Molecular Neurobiology

ISSN0893-7648/94/8(1): 49–64/$4.20

Unifying Features of Systemic and Cerebral Amyloidosis

Jorge Ghiso,*[1] Thomas Wisniewski,[2] and Blas Frangione[1]

[1]Departments of Pathology and [2]Neurology, New York University Medical Center, New York, NY

Abstract

Amyloidosis is a generic term for a group of clinically and biochemically diverse diseases that are characterized by the deposition of an insoluble fibrillar protein in the extracellular space. Over 16 biochemically distinct amyloids are known. Despite this diversity, all amyloids have a particular ultrastructural and tinctorial appearance, a β-pleated sheet structure, and are codeposited with a group of amyloid-associated proteins. The most common amyloidosis is Alzheimer's disease (AD), where Aβ is the main component of the amyloid. Recently it has been found that Aβ exists as a normal soluble protein (sAβ) in biological fluids. This links AD more closely to some of the systemic amyloidoses, where the amyloid precursor is found in the circulation normally. Numerous mutations have been found in the Aβ precursor (βPP) gene, associated with familial AD. Many mutations are also found in some of the hereditary systemic amyloidoses. For example, over 40 mutations in the transthyretin (TTR) gene are associated with amyloid. However, both Aβ and TTR related amyloid deposition can occur with no mutation. The pathogenesis of amyloid is complex, and appears to be associated with genetic and environmental risk factors that can be similar in the systemic and cerebral amyloidoses.

Index Entries: Amyloid β; Alzheimer's disease; prion; chaperones; ApoE; ApoJ.

Introduction

The term amyloidosis comprises a group of diseases of different etiology characterized by the deposition of fibrillar proteins (amyloid) in the extracellular spaces of different tissues that may lead to cell damage, organ dysfunction, and death. There are over 16 biochemically distinct forms of amyloid (Table 1).

The designation "amyloid" was introduced by Virchow in 1854 *(1)* to describe an eosinophilic substance with hyaline appearance under the light microscope, deposited in tissues. A century later, electron microscopic studies revealed that the homogeneous material was actually fibrillar *(2)*. The capability to solubilize the fibrils *(3)* enabled subsequent characterization of their major protein constituents, and in 1971 the first amyloid subunit was biochemically characterized by amino acid sequence analysis *(4)*. Since then, it is recognized that many different proteins are capable of forming amyloid *(5)*. In spite of their biochemical heterogeneity, all types of amyloid share common properties.

This review summarizes some recent advances in the field.

*All correspondence and reprint requests should be addressed to: J. Ghiso, Department of Pathology, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Table 1
Amyloid Diseases, Amyloid, and Their Corresponding Precursor Proteins

Disease	Amyloid	Precursor protein
Primary or idiopathic	AL	Immunoglobulin L-chain
Amyloidosis in senescence-accelerated mice	AApo AII	Apolipoprotein A-II
Secondary amyloidosis	AA	Apolipoprotein SAA
Familial Mediterranean fever	AA	Apolipoprotein SAA
Long-term hemodialysis	Aβ2m	β_2-microglobulin
Systemic senile amyloidosis	ATTR	Transthyretin (or prealbumin)
Familial amyloid polyneuropathy	ATTR	Transthyretin variants
Familial amyloid polyneuropathy	AApoAI	Apolipoprotein A-I
Familial amyloidosis Finnish-type	AGel	Gelsolin variant
Hereditary non-neuropathic systemic amyloidosis	ALys	Lysozyme
Medullar carcinoma of the thyroid	ACal	Calcitonin
Diabetes mellitus—type II	AIAPP	Islet amyloid polypeptide
Atrial amyloidosis	AANP	Atrial natriuretic peptide
Islet amyloid in the degu	AIns	Insulin
Hereditary renal amyloidosis	AFib	α-chain fibrinogen
Alzheimer's disease/Down syndrome	Aβ	β-amyloid precursor protein
Sporadic cerebral amyloid angiopathy	Aβ	β-amyloid precursor protein
Amyloid related to normal aging	Aβ	β-amyloid precursor protein
*HCHWA—Dutch type	Aβ	β-amyloid precursor variant
*HCHWA—Icelandic type	ACys	Cystatin-C variant
Transmissible spongiform encephalopathy	APrP	Protease resistant protein

*Hereditary cerebral hemorrhage with amyloidosis.

Amyloid Fibrils

Although the conditions and the mechanisms of amyloid formation and deposition, as well as their tissue-specific localization, are poorly understood, all types of amyloid share unique physico-chemical properties despite differences in primary structure:

1. Characteristic fibrillar, unbranched appearance on electron microscopy, being 8 to 10 nm thick and up to 100 nm long *(6–8)*. These measurements may vary according to the nature of the protein subunit, and the number of filaments that constitute the fibril *(2)* (it may be composed of two or more filaments arranged into helically twisted structures, i.e., PHF) *(9)*.
2. Special tinctorial properties, widely employed for histopathological diagnosis. These include apple-green birefringence under polarized light and yellow-green fluorescence after Congo red and thioflavin S staining, respectively *(10,11)*. Congo red binding may be dependent on the formation of antiparallel homodimers of the amyloid protein molecules *(12)*.
3. Predominantly β-pleated sheet secondary structure, as demonstrated by X-ray diffraction analyses *(13,14)*. Extensive antiparallel β-sheet strands with their axes arranged perpendicular to the long axis of the fibril have been reported in many amyloids, regardless of the different protein subunits that form the fibril *(8,15,16)*.
4. High degree of insolubility under physiological conditions, which may preclude their complete proteolytic degradation in vivo.
5. Association with presumed chaperone proteins, such as amyloid P-component *(17,18)*, proteoglycans *(19)*, apolipoprotein E *(20,21)*, apolipoprotein J *(22)*, and other serum proteins.

Amyloid Diseases

The distribution of the amyloid deposits varies according to the different types of amyloidosis. It may be systemic or be limited to a single organ. Table 1 lists the best-known amyloid diseases and amyloid proteins as well as the corresponding precursor proteins.

Primary Amyloidosis

AL, primary or idiopathic amyloidosis, is associated in 80% of cases with a monoclonal component that can be identified in serum, urine, or cerebrospinal fluid *(23–25)*. This type of amyloid complicates

multiple myeloma in about 15% of cases, whereas a higher incidence has been reported in light-chain disease (26) and IgD myeloma associated with Bence Jones proteinuria (27). Amyloid deposits in these patients are primarily distributed in the heart, skin, gastrointestinal tract, tongue, peripheral nerves, and ligaments, but the kidney, liver, spleen, and adrenals can also be compromised (23,25,26,28–31). The amyloid subunit for AL was the first amyloid protein biochemically characterized (4). With the exception of two recently reported cases, in which amyloid fibrils are composed of an unusual truncated Gamma 1 heavy-chain (32), the fibrils in all cases of primary and myeloma-associated amyloidosis are composed of either N-terminal fragments of L-chain, or L-chain fragments plus intact L-chain (Mr = 5–23 kDa) (5,33–35). The lambda/kappa ratio among AL proteins is the reverse of normal distribution (36) and certain types of L-chain appear to be preferentially associated with AL amyloidosis, as was shown for the lambda VI subgroup (37).

SAA Related Amyloidoses

AA, secondary or reactive amyloidosis, is a rare complication of common chronic infections (tuberculosis, leprosy, osteomyelitis, bronchiectasis, skin infections associated with parenteral drug abuse), inflammatory disorders (adult and juvenile rheumatoid arthritis), and certain neoplasms (Hodgkin's disease, renal cell carcinoma). The main constituent of the amyloid fibrils is protein AA (Mr = 8500 daltons), a 76-residue N-terminal fragment derived by proteolysis of SAA, a 12,500-dalton, acute-phase reactant. SAA is an apolipoprotein synthesized in the liver that circulates as a high-density fraction (high-density lipoprotein 3 complex) (38–40). Inflammatory stimuli increase the levels of SAA several hundredfold. This increased concentration of SAA appears necessary for AA formation; however, reactive amyloidosis only occurs in a small proportion of individuals with chronic infections, so other factors must also play a role.

In addition to chronic inflammatory conditions, AA is also the amyloid protein found in familial Mediterranean fever (FMF), an autosomal recessive disorder of unknown etiology characterized by recurrent episodes of fever, serositis, and prostration that occurs primarily in the ethnic group of North African Sephardic Jews (41). This disease deserves special consideration, since it has been demonstrated that long-term treatment with colchicine can prevent the development of amyloidosis in high-risk populations and may suppress additional impairment in FMF patients with proteinuria who have not reached the stage of nephrotic syndrome (42). FMF is the only type of human amyloidosis that can be effectively treated at the present time, although the mechanisms by which colchicine controls amyloid deposition are still poorly understood.

AA has been found to be the major amyloid fibril subunit protein in experimentally induced and spontaneous amyloidosis in a number of animals (43–45). Perhaps the best-studied model for AA amyloid formation is the mouse, where amyloidosis can be induced with a variety of antigenic and inflammatory stimuli (43,46). CBA/J and C57BL/6J mice are among the most susceptible strains, whereas the A/J mouse strain is highly resistant, reflecting genetic differences (47–50). AA in this model is a cleavage product of SAA2, one of the three SAA alleles. This suggests that particular SAA amino acid sequences are more amyloidogenic. Furthermore, a recent study has found that CE/J strain mice, which have a unique Apo-SAA isoform and lack SAA2, are exceedingly resistant to amyloidogenesis (51). The SAA isoform found in these mice differs by only six amino acids. Induction of amyloidosis in this model generally involves chronic injections of casein, azocasein, lipopolysaccharides, or silver nitrate, which trigger the production of acute phase proteins, among them, SAA (52,53). The animals develop amyloidosis in 15–21 d, but this period can be drastically shortened (to 1 or 2 d) if mice are pretreated with tissue extracts from amyloidotic organs and an inflammatory agent. These tissues contain a substance called amyloid-enhancing factor (AEF) (54,55) that is mainly associated with adherent cells (macrophages) (56). Except for its dramatic biological effect, AEF has not been well defined.

β_2-Microglobulin Related Amyloid

Aβ_2m, a systemic form of amyloidosis with a predilection for bone and synovium, may complicate the course of patients undergoing long-term hemodialysis. Clinical manifestations include carpal tunnel syndrome (57–59), erosive arthropathy (59), spondyloarthropathy (60), lytic bone lesions, and pathologic fractures (58,59). The disease first appears after 5–6 yr of hemodialysis. After 20 yr of dialysis 80–100% of patients are affected (61). The amyloid fibrils are composed of monomers, dimers,

and polymeric forms of intact or degraded β_2-microglobulin (Mr = 12 kDa) *(62–64)*, a normal protein belonging to the immunoglobulin superfamily *(65)*. β_2-microglobulin is part of the class I histocompatibility complex *(66)*, and its blood concentration is largely derived from cell membrane shedding. High serum levels are found in some chronic inflammatory conditions because of increased synthesis or in renal diseases where its excretion is reduced *(67)*. β_2-microglobulin is not effectively eliminated by conventional cuprophan dialysis membranes *(67)*; as a result, its serum concentration in hemodialyzed patients can be 40–60 times higher than in normal individuals *(68,69)*. Intact β_2-microglobulin is capable of forming amyloid-like fibrils in vitro *(70)*, however, other factors presumably determine the timing and distribution of fibril formation in vivo.

Transthyretin Related Amyloidoses

An expanding spectrum of overlapping clinical syndromes that include senile systemic amyloidosis and several forms of familial amyloid polyneuropathy share the same protein, transthyretin (TTR or prealbumin), as an amyloid subunit. These diseases are also named transthyretin-related amyloidosis, ATTR. TTR *(71)* is a tetrameric serum protein of four identical subunits (Mr = 14 kDa) that transports thyroxine and retinol-binding protein *(72,73)*. Several amino acid substitutions have been detected in the amyloid fibrils isolated from different kindreds *(74–110)*, and the variant molecules are listed in Table 2. In familial amyloid polyneuropathy (FAP), the clinical findings may be restricted to the lower limbs (also known a FAP type I) or to the upper limbs (FAP type II), but in all cases there is an autosomal dominant form of transmission. Senile systemic amyloidosis, a disease of late onset characterized by amyloid deposition predominantly in the heart, causing congestive heart failure and conduction-system disturbances, is also related to TTR *(106,111–115)*. Over 40 different TTR mutations are associated with amyloid deposition, and over seven silent mutations have been reported. The amino acid position of the different mutations does not correlate well with clinical symptoms. Furthermore, the same mutation in one kindred can be associated with different clinical symptoms in another kindred. Normal TTR can also be associated with amyloid deposition. This variability suggests that other factors besides TTR mutations are important in determining the spectrum of disease. Interestingly, TTR amyloid isolated from individuals heterozygote for the Met^{30} mutation contain both normal and mutated TTR *(116)*.

Apolipoprotein AI Related Amyloid

One particular form of familial autosomal dominant amyloid polyneuropathy, AApoAI (FAP type III or Iowa-type), characterized by lower limb polyneuropathy, peptic ulcers, and nephrotic syndrome, is not associated with TTR deposits. The amyloid protein subunit was shown to be the amino terminal portion (residues 1–83) of apolipoprotein AI with an arginine for glycine substitution at position 26 *(117)*.

Familial Amyloidosis, Finnish-Type

Familial amyloidosis, Finnish-type is an autosomal dominant form of systemic amyloidosis characterized by lattice corneal dystrophy, cranial neuropathy, and intermittent proteinuria *(118)*. Amyloid deposits are found particularly in kidney, skin, muscle, nerves, and cornea, and the fibrils are composed of polymeric forms of a protein subunit (Mr = 7–9 kDa) that is a degradation product of plasma gelsolin *(119,120)*. Gelsolin is an actin-modulating protein that severs actin filaments, nucleates actin filament growth, and caps barbed filament ends *(121)*. The amyloid subunit starts at residue 173 of the gelsolin molecule and contains one amino acid substitution at position 187 (AGel Asn^{187}) *(122)* because of a guanine to adenine transition at nucleotide 654 *(123)*. The same phenotype has also been described in Danish and Czech kindreds, but with a guanine to thymidine transversion at nucleotide 654, resulting in tyrosine substituting aspartic acid at codon 187 (AGel Tyr^{187}) *(124)*.

Endocrine Amyloidoses

Amyloidosis restricted to certain tumors of endocrine glands have been described. In medullary carcinoma of the thyroid, the amyloid fibrils (Mr = 5.7 kDa) are composed of procalcitonin (ACalc) *(125,126)*, whereas in patients with islet cell tumors associated with diabetes mellitus type II, the amyloid subunit deposited (AIAPP) has been termed islet amyloid polypeptide, or amylin (Mr = 4 kDa) *(127–129)*.

A form of senile cardiac amyloidosis restricted to the atria (also called atrial amyloidosis) has been described *(130)*. The 3-kDa amyloid subunit, α-atrial natriuretic peptide (AANP), is a 28-amino-acid fragment derived from a larger precursor mole-

Table 2
Transthyretin Variants

TTR variant	Clinical picture	Geographic kindred	Clinical name	Reference
Arg^{10}	LLN, eye, heart	US		74
Met^{30}	LLN, AN, eye	US/Japan Portugal/Sweden	FAPI	75–78
Ala^{30}	Heart, AN	US		79
Leu^{30}	LLN, AN, heart	Japan		80
Ile^{33}	LLN, eye	Israel	Jewish	81
Leu^{33}	LLN, heart	US		82
Pro^{36}	LLN, eye, heart	US		83
Gly^{42}	LLN, AN	Japan		84
Thr^{45}	Heart	US		85
Asp^{45}	Heart, LLN	US		86
Arg^{47}	LLN, AN	Japan		87
Ala^{47}	LLN, heart, kidney	Italy		88
Val^{47}	CTS, LLN	Sri Lanka		89
Ala^{49}	Heart, CTS	France/Italy		90
Arg^{50}	AN, LLN	Japan		84
Ile^{50}	LLN, AN, heart	Japan		91
Pro^{52}	LLN, heart	England		89
Pro^{55}	LLN, AN, heart, eye	US		92
His^{58}	CTS, heart	US	FAPII	93
Arg^{58}	AN, CTS, eye	Japan		94
Ala^{60}	Heart, CTS	US	Appalachian	95
Leu^{68}	Heart	Germany		96
His^{69}	Eye	US		97
Asn^{70}	CTS, eye	US		98
Ala^{71}	LLN, eye	France		99
Tyr^{77}	Kidney, LLN	US		100
Ser^{84}	Heart, CTS, eye	US	FAPII	101
Asn^{84}	Heart, eye	US		102
Glu^{89}	CTS, LLN, Heart	Italy		96
Asn^{90}	Heart, eye, LLN	US		103
Val^{107}	CTS, LLN	US		104
Met^{111}	Heart	Denmark	Danish	105
Cys^{114}	LLN, AN, eye	Japan		84
Ile^{122}	Heart	US	Senile cardiac	106
Ser^{6} Ile^{33}	LLN, eye	Israel		107
Ser^{6} Gly^{54}	LLN	England		89
Met^{30} Met^{119}	LNN	Portugal		108
Gly^{42} Asn^{90}	LLN, AN	Italy		109
Asn^{90} Gly^{42}	Heart, eye, LLN	US		110

Abbreviations: LLN, lower limb neuropathy; FAP, familial amyloidotic polyneuropathy; CTS, carpal tunnel syndrome; AN, autonomic neuropathy.

cule (14 kDa) that has potent natriuretic and diuretic effects.

Cerebral Amyloidosis

The amyloid fibril deposition restricted to the central nervous system is the most frequent form of amyloidosis. Congophilic lesions in the brain have been described in a variety of diseases of different etiology (Table 1).

Aβ and Related Amyloidoses

Alzheimer's disease (AD) (acquired and familial) is the most common form of human amyloidosis and the major cause of dementia, affecting more than 5% of the population over the age of 65 yr

Table 3
Pathogenic βPP Mutations

Codon (APP 770)	Amino acid change	Phenotype	Reference
670, 671	Lys → Asn, Met → Leu	Early-onset FAD	202
692	Ala → Gly	Dementia or stroke	203
693	Glu → Gly	Early-onset AD	204
693	Glu → Gln	Stroke (Dutch variant of AD)	189
713	Ala → Thr	Dementia	205
713	Ala → Val	Schizophrenia	206
717	Val → Ile	Early-onset FAD	207
717	Val → Phe	Early-onset FAD	208
717	Val → Gly	Early-onset FAD	209

(131). AD is mainly characterized by three different pathological changes:

1. Intraneuronal deposits (neurofibrillary tangles, NFT);
2. Neuritic or senile plaques; and
3. Cerebrovascular amyloidosis that affects small and medium vessels of the leptomeninges and cerebral cortex.

NFT deposits are found mainly in pyramidal neurons of the neocortex, hippocampus, amygdala, and basal nuclei *(132)*. They are composed of bundles of twisted fibrils called paired helical filaments (PHF), with a typical periodicity of 60–100 nm with a maximal width of 10 nm at the crossing points *(9,133)*.

Neuritic or senile plaques are spherical lesions of variable size (10–200 μm) found in the cerebral cortex, especially in the associative areas of neocortex and limbic structures. A typical mature plaque is formed of three elements:

1. A central amyloid core;
2. Dystrophic neurites; and
3. Glial cells *(134,135)*.

It is thought that neuritic plaques may evolve from preamyloid deposits (diffuse or very primitive plaques); those are lesions with irregular borders that, unlike senile plaques, do not stain with Congo red or thioflavin S *(136–140)*.

Vascular amyloid is mainly located in the tunica media and adventitia of small arteries and arterioles within the leptomeninges and cerebral cortex *(141–143)*. Under the electron microscope, the amyloid appears composed of typical straight 10 nm nonbranched fibrils.

The major component of the fibrils in neuritic plaques and cerebrovascular amyloid is a unique peptide designated β-protein or Aβ (Mr = 4.2 kDa) *(144–146)*. Whereas Aβ extracted from senile plaques of AD patients contains 42/43 amino acids *(144–146)*, the vascular amyloid is three residues shorter at the C-terminus (39/40 amino acids long) *(147,148)*. Preamyloid deposits also contain Aβ since they are immunoreactive with anti-Aβ antibodies; however, the exact amino sequence of preamyloid Aβ is not known. It has been shown that biochemically extracted material from preamyloid deposits has the same electrophoretic mobility and Western blotting characteristics as synthetic peptides homologous to Aβ1-40 *(149)*.

In virtually all patients with Down's syndrome over the age of 35 yr, senile plaques occur in the brain and are also composed of Aβ *(150)*. In normotensive patients with sporadic cerebral amyloid angiopathy, Aβ deposits are restricted to the leptomeningeal and cortical vasculature, leading to recurrent hemorrhagic and/or ischemic events (accounting for approx 5–10% of all cases of stroke) *(151–157)*.

The initial report of the N-terminal sequence of Aβ allowed four independent groups to isolate and characterize cDNA for the amyloid precursor protein to Aβ, known as the β-amyloid precursor proteins (βPP) *(158–161)*. The predicted structure of βPP is that of a multi domain, transmembrane cell-surface receptor *(158)* (Fig. 1). βPP contains:

1. A cysteine-rich domain, an acidic fragment, and two putative *Asn*-glycosylation sites in the predicted extracellular region;
2. A transmembrane domain; and

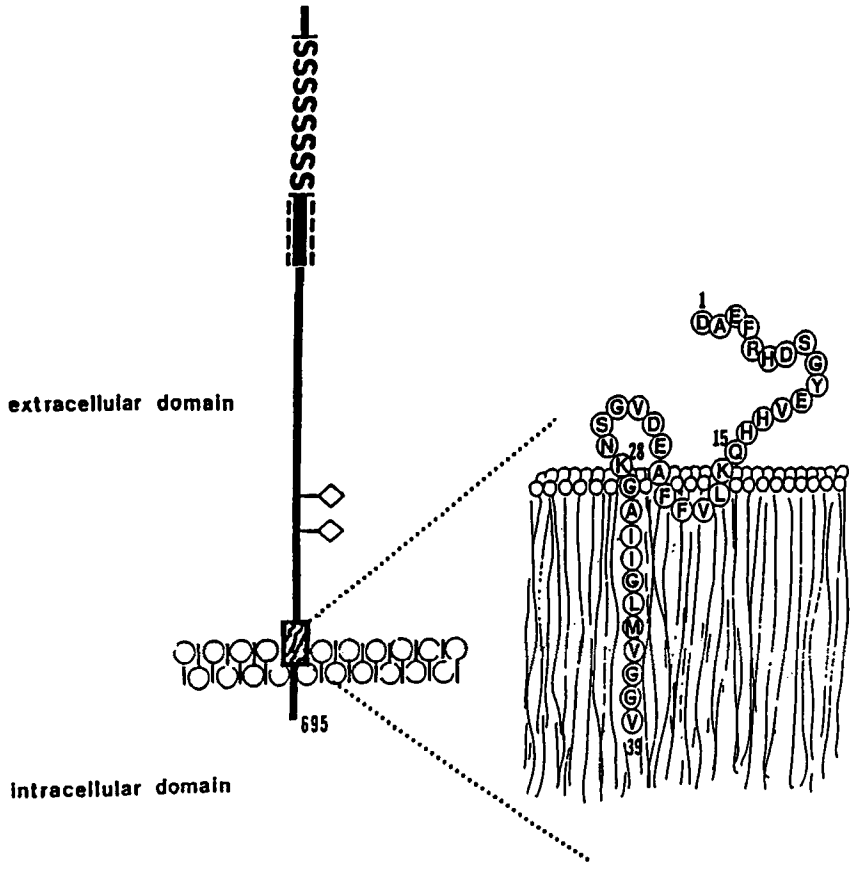

Fig. 1. Schematic representation of the multidomain βPP. NH_2: N-terminus; COOH: C-terminus; SSS: cysteine-rich domain; ===: acidic domain;—◇: glycosylation site. ▨ indicates location of the peptide Aβ: extracellular compartment (first 28 residues); intramembrane (11–14 amino acids). The predicted secondary structure indicates that the molecule contains a short amino-terminal segment in α-helix conformation, followed by two fragments arranged in antiparallel β-sheet configuration.

3. A C-terminus intracellular segment. The amyloidogenic β-peptide (positions 597–635 of $βPP_{695}$) is located in part in the transmembrane domain (11–15 amino acids) and in part in the adjacent extracellular region (28 residues) (Fig. 1).

The βPP gene contains at least 19 exons, spanning more than 190 kb; more than 10 isoforms of βPP may be generated *(158–171)*. Four βPP mRNAs encode proteins of 695, 714, 751, and 770 *(162–165)* amino acids that contain the Aβ peptide. $βPP_{751}$ and $βPP_{770}$ contain a Kuntiz β type protease inhibitor domain. βPP undergoes tyrosine sulfation, N-, and O-glycosylation. βPP has also been shown to have a metalloproteinase inhibitor domain *(172)*. The Aβ sequence arises from portions of exons 14 and 15 (using $βPP_{695}$ numbering or exons 16 and 17 using $βPP_{770}$ numbering). Therefore, Aβ cannot be generated by alternative splicing of βPP, but requires proteolytic cleavage of βPP both at its N- and C-terminals. With these discoveries, a key question becomes how βPP generates Aβ.

The first metabolic pathway of βPP discovered involved a cleavage at Aβ residue 16 by "secretase," releasing a large soluble protein, protease nexin II *(173–177)*. Obviously this pathway precludes amyloid formation. At first it was believed that this was the major normal metabolic pathway for βPP and that Aβ is generated only by an abnormal pathway.

Recent work has found a soluble Aβ-like peptide (sAβ) in media from cell cultures of untransfected and βPP transfected cells, as well as in cerebrospinal fluid (CSF) and plasma from normal and AD patients *(178–181)*. This has been determined using

amino acid sequencing, mass spectroscopy, and immunoprecipitation with specific antisera. Initial amino acid sequence analysis indicated that sAβ is homologous to the amyloid protein extracted from cerebrovascular lesions, Aβ1–40 *(180)*. Further studies using affinity chromatography, sequence analysis, and laser desorption mass spectometry identified sAβ sequences with heterogeneity and length similar to the Aβ components deposited in senile plaques and vascular amyloid *(182,182a)*. Therefore, it is conceivable that these forms of sAβ may represent the immediate precursors of Aβ.

If such a hypothesis is true, AD is linked more closely to several types of systemic amyloidosis, such as amyloid A and immunoglobulin light-chain related amyloidosis, where the soluble amyloid precursor circulates normally *(5)*. This point of view predicts that other β-amyloid related disorders (βAD) *(5)* will be found outside the brain such as inclusion body myositis *(183)*, and that Aβ can be a reactive amyloid induced by an exogenous or endogenous agent. Furthermore, it is likely that the vascular compartment is the origin of Aβ in congophilic angiopathy and preamyloid deposits. The Dutch variant of familial AD is a strong argument in favor of this *(184)*. The latter disease is characterized by the deposit of amyloid mainly in the cerebral vasculature *(185,186)*, with a halo of Aβ immunoreactivity in the parenchyma surrounding many of the vessels, suggesting diffusion of Aβ out of the vascular compartment. This possibility is supported by the recent finding that Aβ1–40 can cross the blood–brain barrier. Classic neuritic plaques do not occur. The amyloid fibrils from this autosomal dominant disease are known to consist of Aβ *(187)* with an amino acid substitution at position 22 (glutamine for glutamic acid) *(188)* that is caused by a single base transition *(189)*.

Unlike the Dutch variant, in sporadic AD it is possible that the origin of the amyloid is mixed, since neuronal cells in culture can also produce sAβ. Whether or not sAβ is the direct precursor of amyloid, it is clear that the same amino acid sequence can have both a fibrillar and soluble conformation. This is similar to the transthyretin and β_2-microglobulin related amyloidoses, where there is an intact circulating amyloid precursor that can polymerize into amyloid fibrils, undergoing a conformational change into β-pleated sheet structure.

A key question in the pathogenesis of AD is what factors determine this conformational alteration. Synthetic peptides Aβ1–40 and their shorter derivatives, SP28(Aβ1–28) and SP14 (Aβ15–28), form fibrils in vitro *(190–194)*. Aggregation of peptides is partially related to concentration. This factor may be important in DS where the circulating levels of βPP are raised *(195)*. Studies using fibrils have also shown that the pH, oxidation, salt, and metal ion concentrations greatly influence fibril formation *(190–194)*. The presence of certain βPP mutations undoubtedly is also important. For example, transfected cells with the Swedish familial AD mutation (at codons 595 and 596 of βPP_{695}) produce higher quantities of sAβ *(196)*. On the other hand, the Dutch familial AD mutation (at codon 618 of βPP_{695}, corresponding to residue 22 of Aβ) accelerates the rate of fibrillogenesis *(197,198)*. This has been documented by electron microscopy of peptides homologous to Aβ in solution, as well as by infrared spectroscopy and circular dichroism studies *(197–199)*. An additional study documented greater stability of Dutch mutation-containing peptides *(200)*. Interestingly, the Dutch mutation-containing peptide fibrils are able to act as instructors or templates speeding fibril formation by nonmutation-containing peptides in vitro *(201)*, correlating with the presence of both mutant and nonmutation-containing Aβ in Dutch vascular lesions *(188)*.

Over 10 different mutations of the βPP gene have been found to be associated with either early-onset FAD or the Dutch variant of AD *(202–209)* (Table 2). Several other clinically silent mutations are also known. The vast majority of AD patients do not have any of these mutations; therefore, other factors must play a role. The known mutations together account for only about 5% of all early-onset FAD cases. Furthermore, many early-onset FAD kindreds have linkage to chromosome 14 *(210,211)* and some late-onset pedigrees have been linked to chromosome 19 *(212)*. The effect of most of these mutations is not known; however, they appear to accelerate a pathological process that can occur without their presence.

Recently the apolipoprotein E (ApoE) gene on chromosome 19 has been linked to sporadic and late-onset AD. There are three common ApoE alleles: E2, E3, and E4 *(213)*. An increased risk of developing late-onset AD is linked to the presence of ApoE4 *(214–216)*. It is not clear how the ApoE4 allele mediates such an effect; however, for some time it has been known that ApoE is associated with all cerebral and systemic amyloidoses by both biochemical and immunohistochemical methods *(20,21,217)*. Furthermore, it is known that ApoE binds to sAβ *(214,218)*, as does another apolipoprotein, ApoJ *(219)*. It has been proposed that ApoE

is a pathological chaperone *(20)* acting to mediate a conformational change to an amyloid fibril. It is possible that ApoE4 in particular plays such a role. Other proteins that bind sAβ may act to inhibit fibril formation *(220)*. From this data a complicated picture emerges, where factors such as the ApoE4 allele and βPP mutations can be regarded as one of many possible risk factors for AD.

Hereditary Cerebral Hemorrhage with Amyloidosis, Icelandic Type

An autosomal dominant form of congophilic angiopathy, ACys, is found in patients with hereditary cerebral hemorrhage with amyloidosis of Icelandic type (HCHWA-I), also named familial cerebral amyloid angiopathy of Icelandic origin *(221)*. The disease affects eight different families in western and southern Iceland, leading to death before the age of 40 yr because of massive intracerebral hemorrhages *(222)*. The pathological findings show cerebrovascular involvement owing to amyloid fibril infiltration of the walls of small arteries and arterioles in the cerebral cortex and leptomeninges, similar to HCHWA-D. Amyloid deposits have recently been detected in other organs, including lymph nodes, spleen, adrenal gland, ovary, testis, appendix, peripheral nerves, and skin *(223)*. The amyloid protein is a degradation product (Mr = 12 kDa) of cystatin C, containing one amino acid substitution (glutamine for leucine) at position 68 and missing 10 residues at the amino terminus *(224,225)*. Cystatin C is a 120-residue basic protein with cysteine protease inhibitory activity *(226,227)*, a property shared by the members of the cystatin superfamily that include stefins, cystatins, and kininogens *(228)*. The protein is normally present in serum and is particularly concentrated in CSF and seminal fluid *(229)*. Patients with HCHWA-I have low levels of cystatin C in CSF *(230)*. DNA studies indicate that the cystatin C variant gene expressed in patients with HCHWA-I contains a single nucleotide change that accounts for the amino acid substitution *(231)*. The presence of the mutation in codon 68 abolishes an Alu I restriction site and can be used as a diagnostic test *(232)*.

Prion-Related Diseases

Transmissible spongiform encephalopathies in humans (also named human prion diseases) are a group of disorders characterized by neuronal spongiform degeneration, astrocytic gliosis, and parenchymal amyloid deposition in the form of amyloid plaques. This group includes Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker disease (GSS), and kuru (for review, *see* ref. *233*). These neurodegenerative diseases have pathologic characteristics similar to those of scrapie, a transmissible disease of sheep and goats *(234)*. The transmissible agent is a proteinaceous particle, named prion, that contains little or no nucleic acid *(235)*. The amyloid fibrils deposited in these human diseases (APrP) derive from a protease-resistant isoform of the prion protein (PrP^{Sc}) *(236)*, a 33–35 kDa neuronal glycoprotein. Amyloid deposition is always present in GSS, but is variably present in other prion-related diseases. Autosomal dominant forms of human prion diseases account for 5–15% of CJD and most of the GSS cases *(237,238)* and several variants have been described *(239)*. Interestingly, two variants of GSS (Indiana and Swedish kindreds) are associated with neurofibrillary tangles similar to that found in AD *(240)*. As in other amyloidoses, different clinical pictures can be associated with the same PrP mutation and the disease can occur without any mutation *(239)*. A key feature of prion-related disease is the conformational change that the PrP^{Sc} can instruct to the native protein. It has also been shown that synthetic peptides corresponding to different mutated regions of the PrP gene have enhanced fibril formation and can also speed fibril formation in nonmutant peptides *(241)*. This is similar to Aβ amyloid where the Dutch mutation, which is endowed with an increased amyloidogenic potential *(197,198)*, can facilitate or instruct *(201)* the conversion of an amyloidogenic normal precursor to amyloid.

Mechanism of Amyloid Fibril Formation

The exact mechanism of amyloid fibril formation and deposition remains unknown. However, in spite of the biochemical diversity of the amyloid proteins, similar factors seem to contribute to fibrillogenesis:

1. Primary structure: Undoubtedly, the amino acid sequence of the amyloid subunit plays a key role in fibril formation. It has been observed that certain proteins derived from very closely related genes are more amyloidogenic than other members of the same family, e.g., the preferential association of the lambda subgroup VI with amyloidosis.
2. Precursor protein: A precursor protein has been demonstrated in all amyloid diseases,

some of which are detected in the circulation. In most of the cases, the amyloid peptide is generated by proteolytic cleavage of the corresponding precursor. There is no specific pattern for cleavage: Some amyloid subunits are N-terminus degradation fragments (L-chains in AL; AA in secondary amyloidosis and FMF), some are C-terminus segments (cystatin-C variant in HCHWA-I), and others are internal proteolytic fragments (Ab in AD and DS; Ab-variant in HCHWA-D; gelsolin variant in FAF). In some cases the precursor protein itself, without degradation, forms the amyloid fibril (b2-microglobulin in long-term hemodialysis; transthyretin monomers in FAP). Increased serum levels of the protein precursor, reflecting overproduction, impaired clearance, or both, is not an infrequent situation, but only a small percentage of patients with high serum levels of amyloid precursors develop amyloidosis.

3. Post-translational modifications: Modification of particular amino acids may be important either in the susceptibility of the precursor to proteolysis and/or polymerization of the resulting peptides. These modifications may include oxidation, phosphorylation, glycosylation, methylation, and so forth. In this regard, oxidized forms of methionine have been reported for the Aβ in AD and in HCHWA-D *(147,188)*.
4. Tissue specificity: It has been shown that different size degradation products may be present in different tissues of the same patient (e.g., AA) *(242)*. Moreover, fibril formation takes place in selected organs in localized forms of amyloidosis. Therefore, tissue-specific enzymes as well as other factors (receptors, amyloid associated proteins, binding sites, and so on) may be responsible for different patterns of amyloid deposition. The presence of Aβ of different length in brain vessel walls and senile plaques in patients with βAD is a good example, and suggests the involvement of enzymes and/or inhibitors characteristic of the organ affected.
5. Amino acid substitutions: Point mutations in genes encoding amyloid precursor proteins have been detected in different types of familial amyloidosis. These mutations may increase the inherent fibrillogenicity of the protein or inhibit normal processing, leading to increased levels. On the other hand, mutations leading to protein variants are not a prerequisite for fibril formation, since in several pathological conditions the primary structure of the amyloid protein appears to be normal. Thus, in conditions where the primary structure of the amyloid precursor protein is normal, other mechanisms (altered expression, post-translational changes, and/or inhibition of normal processing of the amyloid precursor protein) must play a crucial role in the process of amyloid formation.
6. Chaperone proteins: The presence of amyloid-associated or chaperone proteins of a diverse chemical nature within the amyloid deposits may be related to the process of amyloidogenesis. These chaperone proteins include amyloid P component *(17,18)*, α_1-antichymotrypsin *(243)*, glycosaminoglycans *(19)*, apolipoprotein J *(22)*, and apolipoprotein E *(20)*. These proteins may either act to inhibit amyloid formation or to act as pathological chaperones *(20)*, inducing a β-pleated sheet structure. Of interest, two proteins, both apolipoproteins, apolipoprotein E *(214–216,218)* and apolipoprotein J *(219)*, have been shown to bind sAβ. Immunoprecipitation with anti-ApoJ *(219)* or, to a lesser extent, anti-ApoE (unpublished observations) retrieves sAβ from CSF, indicating the existence of a complex between sAβ and these two apolipoproteins. Significantly, the ApoE allele E4 has been linked to late-onset familial and sporadic AD *(214–216)*. Hence, this particular form of ApoE may be important in driving the conformational change from sAβ to amyloid. The other amyloid chaperone proteins are also suspected of playing a role in the conformational state. α_1-Antichymotrypsin has been shown to influence fibril formation by synthetic peptides homologous to Aβ *(244)*. It has been found that fibril formation by synthetic peptides homologous to Aβ 1–40 is inhibited in the presence of cerebrospinal fluid *(220)*, hence in physiological fluids one or more "desaggrins" must exist. On the other hand, glycosaminoglycans have been shown to increase the β-sheet structure of SAA_2 *(51,245)*. This data suggests that the amyloid chaperone proteins play a complex role in determining the balance between a soluble protein and the amyloid fibril.

Future Directions

1. Additional studies using synthetic peptides and sAβ are needed in order to determine the factors necessary for the conformational change that occurs with fibril formation. Such

studies can determine the relationship between conformational changes, amyloid chaperone proteins, and post-translational modifications, as well as their influence on the cytotoxicity of Aβ and other amyloids.
2. Biochemical analysis of the enzymes and pathways involved in precursor degradation may clarify how alterations in the amyloid precursor may influence normal degradation. Once the normal and/or abnormal catabolic pathways are known, new pharmacological approaches will be possible.
3. Application and development of diagnostic tests to determine individuals at high risk of developing amyloidosis, such as screening for ApoE alleles.
4. Development of animal models. The fact that patients with familial amyloidosis express the variant gene, which may accelerate amyloid deposition in transgenic mice, provides an important model to analyze the mechanisms of amyloid formation and test for novel treatments.

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