and C99 by γ-secretase releases the β-amyloid precursor protein intracellular domain (*AICD*) into the cytoplasm.

rites present around the amyloid plaques. Biochemical studies reveal that the tau proteins present in paired helical filaments are hyperphosphorylated, insoluble forms of this normally highly soluble protein (9). The insoluble tau aggregates in the tangles are often complexed with ubiquitin, a feature they share with numerous other intraneuronal protein inclusions in etiologically diverse disorders, such as Parkinson disease and diffuse Lewy body dementia. If this complexing with ubiquitin represents an attempt by the neuron to mark the altered tau proteins for degradation by the proteasome, this seems to be largely without benefit for the patient.

The 2 classic lesions of Alzheimer disease can occur independently in other brain diseases. Tangles composed of tau aggregates that are similar to or indistinguishable from those of Alzheimer disease have been described in about a dozen less common neurodegenerative diseases that lack β-amyloid protein deposits and neuritic plaques. Conversely, diffuse β-amyloid protein deposits can be seen in aged "normal" brains with almost no tangles. The fact that neurofibrillary tangles composed of aggregated forms of tau protein occur in certain diseases in the absence of β-amyloid protein deposition (for example, in frontotemporal dementia, subacute sclerosing panencephalitis, and progressive supranuclear palsy) suggests that tangles can occur in the course of various primary neuronal injuries. As we shall see, growing evidence suggests that the formation of tangles in Alzheimer disease represents a cytologic response by neurons to the gradual accumulation of β-amyloid protein and β-amyloid protein–associated proteins.

β-Amyloid Protein Arises from the Normal Processing of a Receptor-Like Precursor

The purification of the β-amyloid protein from the meningovascular amyloid deposits of Alzheimer disease (1) and the Down syndrome (12) enabled the subsequent cloning of the gene encoding the β-amyloid precursor pro-

tein (2). β-Amyloid protein is derived from β-amyloid precursor protein by sequential cleavages by proteases referred to as β-secretase and γ-secretase (13) (see Glossary) (**Figure 1**). The β-amyloid precursor protein comprises ubiquitously expressed proteins whose heterogeneity comes from the "alternative splicing" together of different protein-coding regions (exons) within the β-amyloid precursor protein gene and also from post-translational modifications, such as the addition of sugar or phosphate groups to the protein backbone. Alternatively spliced forms of β-amyloid precursor protein containing 751 or 770 amino acids are widely expressed in cells throughout the body and also occur in neurons. However, neurons express much higher levels of a 695–amino acid splice form. The difference between the 751-, 770-, and 695-residue forms is the retention in the former of an exon that encodes an amino acid sequence that is homologous to certain inhibitors of serine proteases. The existence of this form suggests one normal function for these longer β-amyloid precursor protein isoforms; indeed, the β-amyloid precursor protein 751 that is in human platelets has been shown to inhibit factor XIa (a serine protease) in the clotting cascade.

Completely deleting the β-amyloid precursor protein gene in mice results in only minor deficits (14), suggesting that its 2 close homologues—amyloid precursor–like proteins 1 and 2 (15, 16)—have functions similar to β-amyloid precursor protein. All 3 members of the β-amyloid precursor protein family are single transmembrane proteins that resemble receptors, with the large amino-terminal region (the ectodomain) projecting from the cell surface or into the lumens of intracellular vesicles (for example, the endoplasmic reticulum, Golgi, trans-Golgi network, and endosomes) and the short carboxy-terminal region projecting into the cytoplasmic (see Glossary for terms) (**Figure 1**, *first line*). The recognition that the last 14 amino acids of β-amyloid protein are derived from the membrane-anchoring portion of β-amyloid precursor protein (**Figure 1**) presents a conundrum: How could β-amyloid protein exist as an intact, free peptide in the extracellular amyloid plaques if this region was normally embedded in cell membranes? It was assumed in this regard that cells must undergo an initial injury to their membranes to allow the unknown protease that creates the C-terminus of β-amyloid protein (nicknamed γ-secretase) to access the intramembrane region of β-amyloid precursor protein (**Figure 1**). This concept means that cerebral β-amyloid protein accumulation could not be considered a truly primary event in Alzheimer disease, since some other process must first injure cell membranes to free the peptide. But in 1992, this notion was dispelled when β-amyloid protein was found to be normally secreted by healthy cells throughout life and to be present in the cerebrospinal fluid and plasma of humans and lower mammals (17–19).

In the normal processing of β-amyloid precursor protein by the 3 secretase enzymes (designated as α-, β-, and γ-secretases), the most common cut occurs between amino acids 16 and 17 of the β-amyloid protein region, that is, 12 residues *N*-terminal to the transmembrane region (**Figure 1**, *third line*) and is carried out by α-secretase. This scission creates a large, soluble ectodomain fragment (β-amyloid precursor protein$_\alpha$) that is released from the cell surface and leaves a C-terminal fragment of 83 amino acids (C83) still embedded in the membrane (**Figure 1**, *third line*). α-Secretases, such as tumor necrosis factor–converting enzyme, are membrane-anchored proteases able to cleave diverse single-transmembrane proteins. Some β-amyloid precursor protein holoproteins that are not subjected to the α-secretase cleavage can instead be cut by β-secretase (**Figure 1**, *fourth line*). β-Secretase (also called β-amyloid cleaving enzyme 1) is a membrane-anchored aspartyl protease with its active site in its ectodomain (20–22). A slightly shorter form of the β-amyloid precursor protein ectodomain (β-amyloid precursor protein$_\beta$) is released from the cell surface, and a C-terminal fragment of 99 amino acids (C99) embedded in the membrane is left. The C99 fragment can subsequently be cleaved by the unusual protease referred to as γ-secretase to create β-amyloid protein. Also, the C83 fragment made by α-secretase can undergo cleavage by the same γ-secretase to generate a small peptide that makes up the latter two thirds of the β-amyloid protein region (designated as p3). In summary, C99 and C83 are the substrates of γ-secretase, which create β-amyloid protein and p3, respectively. In general, a substantially larger portion of total cellular β-amyloid precursor protein molecules is cleaved by α-secretase than by β-secretase.

What are the functional consequences of this complex proteolytic processing of β-amyloid precursor protein? Functions attributed to the secreted β-amyloid precursor protein$_\alpha$ derivative include the inhibition of certain serine proteases, enhancement of cell–cell and cell–substrate adhesion, growth-promoting effects on cells, and neuroprotective properties. As regards the function of full-length β-amyloid precursor protein, the remarkably similar processing of β-amyloid precursor protein and the well-characterized Notch family of cell-surface receptors (discussed later) has suggested that β-amyloid precursor protein itself functions as a receptor. Sequential cuts by the α- and γ-secretases release the β-amyloid precursor protein intracellular domain into the cytoplasm, where some of it can enter the nucleus (with associated proteins) and help regulate the transcription of target genes (23–25). Neither the ligand that binds the β-amyloid precursor protein ectodomain to initiate the α-secretase cleavage nor the downstream genes that are regulated by the β-amyloid precursor protein intracellular domain are defined.

The Genetics of Alzheimer Disease Points to a Central Role for β-Amyloid Accumulation

Estimates of the portion of Alzheimer disease cases that are genetically based vary from 10% to 40%, and

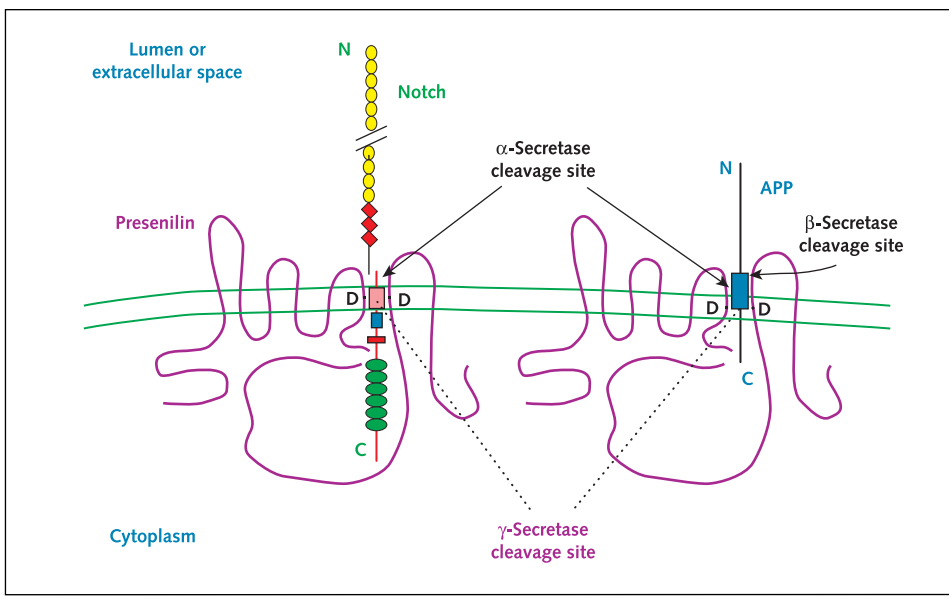
Table. **Genetic Factors Predisposing to Alzheimer Disease: Relationships to the β-Amyloid Phenotype**

Chromosome	Gene Defect	Phenotype
21	β-Amyloid precursor protein mutations	Increased production of all β-amyloid proteins or β-amyloid protein 42
19	Apolipoprotein E4 polymorphism	Increased density of β-amyloid plaques and vascular deposits
14	Presenilin 1 mutations	Increased production of β-amyloid protein 42
1	Presenilin 2 mutations	Increased production of β-amyloid protein 42

some investigators believe that, in time, almost all cases will be found to have some genetic determinants. This question is difficult to answer in a disorder that occurs late in life and was usually not explicitly diagnosed before the last 2 decades. Moreover, the discovery that the apolipoprotein E4 allele is a normal genetic polymorphism that confers an increased risk (but not certainty) of Alzheimer disease (26) indicates that genetic factors predisposing to the disease do not need to occur in a dominant pattern and may thus be hard to recognize in genetic epidemiologic studies.

Currently, 4 genes in which mutations or polymorphisms cause Alzheimer disease have been confirmed (**Table**) (27), and additional candidate genes await confirmation. Missense mutations (see Glossary) in β-amyloid precursor protein account for far less than 0.1% of Alzheimer disease cases, but they have proved to be highly informative about the general mechanism of the disease. Expressing mutant human β-amyloid precursor protein transgenes (see Glossary) in mice provided the first reproducible animal models of the disorder (28). Inheritance of 1 or 2 apolipoprotein E4 alleles is a far more prevalent genetic basis for Alzheimer disease, perhaps accounting for roughly a quarter of cases. Apolipoprotein E4 helps precipitate the disorder primarily in patients who are in their sixties and seventies, thus lowering the typical age of onset of late-life Alzheimer disease (29). Apolipoprotein E4 is a risk factor, not a deterministic gene. Some humans who are homozygous for this allele show no Alzheimer symptoms when they are in their nineties. The third and fourth genes implicated in familial Alzheimer disease are called presenilin 1 and presenilin 2 because missense mutations in these homologous proteins cause a very early onset of Alzheimer disease, usually between 35 and 60 years of age (30–32). To date, more than 100 missense mutations have been identified in presenilin 1 and at least 6 have been identified in presenilin 2. These tend to cluster within and adjacent to the 8 transmembrane domains of this serpentine protein

Figure 2. **Hypothetical model of the role of presenilin in Notch and β-amyloid precursor protein processing based on current information.**



The diagram shows the predicted 8 transmembrane domain topology of presenilin, which occurs principally as a cleaved heterodimer. Some Notch and β-amyloid precursor protein molecules form complexes with presenilin. Two aspartates (*D*) in transmembrane domains 6 and 7 of presenilin are required for the cleavages of Notch and β-amyloid precursor protein within their transmembrane domains, and these may align with the respective sites of cleavage in the 2 substrates. Presenilin directly effects these cleavages in a γ-secretase complex that contains at least 3 other membrane proteins. Several motifs are depicted in Notch: epidermal growth factor–like repeats (*yellow circles*), LNG (lin-Notch-glp) repeats (*orange diamonds*), a single transmembrane (*white box*), the RAM23 domain (*blue square*), a nuclear localization sequence (*red rectangle*), and 6 cdc10/ankyrin repeats (*green ovals*). After the putative intramembranous cleavage is mediated by presenilin, the Notch intracellular domain is released to the nucleus to activate transcription of target genes. β-Amyloid precursor protein contains the β-amyloid protein region (*blue box*), which is released into the lumen after sequential cleavages of β-amyloid precursor protein by β-secretase and then γ-secretase or presenilin. The released β-amyloid precursor protein intracellular domain can reach the nucleus, but its function there is undefined. APP = β-amyloid precursor protein.

(**Figure 2**). Families with Alzheimer disease that show no linkage to the aforementioned 4 genes are being studied. Therefore, several additional genes will probably be implicated, most of them probably acting as polymorphic risk factors (such as apolipoprotein E4) and each explaining a relatively small fraction of Alzheimer disease cases.

Despite the prominence of tau accumulation in the neurofibrillary tangles and dystrophic neurites of Alzheimer disease cases, the tau gene has not been found to be the site of mutations in familial Alzheimer disease. Instead, mutations in tau have been discovered in families with a less common but equally devastating disorder, frontotemporal dementia with parkinsonism linked to chromosome 17 (33–35). The disease is characterized by widespread neurofibrillary tangle formation caused by alterations in the microtubule-binding properties of tau, without amyloid deposits (9, 36). Thus, a primary alteration of tau structure and function can produce a progressive, ultimately fatal neuronal degeneration without leading to secondary accumulation of β-amyloid protein. The latter point addresses a recurring controversy in the study of Alzheimer disease: whether plaques or tangles have temporal precedence in the pathogenesis of the disorder. Both the β-amyloid precursor protein and presenilin mutations in Alzheimer disease and the tau mutations in frontotemporal dementia with parkinsonism linked to chromosome 17 support the conclusion that in Alzheimer disease, β-amyloid protein accumulation leads to tau alterations rather than the reverse.

Genotype-to-Phenotype Conversions in Familial Alzheimer Disease

Both cultured cells and transgenic mice have been used to model the biochemical and neuropathologic effects of each of the 4 genes implicated to date in familial Alzheimer disease (**Table**). The results have been compared with the actual phenotypes observed in the brains of patients carrying the respective gene defects. In all 4 cases, the altered gene products increased the production or deposition of β-amyloid protein in the brain. This work strongly supports the hypothesis that β-amyloid protein accumulation is an early and necessary event in the genesis of Alzheimer disease and is thus worthy of therapeutic inhibition.

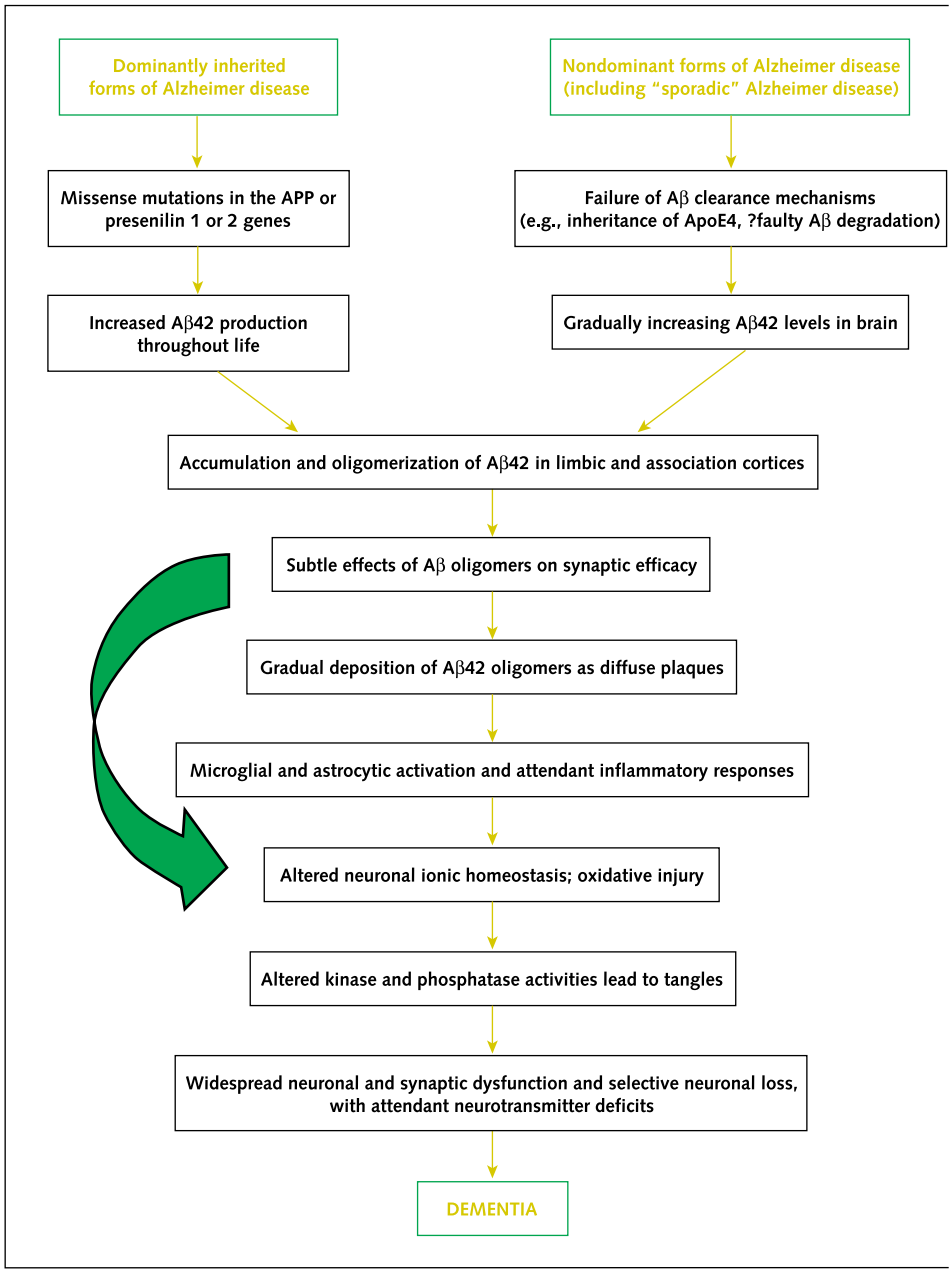
Alterations in the β-amyloid precursor protein gene on chromosome 21 can lead to the Alzheimer disease syndrome in at least 2 distinct ways. β-Amyloid precursor protein can either be overexpressed because of increased gene dosage in trisomy 21 (the Down syndrome) or be the site of missense mutations that increase the β-amyloid protein–generating cleavages of β-amyloid precursor protein (**Figure 1**) or enhance β-amyloid protein aggregation. In patients with the Down syndrome, increased expression of wild-type β-amyloid precursor protein and the resultant overproduction of both β-amyloid proteins 40 and 42 peptides from birth onward leads to the appearance of diffuse β-amyloid protein 42 plaques as early as 10 years of age. Persons with the Down syndrome often display many diffuse β-amyloid protein 42 plaques in their teenage years and twenties, with the subsequent accrual of β-amyloid protein 40 onto these plaques and the appearance of gliosis (see Glossary), neuritic dystrophy (see Glossary), and neurofibrillary tangles occurring when they are in their late twenties and thirties (37). This gradual development of Alzheimer disease-type brain lesions can be associated with progressive loss of cognitive and behavioral functions in patients with the Down syndrome, who are mentally retarded from birth for other reasons.

Most of the β-amyloid precursor protein missense mutations linked to familial Alzheimer disease are located either at the β-secretase cleavage site, thus increasing the cellular production of both β-amyloid proteins 40 and 42, or just after the γ-secretase site, thus selectively increasing β-amyloid protein 42 production (**Figure 1**, *second line*). Several mutations are also located after the α-secretase site within β-amyloid protein (**Figure 1**, *second line*), and they enhance β-amyloid protein aggregation or increase its production (38). Some families with such intra–β-amyloid protein mutations have a phenotype of severe amyloid angiopathy with or without Alzheimer disease.

The Alzheimer disease–promoting effect of inheriting 1 or 2 apolipoprotein E4 alleles seems to involve an enhanced aggregation or decreased clearance of β-amyloid peptides (39–41). When mice transgenic for mutant human β-amyloid precursor protein are crossed with mice in whom the endogenous apolipoprotein E gene is deleted, the offspring show substantially decreased β-amyloid protein plaque burden, and those β-amyloid protein deposits that develop are diffuse (nonfibrillar) (42). Moreover, mice lacking endogenous apolipoprotein E that transgenically express the human apolipoprotein E4 isoform develop far more fibrillar (neuritic) amyloid plaques than do those expressing the apolipoprotein E3 isoform (43).

When presenilin 1 and presenilin 2 were first cloned, the mechanism by which missense mutations in these proteins produced Alzheimer disease was not necessarily expected to involve enhanced β-amyloid protein production. But assays of β-amyloid proteins 40 and 42 in the plasma and skin fibroblast cultures of presenilin mutation carriers soon revealed a selective elevation of β-amyloid protein 42 levels (44). Subsequent modeling of these mutations in cultured cells and transgenic mice has confirmed this result (13, 28). When mice expressing mutant human β-amyloid precursor protein are crossed with mice expressing a human presenilin 1 mutation, the offspring show accelerated β-amyloid protein 42 accumulation and Alzheimer disease–like plaques in the brain (45). Moreover, the ability of presenilin mutations to selectively enhance β-amyloid protein 42 deposition in the human brain has been demonstrated directly in patients (46, 47).

Figure 3. **A hypothetical sequence of the pathogenetic steps of Alzheimer disease based on currently available evidence.**



β-Amyloid protein 42 is the 42-residue form of β-amyloid. Aβ = β-amyloid protein; APP = β-amyloid precursor protein; ApoE4 = apolipoprotein E4.

Presenilins: Mediators of Intramembrane Proteolysis during Development and in Alzheimer Disease

To understand the mechanism by which mutant presenilins increase the γ-secretase cleavage of β-amyloid precursor protein at β-amyloid protein 42, we need to consider the normal biology of presenilin. Presenilin itself undergoes an internal proteolytic cleavage (called endoproteolysis) and thus occurs largely as a dimer of amino-terminal and carboxy-terminal fragments (48) (**Figure 2**). Similar to the β-amyloid precursor protein, presenilins are expressed in all cell types and are highly conserved in evolution. Studies in the roundworm, *Caenorhabditis elegans*, found that its presenilin (called Sel-12) is necessary for the intercellular signaling pathway that involves the cell-surface receptor, Notch (49). Further work in invertebrates and mammals showed that presenilins are actually required for the "intramembranous" (that is, inside the plasma membrane) cleavage of the Notch receptor following binding of its extracellular ligand (50). This cleavage releases the in-

tracellular domain of Notch to the nucleus, where it regulates the transcription of genes important for the proper specification of cell types, both inside and outside the nervous system. Deleting the mouse presenilin 1 gene causes a lethal embryonic phenotype resembling that of deleting the Notch gene (51), and neurons cultured from such mice also have markedly reduced γ-secretase cleavage of β-amyloid precursor protein (52).

These and certain additional findings suggest a provocative hypothesis: Presenilin is the active site of γ-secretase and thus mediates the cleavage of both Notch and β-amyloid precursor protein within the membranes of cells (53) (**Figure 2**). First, β-amyloid precursor protein and presenilin interact directly in cells (54). Second, inhibiting γ-secretase activity with designed compounds that mimic the β-amyloid proteins 40 to 45 region of β-amyloid precursor protein that is cut by this enzyme has revealed that γ-secretase is an aspartyl protease (that is, a protease that uses 2 aspartate residues to cut other proteins, such as the HIV protease) (55). Third, close inspection of the presenilin amino acid sequence reveals 2 aspartates within adjacent transmembrane domains of all presenilins (**Figure 2**). Mutation of either aspartate markedly inhibits the γ-secretase processing of C99 to β-amyloid protein (53). These results suggest that presenilin is an unprecedented type of aspartyl protease that can cleave numerous transmembrane substrates, including β-amyloid precursor protein and Notch, within their membrane domains. The subsequent finding that γ-secretase inhibitors specially designed to mimic the transient complex that β-amyloid precursor protein would form with an aspartyl protease bound directly to presenilins and to no other cellular protein strongly supports this hypothesis (56, 57).

The recognition that presenilin is the active site of γ-secretase provides a linchpin for the "amyloid" hypothesis of Alzheimer disease. The only mutations known to cause dominantly inherited Alzheimer disease occur in either the protease (presenilin) or the substrate (β-amyloid precursor protein) of the reaction that generates β-amyloid protein. Illuminating the role of the presenilins in the processing of β-amyloid precursor protein, Notch and numerous other membrane proteins have suggested a new way of thinking about the origin of Alzheimer disease. Presenilins, with their 2 intramembrane aspartate residues, were conserved during evolution because they mediate the intramembrane cleavage of Notch, whose signaling is required for life in all multicellular animals. Notch may be a favored substrate of the presenilin or γ-secretase (58), but the cleavage of an alternate substrate, β-amyloid precursor protein, can lead to the gradual accumulation of its self-aggregating fragment, β-amyloid protein 42. In short, Alzheimer disease may have occurred in the human population as a side effect of a highly advantageous enzymatic machine that leads to ill consequences only when humans survive well into postreproductive life.

The Complex Alzheimer Disease Cascade: How Does β-Amyloid Accumulation Precipitate Disease?

Although genetic evidence strongly favors the hypothesis that heightened β-amyloid protein accumulation is an early and necessary feature of Alzheimer disease, considerable debate remains about whether this can explain the full Alzheimer phenotype. The β-amyloid protein hypothesis (**Figure 3**) predicts that gradual elevation of β-amyloid protein 42 levels in brain interstitial fluid, and perhaps also inside neurons (59, 60), can lead to the oligomerization of the peptide and eventually to its fibrillization (that is, amyloid formation). β-Amyloid protein accumulation can clearly be initiated by mutations in β-amyloid precursor protein or presenilin, and other genetic polymorphisms (that is, apolipoprotein E4) or mutations could lead to a similar outcome by chronically decreasing clearance of the peptide. There is a growing interest in the possibility that early, diffusible oligomers (for example, dimers, trimers, and tetramers) of β-amyloid protein could themselves be toxic to synapses, whereas the abundant fibrils of mature amyloid plaques represent a relatively inert reservoir of β-amyloid protein that is in equilibrium with the smaller, biologically active oligomers (61, 62). On the basis of the known evolution of β-amyloid protein–related pathology in the Down syndrome and in β-amyloid precursor protein transgenic mouse models, β-amyloid protein 42 oligomerization and its deposition as diffuse plaques seems to be associated with local microglial activation, astrocytosis, and cytokine and acute phase protein release (63). Whether β-amyloid protein oligomers trigger synaptic dysfunction through this intermediate inflammatory process or produce direct synaptotoxic effects by subtly disrupting receptors, channel proteins, and other macromolecules on the plasma membrane (62) may be difficult to sort out because these changes may develop almost simultaneously in vivo.

There is considerable evidence that the effects of a β-amyloid protein–initiated inflammatory and neurotoxic process include excessive generation of free radicals and oxidative injury to proteins and other macromolecules in neurons (64–66). In this context, a therapeutic trial of the antioxidant vitamin E seemed to slow the clinical progression of Alzheimer disease, although actual memory and cognitive deficits did not measurably improve (67). Among the many possible metabolic consequences of β-amyloid protein accumulation and oligomerization, altered ionic homeostasis, particularly excessive calcium entry into neurons, could contribute to selective neuronal dysfunction and death, on the basis of culture studies of the effects of aggregated β-amyloid protein (68–70). Proving that β-amyloid protein accumulation actually triggers the dissociation of the tau protein from microtubules, its excessive phosphorylation, and its assembly into tangles (36, 71) awaits further research. However, when mice transgenic for

mutant human tau were crossed with β-amyloid precursor protein transgenic mice, their offspring showed accelerated accumulation of altered forms of tau, including in tangle-like lesions (72).

Deciphering the Alzheimer Disease Cascade Predicts a New Diagnostic and Treatment Paradigm

Currently, the pharmaceutical treatment of the dementing symptoms of Alzheimer disease is restricted to 2 types of drugs: acetylcholinesterase inhibitors (such as donepezil, galantamine, and rivastigmine) (73) and the recently approved *N*-methyl-D-aspartate antagonist, memantine (74, 75). These agents temporarily relieve some symptoms for a period of time for a subset of patients, but they do not address the underlying pathologic process or substantially slow clinical progression.

Sufficient progress in delineating the disease cascade (**Figure 3**) has been achieved to envision several discrete types of potentially disease-modifying treatments. Inhibitors of β-amyloid protein production, that is, small compounds that cross the blood–brain barrier and decrease (but do not eliminate) either β- or γ-secretase activity, could be therapeutic in the early clinical phases of the disease, particularly in patients with the subtle syndrome of minimal cognitive impairment (mild cognitive impairment) (76, 77), which is an increasingly recognized harbinger of Alzheimer disease. γ-Secretase inhibitors could be designed to decrease β-amyloid protein production by 30% to 40%, without interfering in a quantitatively important way with Notch signaling. Whether such a beneficial therapeutic index for a γ-secretase inhibitor can be achieved by targeting either the active site component (presenilin) or one of its recently identified protein cofactors (such as Nicastrin, Aph-1, or Pen-2) (78) requires further preclinical and clinical research.

As regards β-secretase (β-amyloid–cleaving enzyme 1), far fewer inhibitors of this protease have emerged from high-throughput compound screening on β-amyloid protein–secreting cells than for γ-secretase. This signifies that finding small compounds that adequately block the active site of β-secretase is more difficult. However, the fact that the crystal structure of β-secretase with an inhibitor in place has been solved (79) suggests that designing effective inhibitors is now possible. If so, such inhibitors may have a better therapeutic index than those of γ-secretase, in view of the finding that knocking out the β-secretase gene causes no deleterious phenotype in mice (80).

An alternative approach to secretase inhibition would be to use small molecules to bind β-amyloid protein monomers and prevent their aggregation into potentially neurotoxic oligomers. However, if an antiaggregating compound solely blocked amyloid fibril formation, this could actually allow increased accumulation of intermediates, such as oligomers, and could potentially aggravate neurotoxicity. One advantage of an antioligomerization strategy is that one would be targeting what is believed to be a purely pathologic entity, rather than interfering with normal enzymatic reactions, such as β- or γ-secretase.

An immunologic approach to lowering the levels of β-amyloid protein monomers, oligomers, and higher aggregates is supported by an increasing number of studies in β-amyloid precursor protein transgenic mice. Parenteral immunization of mice with synthetic human β-amyloid proteins 1 to 42 was initially shown to lead to an antibody response associated with striking clearance of β-amyloid deposits, or else their actual prevention if the mice were immunized very early in life (81). Subsequent studies have confirmed and extended this approach by showing that β-amyloid protein immunization can lower brain β-amyloid protein burden and also improve learning deficits in the mice (82, 83). A mucosal (intranasal) route of β-amyloid protein immunization can also yield antibodies to β-amyloid protein and reduce plaque burden and associated neuritic and glial changes (84). Importantly, passive administration of β-amyloid protein monoclonal antibodies can achieve the β-amyloid–clearing effect, potentially obviating the need for active vaccines (85). Three general mechanisms for the beneficial effects of active and passive immunization have been proposed. First, the anti–β-amyloid protein antibodies may cross the blood–brain barrier in small amounts and bind to β-amyloid protein, followed by gradual clearing of the resultant β-amyloid protein antibody complexes by local microglia (85). Second, high titers of anti–β-amyloid protein antibodies in the peripheral circulation may bind and sequester β-amyloid protein in that compartment, resulting in a gradual redistribution of β-amyloid protein from brain parenchyma to cerebrospinal fluid to plasma (86). Third, the anti–β-amyloid protein antibodies might bind to soluble β-amyloid protein oligomers in the brain and neutralize their synaptotoxic effects (87).

No untoward antigen–antibody reactions were reported in active vaccination experiments in mouse models (81, 84). However, the administration of a β-amyloid protein 1 to 42 peptide vaccine (with an adjuvant) to humans with mild to moderate Alzheimer disease resulted in some 6% of patients developing an inflammatory reaction in the central nervous system that resembled a postvaccinal meningoencephalitis (88). Although this adverse event led to cessation of the trial, subsequent follow-up revealed that at least a subset of patients who had developed high titers of antibodies to β-amyloid protein titers seemed to show a slowing of their cognitive and behavioral decline (89). In view of these developments, human trials of passively administered anti–β-amyloid protein antibodies are being initiated.

Another β-amyloid protein–based approach to treating Alzheimer disease is to administer anti-inflammatory drugs that could interfere with aspects of the microglial, astrocytic, and cytokine responses found in the brain of a

patient with Alzheimer disease. The epidemiologic evidence that prolonged use of certain nonsteroidal anti-inflammatory drugs (NSAIDs) (specifically inhibitors of cyclooxygenase-1) is associated with a lower risk for Alzheimer disease could be explained on this basis (90). However, exciting studies in cultured cells and β-amyloid precursor protein transgenic mice suggest that some NSAIDs associated with protection from Alzheimer disease (such as ibuprofen) can actually modulate γ-secretase processing of β-amyloid precursor protein to decrease β-amyloid protein 42 production without lowering overall β-amyloid protein levels or interfering with Notch cleavage (91). This unexpected finding could lead to derivatives of certain NSAIDs being used as a special type of "γ-secretase modulator."

Finally, various antioxidants, free radical scavengers, calcium-channel blockers, metal chelators, or modulators of certain signal transduction pathways might protect neurons from the downstream effects of the accumulation of β-amyloid protein. One problem with such approaches may be that neurons respond to β-amyloid protein and its associated inflammatory process in several ways, and blocking 1 or 2 of these response pathways might not substantially decrease overall neuronal dysfunction and loss. A chelator of copper and zinc ions that may decrease cerebral β-amyloid protein levels (92) is being tested in patients with Alzheimer disease.

Because the success of any of the discussed strategies cannot be predicted and because 2 or more approaches might ultimately be combined, all such efforts and others not reviewed here must be vigorously pursued. Current symptomatic treatments aimed at modulating certain neurotransmitters, including acetylcholine and glutamate, may continue to be useful, even if β-amyloid protein–based treatments aimed at early steps in the disease are forthcoming.

In the future, individuals who are nearly 50 years of age or older will probably be offered a specific risk-assessment profile to determine their likelihood of developing Alzheimer disease. Such an assessment, modeled on that used to predict the risk for serious atherosclerotic disease, could include inquiry about a family history of Alzheimer disease; identification of specific predisposing genetic factors; structural and functional brain imaging to detect evidence of presymptomatic lesions; and measurement of β-amyloid protein 42, tau, and other markers of the neuropathology in cerebrospinal fluid and perhaps (in the case of β-amyloid protein) in blood. On the basis of further epidemiologic experience with such assessment measures in large samples of healthy elderly patients, patients with mild cognitive impairment, and patients with Alzheimer disease, estimating—first crudely and later more accurately—the likelihood that an individual will develop Alzheimer disease should be possible. Those at high risk might be offered preventative treatments with 1 or more of the agents discussed in this paper. Although the achievement of an integrated diagnostic and therapeutic approach to this devastating disorder may seem remote, the current rate of scientific progress and the recent initiation of further clinical trials suggest that some level of practical success may come sooner than one might think.

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