DECIPHERING THE GENETIC BASIS OF ALZHEIMER’S DISEASE

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Abstract A remarkable rise in life expectancy during the past century has made Alzheimer’s disease (AD) the most common form of progressive cognitive failure in humans. Compositional analyses of the classical brain lesions, the senile (amyloid) plaques and neurofibrillary tangles, preceded and has guided the search for genetic alterations. Four genes have been unequivocally implicated in inherited forms of AD, and mutations or polymorphisms in these genes cause excessive cerebral accumulation of the amyloid β-protein and subsequent neuronal and glial pathology in brain regions important for memory and cognition. This understanding of the genotype-to-phenotype conversions of familial AD has led to the development of pharmacological strategies to lower amyloid β-protein levels as a way of treating or preventing all forms of the disease.

INTRODUCTION

Until the past decade, degenerative diseases of the brain were considered to be among the most obscure and intractable disorders in medicine. Alzheimer’s disease (AD) epitomized the mechanistic ignorance and therapeutic nihilism that pervaded the study of neurodegeneration in humans. But research advances in two areas—biochemical pathology and genetics—have coalesced to alter dramatically the hopelessness of the field and bring it to the verge of disease-modifying therapies. In the case of AD, determining the composition of the classical brain lesions and identifying at least four genes that predispose to the disorder have allowed an increasingly clear understanding of the genotype-to-phenotype relationships that underlie inherited forms of the disease. As a result, the once controversial hypothesis that progressive accumulation of the amyloid β-protein (Aβ) in limbic and association cortices underlies the development of dementia has gained substantial, albeit sometimes grudging, acceptance.

For many neurodegenerative disorders, for example, Huntington’s disease, amyotrophic lateral sclerosis, and Friedreich’s ataxia, no compelling clues to the
pathogenesis of the neuronal dysfunction and loss existed prior to the discovery of specific causative genes. In contrast, for AD, detailed knowledge of the biochemical pathology both preceded and assisted the discovery of causative genes. The purification in the 1980s of the neurofibrillary tangles and amyloid plaques that are the diagnostic hallmarks of AD (Figure 1) and the identification of their subunit proteins as the microtubule-associated protein, tau, and the amyloid β-protein, respectively, encouraged targeted searches of the genome for AD-linked gene defects. Indeed, the first mutation implicated in familial AD was found in the β-amyloid precursor protein (APP) gene on chromosome 21 (39). Although mutations in tau have not been observed in AD subjects, they have been found in a less-common dementia that shares certain clinical and pathological features with AD, frontotemporal dementia with parkinsonism on chromosome 17 (FTDP-17) [reviewed in (40)]. Thus, candidate positional gene approaches have yielded some successes in AD, whereas other AD-causing genes, e.g., presenilins 1 and 2, were discovered only after unbiased linkage analysis (102) and positional cloning (73, 97, 111).

The earliest clue to the localization of an AD-causing gene came from the recognition in the 1940s and 1950s that patients with trisomy 21 (Down’s syndrome) invariably develop the classical neuropathology of AD (amyloid plaques and neurofibrillary tangles), but they do so already in early middle age. This observation made chromosome 21 a favored site in the initial search for genes underlying dominantly transmitted forms of AD, the existence of which had been known for many years. Indeed, George Glenner, who first isolated and partially sequenced the Aβ peptide from the cerebrovascular amyloid deposits of both AD and Down’s syndrome patients (37, 38), predicted that a genetic defect causing AD would be localized to chromosome 21. He based this hypothesis in part on the knowledge that certain systemic amyloid deposition diseases (amyloidoses) occur in both inherited and “sporadic” forms. Although this notion seemed reasonable to pathologists and biochemists like Glenner who studied systemic amyloid disorders, neuropathologists and other neuroscientists often expressed skepticism that amyloid could represent the primary pathogenic agent in AD. Thus, a schism emerged between a few early proponents of the concept that Alzheimer’s is, in essence, an amyloidosis of the brain and a larger group of scientists who assumed that amyloid deposits were secondary or tertiary events in pathogenesis—“tombstones” of the process. As we shall see, the application of genetics is helping to resolve this debate.

Defining Alzheimer’s Disease

The insidious and progressive loss of memory, orientation, reasoning, and other cognitive functions that occurs in AD was surely observed in the human population long before the Bavarian psychiatrist, Alois Alzheimer, followed a 51-year-old patient to autopsy and described her neuropathology in 1906. Based on his report, AD was initially thought of as a “presenile” dementia (onset <65 years of age), but by the 1960s, it had become clear that some 50%–70% of patients with the
syndrome of “senile” dementia (onset >65 years of age) were clinically and neuropathologically indistinguishable from those with presenile AD, except for their later age of onset. Indeed, prevalence curves indicate that AD does not occur in a bimodal age distribution, but rather as a continuously rising number of cases with age. It is estimated that <1% of Americans aged 60–64 years have AD, increasing steadily to as many as 35%–40% after age 85 (12). Thus, the vast majority of AD subjects have onset after age 65 [late-onset AD (LOAD)], whereas some 2%–3% have onset before 65 [early-onset AD (EOAD)] (90).

After advanced age, the second most-important risk factor for AD is a positive family history of an AD-type dementia (131). Genetic epidemiological studies have yielded widely varying estimates, ranging from 30%–70%, for that portion of the total population risk for AD that may ultimately be attributable to genetic factors (26, 114). Every-day clinical experience suggests that a positive history of a similar dementing illness in first-degree relatives can be obtained in 40%–60% of patients. However, most such families lack autopsy confirmation of the diagnosis, and a considerably smaller fraction of all AD subjects, perhaps 10%–15%, has a family history consistent with an autosomal dominant trait. The latter cases are sometimes referred to as familial AD and, as expected, have been documented primarily in the EOAD population. Although the late onset of most AD cases makes ascertainment of an unambiguous family history difficult, clinicians frequently obtain informal histories of AD-like dementia in deceased relatives. Occasionally, one encounters a striking family history of LOAD in multiple family members from more than one generation. Therefore, it is likely that a much greater fraction of all cases than is currently believed will ultimately be shown to have genetic determinants. In contrast, environmental factors that unequivocally predispose to the disease have been very difficult to identify.

A Characteristic Pathology Assists the Search for Genetic Factors

In most patients, the dementia progresses rather slowly over 5 to 20 years, gradually leading to a severely impaired state, with behavioral symptoms, major language dysfunction, abnormal gait, incontinence, and complete social dependence. At autopsy, one usually observes cerebral atrophy and the classical histological lesions in the hippocampus, entorhinal cortex, amygdala, cerebral association cortices, and certain subcortical nuclei that project to these areas. Neurofibrillary tangles occur in selected neuronal cell bodies in these areas and are intraneuronal masses of abnormal, helically wound filaments [paired helical filaments (PHF)] that are composed of hyperphosphorylated forms of the tau protein. Amyloid plaques are compacted, spherical deposits of extracellular, ∼8-nm fibrils of Aβ, often intimately surrounded by dystrophic dendrites and axons; such lesions are also referred to as neuritic plaques. However, only a subset of all Aβ deposits in a typical AD brain have this morphology, and there are usually many more plaques that have amorphous, streak-like or crudely spherical form. Such deposits, referred to as diffuse
plaques, appear to contain a range of Aβ assembly forms, including fine granular material and some fine filaments, but they have very few ~8-nm mature amyloid fibrils. Several lines of evidence suggest that some of the diffuse plaques gradually evolve to become neuritic plaques [reviewed in (109)]. Electron microscopy indicates that most diffuse plaques contain very few dystrophic neurites, as if the neuropil is not particularly altered by the local accumulation of nonfibrillar forms of Aβ. Almost all AD brains also have scattered cortical and/or meningeal microvessels bearing fibrillar Aβ deposits in their basement membranes, and in a minority of cases, this vascular amyloidosis is rather severe [congophilic amyloid angiopathy (CAA)].

Although one can find considerable variation in the quantity and quality of plaques and tangles among AD brains, the neuropathology is nonetheless relatively stereotyped. Interest has swirled around descriptions of occasional AD families that show unusual types of amyloid deposits, e.g., large “cotton wool” plaques and/or a dearth of neurite-rich plaques. However, such lesion subtypes do not appear to be unique to a particular familial AD genotype, and indistinguishable lesions are occasionally observed in apparently sporadic, late-onset cases (66). As described in this review, a few APP genotypes are strongly associated with severe CAA involving innumerable cerebral and meningeal microvessels, sometimes accompanied by surprisingly few parenchymal Aβ deposits. Often, such patients have experienced intracerebral hemorrhages.

The existence of such a well-defined and relatively specific pathology is the overriding reason that so much progress in elucidating the pathogenesis of AD has occurred during the past two decades. Information about the complex cellular and molecular alterations now attributed to the disease has gradually emerged from increasingly detailed analyses of the composition and genesis of the two hallmark lesions of AD.

CONFIRMED AD GENES: GENOTYPE-TO-PHENOTYPE RELATIONSHIPS

Missense Mutations in the APP Gene

Once the APP gene was cloned in 1987 and localized to chromosome 21q21.3-q22.05, the occurrence of Alzheimer pathology in Down’s syndrome could be explained by a gene dosage effect. This concept was later solidified by the study of a patient with translocation Down’s syndrome in which the chromosomal breakpoint was localized to 21q22.1, telomeric to the APP gene (93). The patient showed no detectable behavioral decline in late life, in contrast to many trisomic Down’s subjects, and postmortem examination at age 78 revealed no amyloid deposits. Therefore, APP overexpression underlies the development of plaques and tangles in trisomy 21. Indeed, elevated levels of both cerebral and plasma Aβ have been documented in Down’s syndrome early in life (124, 128). These observations also provided evidence that amyloid deposition could precede tangle formation, at
least in Down’s syndrome. Immunohistochemical studies of Down’s subjects of increasing age have shown that diffuse plaques can appear by age 12 or earlier, whereas neuritic plaques and neurofibrillary tangles are usually not detectable until one to two decades later (67). It is hypothesized that a similar sequence of evolution of these lesions occurs in conventional AD.

Based in part on such findings, early linkage studies in EOAD families were focused on chromosome 21q. The first definitive implication of APP in AD-like pathology came from the discovery of a Glu to Gln mutation at position 22 of the Aβ sequence in an extended pedigree with hereditary cerebral hemorrhage with amyloidosis (HCHWA)-Dutch type (71). Here, patients succumb to multiple cerebral hemorrhages caused by hyaline necrosis of small arteries laden with Aβ fibrils. Limited numbers of diffuse plaques, in the virtual absence of neuritic changes and tangles, are observed in the cerebral cortex (87). One of the remaining riddles of β-amyloidosis is precisely why this mutation causes almost exclusively microvascular deposition.

Shortly after this discovery, an EOAD family in England was found to have a Val to Ile mutation five residues after the end of the Aβ42 region of APP, within the transmembrane (TM) domain of this type I integral membrane glycoprotein (39). This finding established the first specific genetic defect in AD. Two additional families with different substitutions (Gly or Phe) at the same codon were soon described [reviewed in (114)]. A Flemish kindred with an intra-Aβ mutation (Ala to Gly) immediately C-terminal to the HCHWA-Dutch switch displayed a mixed phenotype of HCHWA and/or EOAD, and a double mutation (Lys/Met to Asn/Leu) was located immediately N-terminal to the first residue of the Aβ region in a Swedish family with EOAD. Over time, additional pathogenic missense mutations have been discovered exclusively in APP exons 16 and 17 (Figure 3) (Table 1) (see also http://www.alzforum.org/members/resources/app/mutations/app_table.html). At the time of this writing, 13 APP mutations lead to the EOAD phenotype (with or without CAA), whereas 2 mutations result exclusively in a CAA phenotype without AD-type dementia.

The Normal Processing and Function of APP

Right after the discovery of the first APP mutations, evidence about the genotype-to-phenotype mechanisms of such mutations emerged. To understand these mechanisms, we must first review the physiology and processing of the precursor.

APP comprises a group of ubiquitously expressed polypeptides whose heterogeneity arises from both alternative splicing and complex posttranslational processing (Figure 2) [reviewed in (109)]. Deletion of the APP gene in mice results in neither early mortality nor appreciable morbidity; cerebral gliosis and changes in locomotor behavior are observed in adulthood (149), and neurons cultured at birth have diminished viability and retarded neurite outgrowth (91). The rather-benign phenotype of APP(−/−) mice is assumed to relate to the continued expression of the homologous family members, APLP 1 and 2. Indeed,
Figure 2  Schematic diagrams of the β-amyloid precursor protein (APP) and its principal metabolic derivatives. The upper diagram depicts the largest of the known APP 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 (TM) at amino acids 700–723 is indicated by the vertical dotted lines. The amyloid β-protein (Aβ) fragment includes 28 residues just outside the membrane plus the first 12–14 residues of the TM domain. In the middle diagram, the arrow indicates the site (after residue 687) of a constitutive proteolytic cleavage made by protease(s) designated α-secretase that enables secretion of the large, soluble ectodomain of APP (APP\(\alpha\)) into the medium and retention of the 83-residue C-terminal fragment (CTF) (C83) in the membrane. C83 can undergo cleavage by the protease called γ-secretase at residue 711 or residue 713 to release the p3 peptides. The lower diagram depicts the alternative proteolytic cleavage after residue 671 by β-secretase that results in the secretion of the slightly truncated APP\(\beta\) molecule and the retention of a 99-residue CTF. C99 can also undergo cleavage by γ-secretase to release the Aβ peptides. Cleavage of both C83 and C99 by γ-secretase releases the APP intracellular domain (AICD) into the cytoplasm.

APLP2\((−/−)/\text{APP}(−/−)\) mice and APLP2\((−/−)/\text{APLP1}(−/−)\) mice have a lethal phenotype (postnatal day 1), whereas APLP1\((−/−)/\text{APP}(−/−)\) mice are apparently normal (48). These results suggest that APLP2 has the key physiological role among the family members. In accord, mice expressing just a single APLP2 allele \([\text{APLP2}(+/−)/\text{APLP1}(−/−)/\text{APP}(−/−)]\) show perinatal lethality. Deletion of the APP-like gene (Appl) in Drosophila yields a viable fly exhibiting subtle behavioral deficits that can be rescued by the human APP gene (78).

Although a number of activities of holoAPP or its major secreted derivative, APP\(\alpha\), have been inferred on the basis of cell culture studies (109), the specific
### TABLE 1  Missense mutations in the APP gene

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Clinical phenotype</th>
<th>Neuropathology</th>
<th>Biochemistry</th>
<th>AOO (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K/M670/1N/L (Swedish)</td>
<td>AD</td>
<td>typical AD</td>
<td>↑Aβ</td>
<td>44–61</td>
</tr>
<tr>
<td>A692G (Flemish)</td>
<td>AD; CH</td>
<td>CAA, SP, NFT</td>
<td>↑Aβ&lt;sup&gt;N&lt;/sup&gt;; ̸=fib</td>
<td>40–60</td>
</tr>
<tr>
<td>E693Q (Dutch)</td>
<td>HCHWA-D; dementia</td>
<td>CAA, DP</td>
<td>↑Aβ fib; toxic&lt;sup&gt;*&lt;/sup&gt;</td>
<td>45–60</td>
</tr>
<tr>
<td>E693K (Italian)</td>
<td>HCHWA-I</td>
<td>CAA, DP&lt;sup&gt;θ&lt;/sup&gt;</td>
<td≯=fib&lt;sup&gt;θ&lt;/sup&gt;; ↓Aβ</td>
<td>60–70</td>
</tr>
<tr>
<td>E693G (Arctic)</td>
<td>AD</td>
<td>typical AD</td>
<td>↑Aβ PF; ↓Aβ</td>
<td>54–61</td>
</tr>
<tr>
<td>D694N (Iowa)</td>
<td>Dementia; CAA</td>
<td>CAA, SP&lt;sup&gt;θ&lt;/sup&gt;, NFT</td>
<td>↑Aβ fib; toxic&lt;sup&gt;*&lt;/sup&gt;</td>
<td>50s–60s</td>
</tr>
<tr>
<td>T714I (Austrian)</td>
<td>AD</td>
<td>DP&lt;sup&gt;θ&lt;/sup&gt;</td>
<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
<td>~34</td>
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<tr>
<td>V715M (French)</td>
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<td>NA</td>
<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
<td>40–60</td>
</tr>
<tr>
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<td>NA</td>
<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
<td>NA</td>
</tr>
<tr>
<td>I716V (Florida)</td>
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<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
<td>~53</td>
</tr>
<tr>
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<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
<td>39–60</td>
</tr>
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<td>V717F (Indiana)</td>
<td>AD</td>
<td>typical AD</td>
<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
<td>~43</td>
</tr>
<tr>
<td>V717G</td>
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<td>typical AD</td>
<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
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</tr>
<tr>
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<td>NA</td>
<td>↑Aβ&lt;sub&gt;42/40&lt;/sub&gt; ratio</td>
<td>56</td>
</tr>
</tbody>
</table>

Aβ<sup>θ</sup>, amino-terminally truncated Aβ species; AOO, age of onset; CAA, congophilic amyloid angiopathy; CH, cerebral hemorrhage; DP, diffuse plaques; DP<sup>θ</sup>, thioflavin-S negative DP; DP<sup>θ</sup>, DP-containing N-terminally truncated Aβ42 but no Aβ40; fib, fibrillogenesis; ̸=fib, altered fibrillogenesis; ̸=fib<sup>θ</sup>, ̸=fib with short straight fibrils; HCHWA, hereditary cerebral hemorrhage with amyloid (a stroke syndrome); LB, sometimes with Lewy Bodies; NA, not available; PF, protofibril formation; SP, senile plaques; SP<sup>θ</sup>, very large SP; SP<sup>θ</sup>, SP-containing-only Aβ40; toxic<sup>*</sup>, toxic to vascular cells; ↑, increased or enhanced; ↓, decreased.

Function(s) of the molecule in vivo remain undefined. However, the remarkably similar processing of APP and the Notch family of cell-surface receptors (see below) has increasingly suggested that APP functions as a receptor whose sequential proteolysis releases a cytoplasmic fragment that enters the nucleus (with associated proteins, e.g., Fe65) and regulates transcription (16, 57, 99). Both the hypothetical ligand that binds the APP ectodomain and the target genes that may be activated or suppressed remain unknown. No evidence that a fundamental cellular function of APP is missing in AD patients has emerged; instead, APP mutations appear to act by a “toxic gain-of-function” mechanism, namely, the increased production of the potentially cytotoxic Aβ fragment.

Both during and after its trafficking through the secretory pathway to the cell surface, a subset of APP molecules undergoes specific endoproteolytic cleavages,
Figure 3  APP mutations genetically linked to familial Alzheimer’s disease or related disorders. The sequence within APP that contains the Aβ and transmembrane (TM) regions is expanded. The underlined residues represent the Aβ1–42 peptide. The vertical broken lines indicate the location of the TM domain. The bold letters below the wild-type sequence indicate the currently known missense mutations identified in certain patients with familial Alzheimer’s disease and/or hereditary cerebral hemorrhage with amyloidosis (see Table 1). Three-digit numbers refer to the codon number according to the βAPP770 isoform.

most frequently a scission between amino acids 16 and 17 of the Aβ region, i.e., 12 residues N-terminal to the TM sequence (Figure 2). This principal secretory cleavage is affected by a protease(s) designated α-secretase(s). The cut creates a large, soluble ectodomain fragment (APP-α), which is released into vesicle lumens and from the cell surface, and a membrane- retained CTF of 83 amino acids (C83). α-Secretase(s) are membrane-anchored proteases capable of cleaving diverse single TM proteins, and they appear to cleave APP at a specific distance from the outer membrane surface while showing little sequence specificity (112). Both ADAM 17 (TNF-α converting enzyme) and ADAM 10 may be capable of acting as α-secretases for APP (15, 60).

Aβ is secreted constitutively by normal cells in culture and detected as a circulating peptide in the plasma and cerebrospinal fluid (CSF) of healthy humans and other mammals (45). When this unexpected observation was made, it was also recognized that a smaller 3-kDa fragment (designated p3) comprising the latter two thirds of Aβ was constitutively released by APP-expressing cells during normal metabolism (45) (Figure 2). In an analogous fashion, other APP holoproteins are instead cleaved by β-secretase just before the Aβ region to create its amino terminus, followed by cleavage of the resultant 99-residue CTF (C99) by γ-secretase to create Aβ (Figure 2). Although precise quantitation is not available, it appears that a substantially smaller portion of total cellular APP undergoes cleavage by β- rather
than $\alpha$-secretase. Moreover, not all of the resultant C99 and C83 fragments are processed by $\gamma$-secretase to Aβ and p3, respectively; alternative proteolytic pathways can fully degrade these CTFs, probably in late endosomes and lysosomes.

Aβ peptides present in culture medium, human CSF, and the brain amyloid deposits of AD subjects show both N- and C-terminal heterogeneity. The C-terminal heterogeneity of Aβ has special importance for its aggregation. Immunohistochemistry with antibodies that selectively recognize either the valine40 or the alanine42 C-terminus have revealed that the first Aβ form deposited as diffuse plaques in AD and Down’s syndrome brains ends at residue 42 (54). In studies of the temporal progression of plaque formation in Down’s syndrome brains of increasing age, Aβ42 peptides can form numerous diffuse plaques as early as age 12, whereas Aβ40 is first detected in plaques almost two decades later (67). This evidence of initial Aβ42 deposition in AD and Down’s syndrome brains fits well with biochemical studies (55) showing that the Aβ42 peptide, with its two additional hydrophobic residues (Ile and Ala), aggregates far more rapidly into amyloid fibrils, [as well as into intermediate assemblies called protofibrils (46, 135)], than does the Aβ40 peptide.

APP Missense Mutations Enhance the Amyloidogenic Properties of Aβ

The concept that missense mutations in APP cause AD via an amyloidogenic mechanism is strongly supported by the fact that all of the known mutations occur at or near the $\beta$- or $\gamma$-secretase cleavage sites or else in the middle of Aβ, near the $\alpha$-secretase site (Figure 3). No AD-linked mutations have been discovered in any other region of this 770-residue polypeptide. The “Swedish” double mutation found at the P2 and P1 sites for $\beta$-secretase causes more efficient processing of APP by this aspartyl protease, resulting in a several-fold increase in the production of both Aβ40 and Aβ42 (18). On the other hand, the several mutations located C-terminal to the $\gamma$-secretase site increase the ratio of peptides ending at Aβ42 to those ending at Aβ40 (27, 104, 123) (Table 1). Because the hydrophobic Aβ42 peptide is more prone to self-assembly (55), increases in its relative amount can promote the formation of oligomeric intermediates and, ultimately, mature amyloid fibrils. In this regard, there is growing evidence that soluble, diffusible oligomers are the principal biologically active species capable of inducing neurotoxicity (47, 65, 134), whereas insoluble fibrils are relatively inert but constitute a reservoir for smaller, potentially toxic oligomers.

Among the mutations located near the middle of the Aβ sequence, it is possible that some may decrease the efficiency of $\alpha$-secretase cleavage and thus allow relative increases in cleavages by $\beta$-secretase (44). However, there is more evidence that these internal mutations alter the aggregation propensity of the peptide (35, 81, 89, 132) and thus lead to cytopathology (Table 1). Among the mutations associated with prominent microvascular deposition, the E22Q (“Dutch”) peptide aggregates on the surfaces of smooth muscle and endothelial cells and thus
confers local toxicity that could lead to necrosis and vessel rupture (32, 132, 136). It should be noted that all patients with APP mutations are heterozygotes. In accord, microvascular Aβ deposits contain mixtures of peptides derived from the mutant and wild-type alleles.

PRESENILINS 1 AND 2: THE SEARCH FOR AD GENES UNMASKS A MAJOR BIOLOGICAL SWITCH

The discovery of mutations in APP that explain a small fraction of EOAD cases was preceded by the realization that inherited forms of AD were genetically heterogeneous (101, 115). About the time that APP mutations were first observed, genome-wide linkage analyses suggested the presence of an EOAD locus on chromosome 14q (102). Further linkage studies and positional cloning led to the identification in 1995 of a novel gene at 14q24.3 that was initially dubbed S182 (111). It encoded a 467–amino acid protein containing six to eight putative TM segments. The initial report identified five missense mutations that segregated with EOAD in a dominantly transmitted fashion. Shortly thereafter, a highly homologous gene on Ch1q31-42 was discovered to be the site of a missense mutation in a large, American EOAD kindred descended from a Volga German population (72, 73, 97). In view of their considerable homology (∼60%) and the early age of disease onset in mutation carriers, the two genes were renamed presenilin 1 (PS1) and presenilin 2 (PS2). It soon became apparent that missense mutations in PS1 might underlie a sizable fraction of familial EOAD cases. At this writing, more than 80 mutations associated with EOAD have been reported in PS1, and at least 6 have been found in PS2 (see http://www.alzforum.org/members/resources/pres_mutations/index.html). All but two of these mutations are missense. One PS1 mutation (S290C) also changes a splice acceptor site and leads to the deletion of exon 9 in the proximal part of the large cytoplasmic loop, but it is the missense mutation that is responsible for the increased Aβ production (118). PS1 mutations cause the earliest and most-aggressive form of AD, commonly leading to onset of symptoms in the fifth decade of life and demise of the patient in the sixth. PS2 mutations generally have a slightly later and more-variable age of onset. The effort to understand exactly how these mutations cause the AD phenotype has uncovered a novel and fascinating mechanism of membrane protein processing in metazoans.

Presenilin Mutations Increase the Production and Oligomerization of Aβ_{42} Peptides

When PS1 and PS2 were first cloned, the manner in which missense mutations caused the AD phenotype was an open question and was not necessarily expected to directly involve the production of Aβ. But assays of Aβ_{40} and Aβ_{42} in the plasma and cultured skin fibroblast media of humans harboring these mutations soon revealed a selective elevation of Aβ_{42} levels (104).
Subsequent modeling of these mutations in transfected cells and transgenic mice has strongly confirmed this finding [e.g., (11, 19, 30)]. It is important to note that the crossing of APP transgenic mice with mice expressing a PS1 missense mutation leads to increased cerebral Aβ42 levels and an accelerated AD-like neuropathological phenotype in the offspring, with Aβ42 plaques (first diffuse and then mature) occurring as early as 3–4 months of age, versus at ~6–8 months in APP monotransgenics (10, 49). Moreover, direct image analysis of human brain amyloid deposits using Aβ42- and Aβ40-specific antibodies shows that PS mutations lead to a ~1.5–3-fold increase in the relative abundance of plaques containing Aβ42 peptides, compared to the levels of such plaques in sporadic AD cases (68). The increased Aβ42 production seen in cells transfected with AD-causing mutant forms of PS1 or PS2 results in enhanced oligomerization of Aβ42 into stable dimers, trimers, and tetramers (143). Such oligomers have recently been shown to alter hippocampal synaptic plasticity in vivo (134). The molecular mechanism by which PS missense mutations alter the γ-secretase cleavage of C99 to yield more peptides ending at Aβ42 versus Aβ40 is discussed after reviewing current knowledge about the complex biology of the presenilins.

Cell Biology of the Presenilins

Shortly after the PS1 and PS2 genes were cloned, two critical observations about their biology were made. First, the presenilin holoproteins (~45 kDa) were found to undergo constitutive endoproteolysis in many cell types and in the brain and thus exist as stable heterodimers comprising an N-terminal fragment (NTF) and a CTF (125). The very low levels of the PS holoprotein in cells and tissues, its apparent rapid conversion into NTF and CTF within ER vesicles, and the stabilization of these fragments in the Golgi (148) all suggest that the fragments are the principal functional form of presenilins. The constitutive endoproteolytic cleavage site (92, 119) occurs within a hydrophobic portion of the large cytoplasmic loop between the sixth and seventh of the eight putative TM domains (75). Steady-state levels of presenilin NTFs and CTFs are tightly regulated, as overexpression of PS1 in transfected cells or transgenic mice does not significantly increase the total amount of PS fragments; rather, the exogenous fragments appear to “replace” the endogenous fragments (126). Excess PS holoproteins are rapidly degraded, mainly by the proteasome (56, 116). Once formed, PS heterodimers can associate into higher molecular mass (~100–180 kDa) complexes that may represent the principal form in which presenilin functions in cells (17, 147).

The second major observation was the identification of the homolog of presenilin in Caenorhabditis elegans, a gene designated sel-12 (70). sel-12 was identified in genetic screens as a facilitator of the worm homologue of Notch, lin-12. The existence of mutations in sel-12 that decrease or eliminate its function has enabled the use of the nematode as a model system for studying the function of the human presenilins (69). Other transmembrane proteins that interact genetically with sel-12 have been identified in C. elegans, including APH-2 (41a) and APH-1.
(41b) (APH, anterior pharynx defective). The mammalian homolog of APH-2 is Nicastrin, which interacts with APP and modifies the $\gamma$-secretase cleavage (147a).

In addition, the use of the yeast two-hybrid system has led to identification of several novel or known mammalian proteins that appear to interact with presenilin. Prominent among these are members of the Armadillo family, called the catenins, including an apparent neuron-specific member of this family, designated $\alpha$-catenin (150). Both $\beta$- and $\beta$-catenins coimmunoprecipitate with PS1. The catenin-binding site appears to be in the distal portion of the cytoplasmic loop between TM domains 6 and 7. This region appears to be dispensable for the function of presenilin in the $\gamma$-secretase mechanism (i.e., in A\textsubscript{B} generation) (59); therefore, the interaction with the catenins may not have pathogenic relevance to AD. Furthermore, the fact that mutations of residues conserved in PS1 and PS2 can elevate $A\textsubscript{B}42$ production and are linked to familial AD suggests that sequences that diverge between the two homologues (such as the region of the PS1 loop that binds the catenins) are less likely to be required for the stabilization of PS heterodimers and for their AD-promoting activity than are highly conserved sequences, such as their C-termini (129). Indeed, the latter site is a good candidate for the binding of the currently unknown cellular factors that regulate PS endoproteolysis and stabilize the heterodimers. Presenilins have also been shown to participate in multiprotein complexes at the cell surface that include the cadherins, important molecules mediating cell-cell adhesion (36).

The loss of function of PS1 produced by deleting its gene in mice leads to markedly abnormal somitogenesis and axial skeletal development, with shortened body length and cerebral hemorrhages (110, 141). In addition, these mice, which die just prior to or at birth, show abnormal embryonic neurodevelopment in the forebrain marked by premature loss of neuronal precursors (110). Deletion of just one PS1 gene in the mouse (PS1$^{+/−}$) has not been associated with notable developmental abnormalities. An important functional insight has been gained by complementation studies in PS1$^{−/−}$ mice. Crossing PS1$^{+/−}$ mice with mice transgenic for AD-causing mutant PS leads to some offspring that have no endogenous (mouse) presenilin but express the mutant human form. Such mice survive and do not have the devastating phenotype found in PS1$^{−/−}$ mice, although they may have subtle abnormalities (25, 94). Therefore, missense mutations in the human presenilins that cause EOAD appear to act as gain- rather than loss-of-function mutations.

**Presenilins and the $\gamma$-Secretase Cleavage of APP**

PS1$^{+/−}$ mice are also critical for deciphering the role of presenilins in APP metabolism. Such mice show normal levels of APP holoproteins and processing by $\alpha$- and $\beta$-secretases but grossly abnormal $\gamma$-secretase function (29). Neurons cultured from these mice accumulate high levels of the $\gamma$-secretase APP substrates, C83 and C99. There is a corresponding substantial decrease ($\sim$70%) in the production of both $A\textsubscript{B}40$ and $A\textsubscript{B}42$ (29). This evidence that PS1 plays a required role in the $\gamma$-secretase mechanism has received substantial support from several
types of experiments. Even prior to the realization that presenilin is necessary for proper \(\gamma\)-secretase cleavage of APP, PS was shown bind to and immunoprecipitate with full-length APP molecules in several cell types (137, 145). This interaction does not require the cytoplasmic tail of APP (145). Because presenilins have very small ectodomain loops, it was unlikely that presenilin and APP would interact via their respective ectodomains. This left the TM domains as the likely site of interaction, a hypothesis that has recently received direct experimental support (1). However, the initial evidence for co-immunoprecipitation of presenilin and APP was challenged by investigators who observed no such interaction (127). From this controversy arose two broad hypotheses for the mechanism of PS in \(\gamma\)-secretase-mediated APP processing. The first, based on the ability to co-precipitate the proteins, suggested that PS participates directly in the \(\gamma\)-secretase mechanism, i.e., is part of the catalytic complex, presumably as a co-factor (145). The alternate hypothesis argued that PS and APP do not physically interact; rather, PS regulates the membrane trafficking of certain proteins, including the components of the \(\gamma\)-secretase reaction (the protease and/or APP), in a way that allows them to come together (85, 127). In the authors’ laboratory, repeated confirmation of the PS-APP interaction, their co-localization to the same vesicle fractions, and the lack of altered subcellular distribution of C83 and C99 (the \(\gamma\)-secretase substrates) in PS1\(^{-/-}\) mice (144, 148) suggested that PS participated physically in \(\gamma\)-secretase cleavage rather than indirectly via a membrane-trafficking function.

While this biochemical evidence was accruing, the study of peptidomimetic transition state analogs designed to inhibit the unknown \(\gamma\)-secretase showed that certain difluoroalcohol-containing compounds mimicking the A\(\beta_{40-45}\) region decreased A\(\beta\) production and raised cellular levels of C83 and C99 (138, 139). The chemical structure of the inhibitory moiety strongly predicted that \(\gamma\)-secretase was an aspartyl protease (139). This concept was supported by evidence that the cellular generation of A\(\beta\) appeared to require a mildly acidic pH. Indeed, cathepsin D, a well-characterized aspartyl protease, had been considered a candidate for \(\gamma\)-secretase, until it was shown that deletion of its gene in mice did not alter A\(\beta\) production.

The combined findings that PS was physically inseparable from the \(\gamma\)-secretase reaction and that \(\gamma\)-secretase had the properties of an aspartyl protease led to the observation of two unusual intramembranous aspartates near the middle of the predicted TM6 and TM7 domains of all presenilins (140). Mutation of either of these conserved aspartates in PS1 to alanine or glutamate revealed that the mutant holoprotein could no longer undergo endoproteolysis, signifying an essential role for both intramembranous aspartates in this reaction. Furthermore, mutation of either TM aspartate markedly reduced A\(\beta_{40}\) and A\(\beta_{42}\) production and elevated the C83 and C99 substrates, in a fashion essentially indistinguishable from the effects of deleting the PS1 gene (29). When these two phenomena (abrogation of PS1 endoproteolysis and \(\gamma\)-secretase cleavage) were examined together by placing an aspartate-to-alanine mutation in the natural variant of presenilin that lacks exon 9
(and therefore the site of PS1 endoproteolysis), this mutant holoprotein still prevented γ-secretase processing of APP (140). This result indicates that, even in a presenilin isoform that cannot undergo endoproteolysis, the TM aspartates are still required for proper γ-secretase processing of C83 and C99. Furthermore, expression of either wild-type or aspartate-mutant PS1 in microsomes showed that the former allowed de novo Aβ generation from C99, whereas the latter did not, and Aβ generation in the presence of the former occurred at mildly acidic, not neutral, pH (140).

The interpretation of the findings just summarized has led to some controversy. One interpretation is that presenilin is required as a “diaspartyl” co-factor for γ-secretase and that mutation of either aspartate prevents that function. The alternate interpretation is that the two PS aspartates actually constitute the active site of γ-secretase, a novel intramembranous aspartyl protease activated by autoproteolysis (139). Recent evidence consistent with either hypothesis has come from subcellular fractionation experiments in which C83 and C99, the substrates of γ-secretase, co-precipitated with PS heterodimers in Golgi- and TGN-like membrane vesicles, whereas the APP holoprotein co-precipitated with PS in an earlier, ER-rich vesicular compartment (142). These data confirm a direct interaction of the APP γ-secretase substrates with PS. Furthermore, the vesicles containing such complexes have substantial steady-state levels of Aβ when PS is wild type but not when it contains the TM aspartate mutations, and new Aβ can be generated in a cell-free reaction from the former but not the latter vesicles (142). It is interesting to note that simultaneous expression of TM aspartate-to-alanine mutations in both PS1 and PS2 drops cellular Aβ production to undetectable levels, suggesting an absolute requirement for functional presenilins (and their TM aspartates in particular) to generate Aβ (58).

At present, absolute resolution of whether presenilin serves either as γ-secretase or as a necessary co-factor has not been achieved. However, the availability of γ-secretase inhibitors—either rationally designed or emerging from high-throughput screens—has allowed biochemical identification of their cognate cellular target and is helping to resolve this issue. Active-site-directed compounds bind selectively and specifically to PS heterodimers (33, 76). This finding is tantamount to proving that presenilin contains the active site of γ-secretase. Although it remains formally possible that an unidentified protease is so intimately associated with PS that the inhibitors can bind its active site while simultaneously binding to PS, the fact that some of these inhibitors are aspartyl protease transition-state mimics that closely resemble the substrate (the Aβ region of APP) (33) makes it highly likely that the protein to which the inhibitor binds (PS) is the actual aspartyl protease. Additional support for this conclusion has come from the discovery that presenilins share a motif around one of their critical aspartates (D385) with a known family of bacterial aspartyl proteases (117). Although PS may thus represent the active-site component of γ-secretase, recent evidence indicates that additional membrane proteins are required for activity, including nicastrin/APH-2 and APH-1 (see 33a, 60a).
Presenilins as Key Mediators of Notch Signaling

As this work on the role of PS in APP processing was unfolding, further analyses of presenilin/sel-12 function in Drosophila, C. elegans, and mouse led to the crucial insight that presenilin was absolutely required for proper Notch signaling (28, 122, 146). Signal transduction mediated by the cell-surface receptors Notch in Drosophila and lin-12 and glp-1 in C. elegans are essential for a large variety of cell-fate decisions during development [reviewed in (3)]. The importance of cell-cell interactions controlled by the lin-12/Notch pathway for proper development of vertebrates and invertebrates is clear from many genetic analyses, but the biochemical mechanism by which these receptors transmit surface signals to the nucleus to alter expression of various downstream genes has been poorly understood. One model for signal transduction by ligand-activated Notch receptors involved proteolytic processing of this type 1 membrane protein to release its intracellular domain (NICD) to the nucleus (61). Mutation of an amino acid at the putative cleavage site, located within the predicted TM domain of Notch, markedly decreased Notch signaling in vitro and in vivo, thus linking the intramembrane proteolysis of Notch with its function in activating transcription of nuclear genes (52, 106). Very small amounts of NICD appear to reach the nucleus, making this fragment difficult to detect immunochemically (106). Either during or after nuclear entry of NICD, it can bind to and activate members of the CSL (CBF-1, Su(H), and Lag-1) family of downstream Notch effectors.

Compelling evidence that presenilin is an essential participant in this cleavage event has arisen from studies in flies and mammalian cells (Figure 4). Lethal loss-of-function mutations in the Drosophila presenilin abolish Notch signaling by preventing NICD from being released to the nucleus (122). The PS null mutations produce a somatic and neural phenotype in fly that is highly similar to that of flies lacking functional Notch. Moreover, mouse cells devoid of PS1 undergo markedly decreased proteolytic release of NICD from a Notch construct (28, 113). A series of peptidomimetic compounds designed to inhibit the γ-secretase processing of APP (139) show the same rank order of potency in inhibiting the intramembranous cleavage of Notch (28). Moreover, the PS aspartate mutations, which block γ-secretase cleavage of C83 and C99, also inhibit the release of NICD and its translocation to the nucleus (6, 95). Therefore, presenilin serves either as an essential co-factor in the γ-secretase cleavage of Notch or, more likely, as the protease itself (Figure 4). It appears that Notch and PS can interact at or close to the cell surface because biotinylation of each protein has been observed and biotinylated Notch can be recovered by immunoprecipitating PS heterodimers (95). Although some studies conclude that PS1 is essentially restricted to the cis-Golgi and earlier compartments (2, 24, 62), there is now compelling evidence that PS heterodimers can reach the cell surface (36, 63, 107), where they mediate the processing of Notch, APP, APLPs, ErbB4 (88), and, perhaps, numerous other type 1 membrane proteins (121). The PS-mediated cleavage of substrates within the phospholipid bilayer has provided one of the first and clearest examples of the
phenomenon of regulated intramembrane proteolysis (RIP) (13). Thus, research originally intended to identify AD genes has uncovered a novel and far-reaching mechanism in protein biology.

The exciting progress in Notch and presenilin biology allows one to place the emerging public health catastrophe of AD into a new perspective. It appears that the principal function of the presenilins is to mediate the final proteolytic cleavage that enables Notch signaling, thereby conferring great developmental advantage during the evolution of multicellular organisms. However, the survival of large numbers of humans far beyond reproductive age due to advances such as antibiotics has increasingly permitted a kinetically less-favored substrate of this reaction (APP) to be converted to a highly stable and self-aggregating product (Aβ42) that can lead to progressive neurodegeneration. Recent quantitative comparisons of Notch and APP as substrates of presenilin support this hypothesis (5). We speculate that partial loss-of-function mutations in human PS1 may exist that decrease the efficiency of the processing of APP to Aβ42, just as AD-causing gain-of-function mutations increase it. Such mutations might ultimately be discovered in very old humans who show little or no age-related Aβ accumulation and have thus escaped AD. It would be particularly interesting to search for such “escapees” among centenarians who carry one or two ApoE4 alleles but nevertheless show no clinical or neuropathological evidence of AD.

APOLIPOPROTEIN E: A POLYMORPHIC RISK FACTOR FOR AD HELPS REGULATE CEREBRAL Aβ LEVELS

The discovery that inheritance of the ε4 allele of apolipoprotein E predisposes to AD provided the first genetic risk factor for the disorder in the typical late-onset period. A biochemical screen for proteins in CSF that bind synthetic Aβ led to the identification of ApoE as such a protein and to the subsequent recognition that its gene was in a region of chromosome 19q previously linked to AD in some late-onset families (120). Even before this study, ApoE immunoreactivity had been reported in a high percentage of Aβ deposits in typical AD brains (84). Further association analyses soon demonstrated that the ε4 allele of ApoE is substantially overrepresented in AD patients. Inheritance of one or two ε4 alleles heightens the likelihood of developing AD and results in an earlier mean age of onset than occurs in subjects harboring only ε2 and ε3 alleles (22). ApoE4 appears to precipitate the disorder primarily in subjects in their 60s and 70s and may be capable of accelerating the age of onset by as much as 15 years (26). Inheritance of a single ε4 allele has been variably estimated to increase the likelihood of developing AD by 2–5-fold or more, and two E4 alleles may increase risk by 5–10-fold or more (8). Conversely, inheritance of the ε2 allele has been found to confer protection against the development of AD by lowering the risk and increasing the age of onset (21, 26). Nonetheless, ApoE4 is a risk factor for, not an invariant cause of, AD. Some humans homozygous for the E4 isoform show no Alzheimer symptoms...
in their ninth decade of life and beyond. Conversely, many humans develop AD without harboring E4 alleles. The recognition that inheritance of ε4 predisposes to AD provided one of the first polymorphic genetic risk factors for a common late-onset disease.

After the genetic connection between AD and ε4 was made, immunohistochemical studies showed that inheritance of this allele was associated with a significantly higher Aβ plaque burden than occurred in patients lacking it (96, 105). Although some brains of ApoE4 allele carriers showed higher neurofibrillary tangle densities, this change did not always reach the statistically significant level of elevation invariably observed for Aβ deposits. It is important to note that, in aged humans who died without symptoms of AD, ApoE4 genotype was still associated with enhanced amounts of diffuse Aβ12 plaques in the brain, suggesting that the Aβ-elevating effects can be observed presymptomatically as well as in hosts who may not develop AD (7). Of interest, polymorphisms in the promoter region of the ApoE gene have also been associated in some studies with an increased risk for AD.

The biochemical mechanism by which the presence of the ApoE4 protein leads to increased Aβ deposition has been difficult to pinpoint. Humans bearing ApoE4 alleles show no evidence of a general increase in Aβ production, and co-expression of APP with each of the human ApoE isoforms causes no detectable change in cellular Aβ generation (9). Instead, human neuropathological analyses and mouse modeling suggest that inheritance of ApoE4 leads to a rise in the steady-state levels of Aβ in the brain, presumably by enhancing its fibrillogenic potential and/or decreasing its clearance from the brain’s extracellular space. In vitro studies quantifying synthetic Aβ fibrillogenesis suggest that the addition of purified ApoE4 protein results in more fibrils, compared to levels obtained in the presence of ApoE3 (34, 79). In this paradigm, ApoE4 may be serving as a less-effective inhibitor of Aβ fibrillogenesis rather as a more-potent stimulator (34). An alternative mechanism for the AD-promoting effect of ApoE4 inheritance emerges from studies of the neuronal effects of either the E4 or the E3 human protein. The neurons of mice expressing E4 show decreased neuritic outgrowth and decreased maintenance of established neurites (14, 133). Related results have been obtained with ApoE in culture (86) and suggest that ApoE4 protein is less supportive of normal neuronal form and function than are ApoE3 or E2 proteins. However, this enhanced neuronal vulnerability may not be responsible for the ApoE4 effect in AD because Aβ deposition in meningeal blood vessels (i.e., CAA) is also strongly increased by E4 gene dosage, even in the absence of Alzheimer-type neuropathology and clinical AD (42). The fact that ApoE4 enhances Aβ deposition not only in cerebral plaques but also in microvessels outside the brain parenchyma—and in the absence of AD—potentially separates the ApoE4 effect of promoting the AD amyloid phenotype (i.e., more CAA and plaques) from any deleterious effects ApoE4 might have on neuronal/neuritic vulnerability. In other words, the most-parsimonious explanation for the ApoE4 effect in AD is that this isoform acts to decrease Aβ clearance and/or enhance its aggregation in both the cerebral cortex and the brain’s microvasculature.
Such a mechanism is supported by studies in which mice transgenic for mutant human APP are crossed with mice in which the endogenous ApoE gene is deleted. The resultant offspring show substantially decreased Aβ burden compared to that seen in the parental APP line, and the Aβ deposits that develop are overwhelmingly diffuse (nonfibrillar) (4). Moreover, mice lacking endogenous ApoE that transgenically express human ApoE3 or E4 (plus human APP) initially show even less Aβ deposits than mice expressing no ApoE at all (51). But as they develop deposits with age, the presence of the E4 isoform leads to far more fibrillar (neuritic) plaques than does the E3 isofrom (50).

An important caveat about in vitro studies attempting to elucidate the mechanisms by which the ApoE proteins induce such effects is that they need always to be conducted in the presence of lipid, i.e., where ApoE is assembled into lipoprotein particles. There is currently no evidence that any significant portion of ApoE proteins occurs as free polypeptides in brain or other tissues. As a result, early studies examining the effects of pure ApoE on Aβ in vitro are difficult to interpret. Further in vitro and in vivo experiments will clarify whether ApoE4 increases Aβ steady-state levels in the brain by less efficiently preventing its aggregation, inhibiting its re-uptake into cells, slowing its proteolytic degradation, or other effects on its clearance.

THE SEARCH FOR NEW GENES IN LATE-ONSET AD

The four known genes we review in detail above are variably estimated to account for 20%–50% of the total genetic variance (heritability) of AD. ApoE appears to account for most of this fraction, as APP and PS mutations are rare. Although as many as 40% of all AD cases carry at least one apoE4 allele (100), one cannot assume that this allele is solely responsible for precipitating the disorder in all these carriers, as even homozygosity for apoE4 does not necessarily result in clinical AD. Therefore, one or more additional major genes and perhaps several minor ones are likely to contribute to the total heritability of the disease. In this regard, an oligogenic segregation analysis of 75 LOAD families (742 individuals) using the Monte Carlo Markov chain method estimated that four unidentified loci make a contribution to the variance in age of onset that is similar or greater in magnitude than that of ApoE (26). One of these hypothetical loci alone accounted for >50% of onset age variation, exceeding the effect attributable to ApoE4 (~9% in this study) by sixfold.

The search for new genes is appropriately focused on LOAD, where the vast majority of cases occur. As in other genetically complex and heterogeneous disorders, progress has arisen from a combination of two broad approaches: genetic linkage studies and analyses of genetic association/linkage disequilibrium. The latter approach includes both typical case-control studies, which compare genetic polymorphisms in unrelated cases and controls, and family-based association methods, in which cases and controls come from the same family [reviewed in (8)].
At this writing, five genome-wide scans in LOAD have been completed. The only chromosomal region that showed clear evidence of linkage to AD in all of the studies was the region around ApoE on chromosome 19. However, at least four other chromosomal regions were implicated in two or more of the studies, and these include regions on chromosomes 12, 10, 9, and 6 [reviewed in (83)]. Some of these regions have been positive in subsequent focused linkage studies and association analyses, supporting the presence of LOAD genes. We briefly review the evidence for and against several candidate genes located within or near these linkage regions that have become the object of particularly intensive scrutiny.

Chromosome 12: α2M, LRP, and CP2

α2-macroglobulin (α2M) and the low-density lipoprotein receptor-related protein (LRP) are two chromosome-12 gene products known to interact functionally. α2M is an abundant pan-protease inhibitor in serum that plays a role in the binding, clearance, and/or degradation of Aβ. LRP is a large cell-surface receptor that can bind and perhaps internalize several proteins implicated in AD, including ApoE, α2M, and the KPI-containing forms of APP [reviewed in (53)]. Thus, one or both of these candidate gene products could be involved in the normal clearance of extracellular Aβ in the brain. The two genes are located some 50 cM apart. Presumably, they are too widely spaced to be in linkage disequilibrium, but it remains unclear whether there are two separate LOAD genes on chromosome 12. Although some association studies examining haplotypes of multiple polymorphisms in each of these genes support their respective associations with AD [reviewed in (8)], there have also been a large number of negative association studies. The chromosome 12 gene encoding the transcription factor, CP2/LBP-1c/LSF (CP2), has recently been associated with AD, and it regulates the expression of numerous genes, including Fe65, α2M, and interleukin-1α (64). It lies just 6 cM proximal to LRP and may therefore be in linkage disequilibrium. Taken together, the available data point to at least one LOAD gene on chromosome 12, but its identity remains enigmatic.

Chromosome 10: IDE and uPA

Several linkage and association studies have provided evidence for the existence of one or possibly two LOAD genes on chromosome 10q [reviewed in (83)]. One region (~80 cM) came up not only in an AD sibling-pair genome scan, but also in a linkage study that used elevated plasma Aβ42 levels as a quantitative trait locus. A second region (~115–130 cM) was identified by both linkage and family-based association analyses that focused on insulin-degrading enzyme (IDE) as a candidate gene. IDE had previously emerged from an unbiased screen for Aβ-degrading proteases in cultured cells and degrades natural Aβ40 and Aβ42 produced by microglial cells and neurons [reviewed in (108)]. An intriguing finding is that another protease, urokinase-type plasminogen activator (uPA), is located within the first linkage region, and it has been implicated in Aβ degradation via the plasmin system (130). Thus, two loci for LOAD could conceivably exist on chromosome
10q, and they contain candidate genes whose dysfunction could elevate brain Aβ levels.

Chromosome 9

At present, three genome-wide screens have reported linkage of LOAD to anonymous markers on chromosome 9 [reviewed in (8)]. However, the two broad regions identified in these scans are widely separated (~30 and ~100 cM), and there has been little examination, so far, of positional candidate genes localized to these two regions. Thus, the situation on chromosome 9 is even less well resolved than those on Ch12 and 10.

Chromosome 6: TNFα

Three of the reported genome-wide scans have suggested linkage to chromosome 6 but to distinct regions that may or may not represent one broad linkage peak [reviewed in (83)]. A subsequent study reported linkage of several markers flanking the tumor necrosis factor-α (TNFα) gene in an apoE4-positive subset of LOAD families (20). TNFα is located close to one of the original linkage regions. This study also found an association of LOAD with haplotypes of several polymorphisms within the TNFα gene, using the sibling disequilibrium test. At least two other association studies have likewise implicated TNFα. As a multifunctional pro-inflammatory cytokine that also has effects on lipid metabolism and insulin resistance, TNFα could play a pathogenic role in the complex microglial and astrocytic responses to Aβ deposition that have been described in AD (98).

Some Other Candidate Genes

Among the numerous AD candidate genes that are on chromosomes other than those identified in the genome-wide screens, the interleukin-1 genes (IL-1A and IL-1B) are of particular interest. Several studies have now associated polymorphisms in regulatory regions of the IL-1A or IL-1B genes on chromosome 2q with EOAD [reviewed in (43)]. As such, this work provides the first evidence for genes potentially modifying susceptibility to, and age of onset of, EOAD. IL-1 is a pro-inflammatory cytokine that appears to be abundantly present in peri-plaque microglia in both EOAD and LOAD brains. The IL-1A and IL-1B genes are located in a cluster with two IL-1 receptors and certain IL-1 receptor antagonists. One can speculate that increased expression of IL-1α and IL-1β (and perhaps misregulation of other genes in the cluster) could accelerate the inflammatory cascade that accompanies Aβ accumulation, thus making clinical (symptomatic) AD appear earlier in life. In this manner, IL-1 polymorphisms could enhance susceptibility to both EOAD and LOAD, which are in any case distinguished by artificial age cutoffs. Indeed, a recent study reports a correlation between IL-1A alleles and the rate of cognitive decline in AD (82).
Finally, several association studies have examined the gene for cystatin C (chromosome 20p11.2) in AD, with both positive and negative results. This circulating inhibitor of extracellular cysteine proteases has been immunohistochemically localized to amyloid plaques in the brains of both AD patients and APP transgenic mice.

UNRAVELING THE MECHANISMS OF AD GENES POINTS TO A COMMON PATHOGENIC CASCADE

As in the case of the four confirmed genes, each new gene found to predispose to AD will need to be analyzed as to its effects on the production, deposition, clearance, or cytotoxicity of Aβ. We will thus learn how many genetic forms of the disorder involve an insidious rise in the steady-state levels of Aβ that could precipitate the inflammatory and neurodegenerative features of the syndrome (Figure 5). Although we have discussed some of the ways in which genetic alterations can chronically elevate Aβ concentrations, the putative downstream effects of this accumulation remain the subjects of intensive study and lively debate. It is widely assumed that the chronic elevation of Aβ42 in brain extracellular fluid and inside neurons caused by APP or presenilin mutations slowly leads to the oligomerization and, eventually, fibrilization of the peptide and its deposition as diffuse and, later, mature plaques. Based primarily on analyses of Down’s subjects and mice expressing mutant APP and/or PS1, it is hypothesized that Aβ42 accumulation and diffuse plaque formation is followed by local microglial activation, cytokine release, reactive astrogliosis, and a multiprotein inflammatory response, including the triggering of the classical complement cascade (31, 98). Such a glial-mediated inflammatory process as well as any direct neurotoxic effects of oligomeric Aβ could produce the multifaceted biochemical and structural changes in surrounding axons, dendrites, and neuronal cell bodies that characterize the limbic and association cortices in AD. Considerable evidence has accumulated that the effects of an Aβ-initiated inflammatory and neurotoxic process include excessive generation of free radicals and peroxidative injury to proteins and other macromolecules in neurons. Among the numerous possible metabolic consequences of Aβ accumulation and oligomerization, altered ionic homeostasis, particularly excessive calcium entry into neurons, may well contribute to selective neuronal dysfunction, tangle formation, and cell death [see, e.g., (77, 80)]. Establishing precisely how Aβ accumulation triggers neuronal changes that lead to the hyperphosphorylation of tau and its decreased binding to microtubules (40) must await further studies of transgenic mice that overexpress both mutant APP and mutant human tau, i.e., a “plaque plus tangle” phenotype (41, 74).

Although substantial evidence supports the Aβ-mediated cytopathological cascade summarized briefly in the preceding paragraph (Figure 5), many questions remain unanswered. First, what are the relative contributions of Aβ accumulation extracellularly versus intraneuronally in initiating the neurotoxic response?
A Hypothetical Sequence of the Pathogenetic Steps of Familial Forms of Alzheimer’s Disease

Missense mutations in APP, PS1, and PS2 genes

- Altered proteolysis of APP
- Increased production of Aβ42

Progressive accumulation and aggregation of Aβ42 in brain interstitial fluid
- Deposition of aggregated Aβ42 as diffuse plaques (in association with proteoglycans and other amyloid-promoting substrates)
- Aggregation of Aβ40 onto diffuse Aβ42 plaques
- Accumulation of certain plaque-associated proteins (complement c1q, etc.)

“Inflammatory” response:
- Microglial activation and cytokine release
- Astrocytosis and acute phase protein release

- Progressive neuritic injury within amyloid plaques and elsewhere in the neuropil

Disruption of neuronal metabolic and ionic homeostasis; oxidative injury
- Altered kinase/phosphatase activities → Hyperphosphorylated tau → PHF formation

- Widespread neuronal/neuritic dysfunction and death in hippocampus and cerebral cortex with progressive neurotransmitter deficits

DEMENTIA

Figure 5 A hypothetical sequence of the pathogenetic steps of early-onset familial AD.
Second, are Aβ oligomers, not monomers or large polymers (amyloid fibrils), the principal cytotoxic moiety in the disease (134)? Third, does apoptosis of neurons play an important role in producing AD brain dysfunction (23)? Finally, how can one explain the selective vulnerability of neuronal populations in AD? For example, do brain-region-specific factors (e.g., pro- or antiaggregating proteins) regulate the degree of local Aβ oligomerization? And even in the immediate vicinity of oligomeric and fibrillar Aβ, why do some neurons and neurites undergo injury while neighboring cells survive largely unscathed?

POTENTIAL THERAPEUTIC STRATEGIES

Despite these and other unresolved questions, sufficient progress in delineating the disease cascade has been achieved to envision several discrete targets for treatment. Inhibitors of Aβ 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 phases of the disease, particularly in patients with minimal cognitive impairment, and in nondemented subjects. In the case of γ-secretase inhibitors, these compounds would need to be administered to decrease Aβ production by some 30% or so without interfering in a meaningful way with the processing of Notch and other PS substrates. An alternate approach would be to use small molecules to bind Aβ monomers and prevent their assembly into potentially neurotoxic oligomers. However, if such antiaggregating compounds only blocked polymer formation, this could allow increased accumulation of metastable intermediates such as oligomers that might aggravate the disease. Of particular interest are the current approaches to active and passive Aβ immunization, which may operate to decrease both monomer and oligomer levels (103).

A third strategy would be to administer antiinflammatory drugs that interfere with the microglial, astrocytic, and cytokine responses. Epidemiological evidence that consumption of nonsteroidal antiinflammatory drugs for other purposes is associated with a somewhat lower likelihood of developing AD could potentially be explained on this basis. Finally, one could use a variety of antioxidants, free radical scavengers, calcium channel blockers, and modulators of certain signal transduction pathways that might protect neurons from the downstream effects of Aβ accumulation. The problem here is that there are probably multiple ways in which neurons respond to Aβ and its associated inflammatory process, and blocking one or two of these might not significantly diminish overall neuronal dysfunction and loss. One can also envision the use of neurorestorative factors, e.g., neurotrophins and small compounds mimicking their actions, but these would operate in the face of ongoing new injury from the cytotoxic effects of Aβ.

A new diagnostic and treatment paradigm is emerging from the very substantial progress in elucidating the functions and dysfunctions of gene products implicated in AD. In the future, individuals reaching their 50s or beyond may be offered a specific risk-assessment profile to determine their likelihood of developing AD,
modeled on that now widely used to judge the risk of serious atherosclerotic disease. Such an assessment would include inquiry about a positive family history of AD or a related dementia; identification of specific predisposing genetic factors; structural and functional brain imaging to detect evidence of presymptomatic disease; and measurement of Aβ42, tau, and other markers of the neuropathology in CSF and (in the case of Aβ) blood. Based on further epidemiological experience with such assessment measures in large populations of healthy elderly and AD subjects, it should be possible to estimate—first crudely and later more accurately—the likelihood that an individual will develop AD. If this can be accomplished, then those at risk could be offered preventative treatments with one or more of the agents contemplated above. Although the achievement of an integrated diagnostic and therapeutic approach to this complex and devastating disorder may seem remote, the current rate of scientific progress and the emergence of novel clinical trials indicate that some level of practical success may come sooner than one might think.

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Figure 1  High-power photomicrograph of a section of the amygdala from an Alzheimer’s patient showing the classical neuropathological lesions of the disorder. The modified Bielschowsky silver stain demonstrates two senile (neuritic) plaques consisting of compacted, spherical deposits of extracellular amyloid surrounded by a halo of silver-positive dystrophic neurites, which can include both axonal terminals and dendrites. Some of the pyramidal neurons in this field contain neurofibrillary tangles, darkly staining masses of abnormal filaments occupying much of the perinuclear cytoplasm. Electron microscopy of such neurons usually reveals large, nonmembrane-bound bundles of paired helical filaments.
Figure 4  Hypothetical model of the role of presenilin (PS) in Notch and APP processing based on current information. The diagram shows the predicted 8-TM domain topology of PS, which occurs principally as a cleaved heterodimer. Some Notch and APP molecules form complexes with PS. Two aspartate residues (D) in TM6 and TM7 of PS are required for the cleavages of Notch and APP within their TM domains, and these are predicted to align with the respective sites of cleavage in the two substrates. It is likely that PS directly affects these cleavages, but it remains possible that a still-unidentified aspartyl protease (γ-secretase) present in the complexes does so. PS-mediated proteolysis of both Notch and APP is preceded by ectodomain shedding that is mediated by certain ADAM proteases. Alternatively, a subset of APP holoproteins can undergo ectodomain shedding by β-secretase. Several motifs are depicted in Notch: EGF-like repeats (yellow circles), LNG repeats (red diamonds), a single TM (orange box), the RAM23 domain (blue square), a nuclear localization sequence (red rectangle), and six cdc10/ankyrin repeats (green ovals). Following the putative intramembranous cleavage mediated by PS, the Notch intracellular domain is released to the nucleus to activate transcription of target genes. APP contains the Aß region (light blue box), which is released into the lumen after sequential cleavages of APP by β-secretase and then γ-secretase/PS. The APP intracellular domain also is released to the nucleus.