Amyloid β-Protein and the Genetics of Alzheimer’s Disease*

Dennis J. Selkoe
From the Department of Neurology and Program in Neuroscience, Harvard Medical School and Center for Neurologic Diseases, Brigham and Women’s Hospital, Boston, Massachusetts 02115

The effort to decipher the mechanism of Alzheimer’s disease (AD) has attracted the interest of investigators from diverse biological disciplines, including biochemistry, cell biology, molecular genetics, neuroscience, and structural biology. The eclectic nature of research approaches to AD and the intensity of scientific interest in the problem have made it increasingly likely that AD will become a premier example of the successful application of biological chemistry to the identification of rational therapeutic targets in a major human disease. Much of the recent progress in elucidating the pathogenesis of AD has centered on the apparent role of the 40–42-residue amyloid β-protein (Aβ) (1) as a unifying pathological feature of the genetically diverse forms of this complex disorder.

Biochemistry of Cerebral β-Amyloidosis, a Route to Genetic Insights

It has been known since the time of Alzheimer (2) that insidiously progressive loss of memory, cognition, and behavioral stability in older humans can be associated with the development of innumerable intraneuronal and extracellular filamentous lesions in the limbic and cerebral cortices. Inside neurons, bundles of abnormal 10–20-nm cytoplasmic fibers (paired helical filaments) occur both in neuronal cell bodies (comprising neurofibrillary tangles) and in axons and dendrites (referred to collectively as dystrophic neurites). In addition to this filamentous degeneration of selected neurons and their processes, AD is characterized by abundant extracellular masses of 8–9 nm filaments composed of Aβ. These spherical deposits of Aβ fibrils are plaques, plaques are often intimately associated with dystrophic axons and dendrites (some of which contain paired helical filaments) as well as with activated microglia and reactive astrocytes. The presence of such “neuritic plaques,” together with numerous neurofibrillary tangles, in the hippocampus, amygdala, cerebral cortex, and certain other brain regions serves as the basis for a definitive pathological diagnosis of AD. It should be noted that the amyloid β-protein of AD is only one of several different proteins that can accumulate excessively in the extracellular spaces of various tissues and produce distinct human diseases (collectively called amyloidoses).

Although arguments were once raised that studying the biochemistry of the plaques and tangles would be unlikely to lead to insights into critical events in AD pathogenesis, this has not turned out to be the case. Immunocytochemical and biochemical analyses of the intraneuronal neurofibrillary tangles conducted during the last decade have led to the conclusion that the microtubulate-associated phosphoprotein, tau, is the major or, more likely, the sole subunit of the paired helical filaments found in both the tangles and in many of the dystrophic neurites observed in AD cortex (3, 4). Extensive studies by numerous laboratories have shown that tau protein, which normally enhances the polymerization of tubulin into microtubules and stabilizes these organelles in neurons, becomes excessively phosphorylated, apparently due to a combination of enhanced activity of certain kinases and decreased activity of certain phosphatases. Thus, the modification of this normally soluble neuronal protein into an insoluble filamentous polymer seems to involve a disruption of cytoplasmic phosphorylation/dephosphorylation cascades, but the factors that trigger this imbalance are poorly understood. Importantly, neurofibrillary tangles composed of paired helical filaments containing hyperphosphorylated tau molecules are found in a variety of etiologically diverse neurological diseases besides AD, strongly suggesting that this cytoskeletal alteration can develop as a secondary (albeit important) response to a variety of cerebral insults. In accord with this view, the gene on human chromosome 17 that encodes the tau polypeptides is not known to be a site of disease-causing mutations in familial forms of AD.

In contrast, studies of Aβ, which is the subunit of the amyloid fibrils found in neuritic plaques and in some meningeal and cerebral blood vessels, resulted in the identification of the first specific genetic cause of AD. The purification of both plaque and vascular amyloid deposits and the isolation of their 40–42-residue constituent peptide (Aβ) led to the cloning of the type 1 integral membrane glycoprotein from which Aβ is proteolytically derived, namely the β-amyloid precursor protein (βAPP) (5). The localization of the βAPP gene to chromosome 21q appeared to explain the observation that patients with trisomy 21 (Down’s syndrome) incur β-amyloid deposits in late childhood or young adulthood and subsequently develop the classical neuropathological features of AD in their forties (6–9). This realization led in turn to a specific search for families with autosomal dominant AD who had genetic linkage to chromosome 21, resulting ultimately in the identification of six different missense mutations in βAPP, five associated with familial AD (10–14) and one with the neuropathologically related syndrome of hereditary cerebral hemorrhage with amyloidosis of the Dutch type (15). Although βAPP mutations, all of which clustered in the βAPP region of the precursor, have proven to be a very rare cause of familial AD, representing less than 1% of such cases, they are important for understanding the mechanism of the progressive cerebral Aβ deposition that occurs in all cases of AD.

Most of the βAPP missense mutations have now been examined in transfected human cell lines, and their phenotypic effects have also been studied directly in primary skin fibroblasts and plasma obtained from subjects harboring these mutations. In each example studied to date, the missense mutations have been shown to alter the proteolytic processing of the precursor (see below) in a way that results in increased production of Aβ peptides, particularly of the highly hydrophobic (and thus amyloidogenic) 42-residue form of Aβ (Aβ42) (16–20). For example, one of the disease-causing missense mutations occurs immediately N-terminal to the start of the Aβ region, i.e. at the P1′ position for the protease (called “γ-secretase”), which generates the N terminus of Aβ (Fig. 1). Other mutations occur 4 residues C-terminal to the end of the Aβ region and lead to increased cleavage of βAPP by the protease (called “γ-secretase”) that generates the C terminus of the Aβ40 peptide (Fig. 1). The elucidation of the effects of the βAPP mutations on cellular Aβ production was made possible by the discovery in 1992 that Aβ is proteolytically generated from βAPP under normal metabolic conditions and is constitutively secreted by essentially all neural and non-neural cells that express the precursor (21–23). The heightened cerebral deposition of Aβ that results from the effects of the βAPP missense mutations can be viewed as loosely analogous to the excessive tissue deposition of another normal metabolic, cholesterol, in subjects with various forms of familial hypercholesterolemia. It remains unclear as to why Aβ, although secreted by many cell types throughout the body, is overwhelmingly deposited in the brain in AD, with only miniscule amounts of non-fibrillar Aβ deposits observed in some peripheral tissues (24–26).

Polymorphism of Apolipoprotein E Influences the Incidence and Neuropathology of AD

Because the βAPP mutations explained such a small percentage of familial AD, the search continued for other genetic loci that could

---

* This minireview will be reprinted in the 1996 Minireview Compendium, which will be available in December, 1996.

* The abbreviations used are: AD, Alzheimer’s disease; Aβ, amyloid β-protein; βAPP, β-amyloid precursor protein.
Minireview: Amyloid β-Protein and Genetics of Alzheimer’s Disease

Fig. 1. The β-amloid precursor protein and its principal metabolic derivatives. The upper diagram depicts the largest βAPP alternate transcript, comprising 770 amino acids. A 17-residue signal peptide occurs at the N terminus. 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). Two sites of N-glycosylation (CHO) are found at residues 542 and 571. A single membrane-spanning domain at amino acids 700–723 is indicated by the vertical hatched bar. The Aβ fragment (hatched box) includes 28 residues just outside the membrane plus the first 12–14 residues of the transmembrane domain. In the middle diagram, the left arrow indicates the site (after residue 687) of a constitutive proteolytic cleavage, made by an unknown protease(s) designated α-secretase, that enables secretion of the large, soluble ectodomain of APP (α-APP) into the medium and retention of the 83-residue C-terminal fragment (~10 kDa) in the membrane. The 10-kDa fragment can undergo cleavage(s) by an unknown protease(s) called γ-secretase(s) at residues 711 or 713 to release the p3 fragment in the presence of apoE4. Alternative proteolytic cleavage, after residue 671 by an unknown enzyme(s) called β-secretase that results in the secretion of the truncated β-APPα molecule and the retention of a 99-residue (~12 kDa) C-terminal fragment. The 12-kDa fragment can also undergo cleavage(s) by γ-secretase(s) to release Aβi.

Genetic factors predisposing to Alzheimer’s disease

TABLE I

| Chromosome | Gene defect | Age of onset | Aβ phenotype |
|------------|-------------|--------------|--------------|
| 21         | βAPP mutations | 50s          | Production of total Aβ and/or Aβ42 peptides or Aβ40 peptide(s)  |
| 19         | ApoE4 polymorphism | 60s and older | Density of Aβ plaques and/or vascular deposits |
| 14         | presenilin 1 mutations | 40s and 50s | Production of Aβ40 and/or Aβ42 peptides |
| 1          | presenilin 2 mutations | 50s          | Production of Aβ42 peptides |

Positional cloning led to the identification in 1995 of two highly homologous familial AD genes, currently termed presenilin 1 and presenilin 2 (40–42). These genes encode 467- and 448-residue polypeptides whose sequences and hydrophathy profiles suggest that they contain 7–9 transmembrane domains. The normal functions of these highly expressed proteins are not yet known. Accordingly, the mechanism by which missense mutations, of which 27 have already been reported (25 in presenilin 1 and 2 in presenilin 2), cause early-onset familial AD remains to be elucidated by studies in transfected cell lines and transgenic animals. However, a major due to their pathogenic effect has come from recent analyses of Aβ levels in plasma and the conditioned media of skin fibroblasts obtained from carriers of mutant presenilin genes. Sensitive enzyme-linked immunosorbent assays show that Aβ peptides ending at residue 42 (Aβ42) and Aβ40 (Aβ40) are selectively augmented by at least some missense mutations in presenilin 2, whereas levels of the major form of Aβ (Aβ40) are largely unchanged (43). Aβ42 peptides have been found to have a markedly enhanced rate of aggregation into amyloid fibrils in vitro, compared to that of Aβ40 peptides (44). Moreover, immunocytochemical studies using antibodies specific to the two Aβ C termini reveal that Aβ42 peptides are the initially deposited species in the earliest (“diffuse”) plaques in both AD and Down’s syndrome brains (8, 9, 45, 46).

In summary, four genes have been implicated to date in familial forms of AD: three that, when mutant, cause autosomal dominant forms of the disease (βAPP, presenilin 1, and presenilin 2) and one in which a naturally occurring polymorphism (apoE4) represents a major genetic risk factor for the development of the disease (Table I). Available evidence strongly suggests that each of these four genes predisposes to the AD phenotype by enhancing the production and/or the deposition of Aβ peptides (or in the case of apoE4, perhaps by decreasing its clearance from tissue).

The Expression and Post-translational Processing of βAPP

Growing evidence implicating extracellular Aβ42 accumulation and resultant amyloid plaque formation as early and necessary steps in the pathogenesis of the known forms of heritable AD has heightened interest in understanding the details of the trafficking and proteolytic processing of βAPP (reviewed in Ref. 47). This protein occurs in numerous different isoforms, which arise from alternative splicing of a single gene. The shortest of the major isoforms (695 amino acids) is expressed almost exclusively in neurons, whereas the other two common forms (751 and 770 amino acids, respectively) are expressed both in neural and non-neural cells. Additional heterogeneity of the βAPP poly peptides arises from their complex post-translational modifications, including sulfation, phosphorylation, and both N- and O-linked glycosylation (e.g., Refs. 48–50). These modifications occur during the trafficking of the protein through the secretory pathway. βAPP is co-translationally translocated into the endoplasmic reticulum via its signal peptide and then matured during passage through the Golgi by acquiring sulfate, phosphate, and sugar groups, following which a minor percentage of mature molecules is transported to the plasma membrane via secretory vesicles (47). At the cell surface, some βAPP molecules undergo proteolysis by an unidentified protease designated “γ-secretase,” which cleaves between lysine 687 and leucine 688, i.e. between residues 16 and 17 of the Aβ region, releasing the large, soluble ectodomain (referred to as α-APP) into the medium (51) (Fig. 1). Alternatively, undeaved surface βAPP
molecules can undergo endocytosis via clathrin-coated vesicles, apparently mediated by a YENPTY signal sequence in the distal cytoplasmic tail (52), following which the full-length precursor is trafficked to late endosomes and lysosomes for apparent degradation (53, 54) or is rapidly recycled within early endosomes to the cell surface (55). The latter pathway has been shown to be a principal site for the two proteolytic cleavages that generate the Aβ peptide (56). A protease termed “β-secretase” initiates Aβ generation by cleaving βAPP after methionine 671, creating a 99-residue (~12 kDa) membrane-retained C-terminal fragment having residue 1 (aspartate) of Aβ as its N terminus (57), and this results in the secretion of a truncated APPs molecule, called β-APPs, into the medium (58) (Fig. 1). The 12-kDa fragment may then undergo mechanistically enigmatic “γ-secretase” cleavages within the hydrophobic transmembrane domain at either valine 711 or isoleucine 713 that release the 40- or 42-residue Aβ peptides, respectively, into the medium. It appears that only a minority of all β- or γ-synthesized βAPP molecules undergoes either the α-secretory or the β-secretory fate; many full-length precursor molecules remaininserted into internal membranes, particularly in the Golgi.

The diverse metabolic fates of βAPP just summarized are under complex regulation. For example, several first messengers, including cholinergic agonists and other neurotransmitters that can activate the phospholipase C/protein kinase C-dependent pathway, can enhance α-secretase deavage of βAPP and the consequent release of α-APPs into the extracellular fluid (e.g. Refs. 59 and 60). The mechanism by which this enhancement is brought about is unclear. It does not involve a direct change in the phosphorylation state of βAPP (50) and may involve instead the phosphorylation of α-secretase or an enhancement of the trafficking of Golgi-derived vesicles containing βAPP to the cell surface (61), where α-secretase is known to be active (62). In addition to the regulated processing of βAPP through the α-secretary pathway, the amyloidogenic processing of βAPP (i.e. β-followed by γ-secretase cleavages) can be enhanced, for example by increases in intracellular free calcium levels (63).

βAPP Function and Dysfunction

Although the foregoing summary demonstrates that there has been considerable progress in delineating factors that regulate the processing of βAPP, the functional implications of these varied effects remain unclear. The physiological consequences of the enhanced secretion of either α-APP, or Aβ have not been defined. In particular, a specific receptor for α-APP, is not known, although this possibility has been suggested, and βAPP transgenic mice appear to have a supraphysiological dose of synthetic Aβ1–40, whereas β-amyloid deposition in vivo evolves very slowly from peptides with heterogeneous N and C termini that are secreted at picomolar to low nanomolar concentrations by neurons, astrocytes, microglia, and endothelial cells (83) in culture. However, such in vitro experiments necessarily employ short-term exposure of cells to supraphysiological doses of synthetic Aβ1–40, whereas β-amyloid deposition in vivo evolves very slowly from peptides with heterogeneous N and C termini that are secreted at picomolar to low nanomolar concentrations by neurons, astrocytes, microglia, and endothelial cells (83) in culture.

A more compelling model of the consequences of excessive Aβ accumulation has come from the production of a transgenic mouse that markedly overexpresses an AD-linked mutant form of βAPP in selected neurons (84). These animals secrete large amounts of both Aβ12 and Aβ42 peptides into the extracellular fluid of brain and begin to accumulate diffuse and compacted (fibrillar) Aβ deposits after about age 5–6 months. Many of these amyloid plaques are intimately associated with dystrophic neurites, activated microglia and reactive astrocytes, and smooth muscle cells in the brain. In situ hybridization and immunocytochemistry for phosphorylated tau and neurofilament proteins, suggest that they are undergoing AD-like cytoskeletal alterations (85).

The substantial similarity of the neuropathology in this model to that of AD strongly supports the hypothesis that excessive accumulation of first soluble and then aggregated Aβ12 and Aβ42 peptides can initiate Alzheimer-type neuronal and glial changes. This and other animal models of neurofibrillary tangles have not been described to date, but some cerebral neurons develop immunoreactivity for phosphorylated tau and neurofilament proteins, suggesting that they are undergoing AD-like cytoskeletal alterations (85).

The concept that alterations in several distinct genes (four of which have been identified to date) can lead by different mechanisms to a chronic imbalance between Aβ production and clearance that results in aggregation of first the 42-residue and then the 40-residue peptide into cytotoxic plaques is now supported by multiple lines of evidence. Exactly how aggregated Aβ and the locally secreted and blood-borne proteins that become associated with it in plaques (e.g. heparan sulfate proteoglycan (86), α1-antichymotrypsin (87), apolipoprotein E (88), complement components (89, 90), serum amyloid P component (91), and cytokines (92)) exert toxic effects on surrounding cells is an area of intensive study. It appears that aggregated but not monomeric Aβ peptides can induce cell dysfunction and death in vitro by a range of presumably interrelated mechanisms that include oxidative injury (83, 93), alterations in intracellular calcium homeostasis (38, 39), and cytoskeletal reorganization (64). Sufficient knowledge of some of the principal elements of the amyloid-induced cascade has emerged that the process of identifying small molecules which could inhibit one or another step is now well under way. Of particular therapeutic interest are attempts to design protease inhibition of Aβ peptides from neuronal and glial cells, something which may be possible to accomplish prior to knowing the identities of the requisite proteases. Interfering with the aggregation of Aβ12 and Aβ42 peptides or
inhibiting the toxicity that these extracellular aggregates produce on neurons, their processes, and glial cells are also of great therapeutic relevance. Finally, controlling the specialized inflammatory response that appears to be triggered by aggregated Ab (including microglial stimulation, activation of the classical complement cascade, cytokine release, and reactive astrocytosis) should also prove to be of benefit to patients with this disease. Given the accelerating pace of progress, there will be little doubt that further biochemical and pharmacological research will lead to a range of therapeutic options during the next several years.

REFERENCES

1. Glenner, G. G., and Wong, C. W. (1984) Biochem. Biophys. Res. Commun. 120, 885–890.
2. Alzheimer, A. (1907) Centralblatt f. Nervenheilkunde und Psychiatrie 30, 18298.
3. Goedert, M., Trojanowski, J. Q., and Lee, V. M.-Y. (1996) In The Molecular and Genetic Basis of Neurological Disease (Rosenberg, R. N., Prusiner, S. B., DiMaura, S., and Barchi, R. L., eds) 2nd Ed., Butterworth-Heinemann, Stoneham, MA.
4. Levy, E., Carman, M. D., Fernandez-Madrid, I. J., Power, M. D., Lieberburg, I., et al. (1996) Neurobiol. Dis. 3, 16–32.
5. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., et al. (1996) Nature 384, 704–706.
6. Murrell, J., Farlow, M., Ghetti, B., and Benson, M. (1991) Science 250, 97–99.
7. Murrell, J.-C., Maruyama, K., and Macklis, J. D. (1996) Brain Res. 718, 117–179.
8. Citron, M., Vigo-Pelfrey, C., Teplow, D. B., Miller, C., Schenk, D., et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 11399–11403.
9. Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., et al. (1991) Nature 356, 567–571.
10. Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., et al. (1991) Nature 356, 567–571.
11. Kalaria, R. N. (1992) J. Neurol. Sci. 112, 575–580.
12. Murrell, J., Farlow, M., Ghetti, B., and Benson, M. (1991) Science 250, 97–99.
13. Murrell, J.-C., Maruyama, K., and Macklis, J. D. (1996) Brain Res. 718, 117–179.
14. Hendriks, L., van Duijn, C. M., Cras, P., Cruts, M., Van Hul, W., et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 150, 799–804.
15. Levy, E., Carman, M. D., Fernandez-Madrid, I. J., Power, M. D., Lieberburg, I., et al. (1996) Science 270, 218–221.
16. Citron, M., Olsfetter, T., Haass, C., McConlogue, L., and Hung, A. Y., et al. (1992) Nature 360, 672–674.
17. Cai, X.-D., Golde, T. E., and Younkin, G. S. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 1122–1124.
18. Citron, M., Olsfetter, T., Haass, C., McConlogue, L., and Hung, A. Y., et al. (1992) Nature 360, 672–674.
19. Cai, X.-D., Golde, T. E., and Younkin, G. S. (1993) Science 258, 1336–1340.
20. Citron, M., Vigo-Pelfrey, C., Teplow, D. B., Miller, C., Schenk, D., et al. (1996) Proc. Natl. Acad. Sci. U.S.A. 91, 11399–11403.
21. Haass, C., Hung, A. Y., Selkoe, D. J., and Teplow, D. B. (1994) J. Biol. Chem. 269, 17741–17748.
22. Suzuki, N. C., Claire, T. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., et al. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 763–767.
23. Citron, M., Olsfetter, T., Haass, C., McConlogue, L., and Hung, A. Y., et al. (1992) Nature 360, 672–674.
24. Saitoh, T., Sunsdmo, M., Roch, J.-M., Kimura, N., Cole, G., et al. (1994) J. Neurosci. 14, 180–184.
25. Mastroeni, P., Rocchi, G., D’Andrea, F., and De Vizio, D. (1992) Cell 75, 91–97.
26. Evrard, P., Bélanger, P., and Belanger, A. (1994) Neurobiol. Dis. 1, 253–259.
27. Mastroeni, P., Rocchi, G., D’Andrea, F., and De Vizio, D. (1992) Cell 75, 91–97.
28. Corder, E. H., Saunders, A. M., Schmechel, D., Pericak-Vance, M. A., Eng, J. H., et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 1977–1981.
29. Corder, E. H., Saunders, A. M., Smith, M., Schmechel, D., Egan, W. H., et al. (1993) Science 261, 921–925.
30. Corder, E. H., Saunders, A. M., Risch, N. J., Appassionata, J., Schmechel, D. E., Egan, W. H., et al. (1994) Nat. Genet. 7, 180–184.
31. Schmechel, D. E., Saunders, A. M., Crain, B. J., Hulte, C. M., et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 9649–9653.
32. Rebeck, G. W., Wszolek, Z. K., and Hardy, J. A. (1996) J. Neurosci. 16, 575–580.
33. Keen, I., and Karran, E. (1996) Brain Res. 725, 185–191.
34. Greenberg, S. M., Rebeck, G. W., Vassella, G., and Younkin, G. S. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 3586–3590.
35. Polvks, T., Suvina, R., Hafla, M., Kainlaine, K., Vuorio, A., et al. (1995) Neurobiol. Aging 16, 12243–12247.