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Molecular Biology of Alzheimer's Amyloid—Dutch Variant

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

Departments of [1]Neurology and [2]Pathology, New York University Medical Center, 550 First Avenue, New York, NY 10016

Contents

Abstract

Hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D) (or familial cerebral amyloid angiopathy) and familial Alzheimer's disease (FAD) share several properties. Both are autosomal dominant forms of cerebral amyloidosis characterized by β-amyloid (Aβ) deposition. In HCHWA-D the Aβ is predominantly found in blood vessels and in early parenchymal plaques, whereas in AD parenchymal Aβ deposits in the form of senile plaques and neurofibrillary tangles are a more prominent finding. Point mutations in the amyloid precursor protein (APP) have recently been described, in both conditions. A G to C transversion at codon 618 (extracellular portion of APP_{695}), producing a single amino acid substitution of glutamine instead of glutamine acid, occurs in HCHWA-D; whereas mutations at codon 642 in the intramembrane region of APP_{695} (phenyl-alanine, isoleucine, or glycine instead of valine) are associated with early onset FAD. This suggests that the site of particular mutations in the APP gene and the type of amino acid substitution in the APP holoprotein are more important in determining clincopathological phenotype and age at which Aβ is deposited. Thus FAD and HCHWA-D can be regarded as two sides of the same coin.

Index Entries: Alzheimer's disease, β-amyloid, amyloid, familial Alzheimer's disease, Hereditary Cerebral Hemorrhage with Amyloidosis, Dutch type.

*Author to whom all correspondence and reprint requests should be addressed.

Introduction

Hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D) is a well characterized, albeit rare autosomal dominant form of cerebral amyloidosis (Wattendorf et al., 1982; Luyendijk et al., 1988). Recent findings into the molecular biology and protein chemistry of this disease have provided insights into a closely related and very common disorder, Alzheimer's disease (AD). HCHWA-D was first described in four large families from two coastal villages in the Netherlands. Three families (136 patients) were from Katwijk, and one family (14 patients) was from Scheveningen (Wattendorf et al., 1982; Luyendijk et al., 1988). More than 500 individuals are at risk for developing the disease in Holland (Hann et al., 1989).

Clinically, HCHWA-D presents between the ages of 45 and 65 with a stroke (Wattendorf et al., 1982; Luyendijk et al., 1988) that is fatal in approx 50% of patients (Luyendijk et al., 1988; Haan et al., 1989). The remainder go on to have further strokes. The latter patients, on neuropsychiatric evaluation, show cognitive deficits, which in a majority, are sufficient to be diagnosed as dementia (Haan et al., 1990). Although multiple strokes, with resultant loss of brain tissue are the major cause of the dementia, some patients also have a progressive impairment of mental function unrelated to stroke (Haan et al., 1991). Neuropathologically, there is extensive deposition of amyloid in the small cerebral arteries and arterioles (Figs. 1A,B). In addition, there are deposits of amyloid in the parenchyma that resemble the early, preamyloid, or diffuse plaques of AD (Timmers et al., 1990).

Mature plaques with an amyloid core are absent. Neurofibrillary tangles, an important feature of AD, are also absent; however, occasional ubiquitin immunoreactive profiles suggestive of dystrophic neurites are present in the vicinity of amyloid deposits (Fig. 1C). The amyloid fibrils in HCHWA-D leptomeningeal vessels are a 39 amino acid peptide (van Duinen et al., 1987) that is similar to the β-protein (Aβ) found in cerebrovascular amyloid deposits in AD, Down's syndrome (DS), and cerebral congophilic angiopathy (Glenner and Wong, 1984; Masters et al., 1985; Wong et al., 1985; Selkoe et al., 1986; Prelli et al., 1988). It is apparently a few amino acids shorter at the carboxyl terminus than Aβ isolated from senile plaques in AD (Prelli et al., 1988).

Aβ and Amyloid Precursor Protein

Aβ is a 4 kDa (Glenner and Wong, 1984; Masters et al., 1985; Wong et al., 1985; Selkoe et al., 1986; Prelli et al., 1988) internal proteolytic fragment of a larger amyloid precursor protein (APP), a putative membrane-spanning glycoprotein encoded by a gene located on chromosome 21 (Goldgaber et al., 1987; Kang et al., 1987; Robakis et al., 1987; Tanzi et al., 1987). The APP gene contains at least 18 exons, spanning more than 170 kb (Lemaire et al., 1989; Yoshikai et al., 1990). Thus far, the APP gene has been found to encode six different mRNAs (Kitaguchi et al., 1988; Ponte et al., 1988; Tanzi et al., 1988; De Sauvage and Octave, 1989; Golde et al., 1990; Jacobsen et al., 1991); all of these produce proteins identical at the amino terminus. Two of the mRNAs encode proteins of 365 (Jacobsen et al., 1991) and 563 (De Sauvage and Octave, 1989) amino acids that do not contain the Aβ peptide. The other four known APP mRNAs (Kitaguchi et al., 1988; Ponte et al., 1988; Tanzi et al., 1988; Golde et al., 1990) encode proteins of 695, 714, 751, and 770 amino acids. The 751 and 770 proteins contain a 56 amino acid domain that has 50% homology to the Kunitz family of serine protease inhibitors. The 714 and 770 proteins have a 19 amino acid domain homologous to the MRC OX-2 antigen found on the membranes of neurons and thymocytes (Weidemann et al., 1989). The Aβ sequence arises from portions of exons 14 and 15 (using APP695 numbering or exons 16 and 17 using APP770 numbering); hence, Aβ cannot be generated by alternative splicing and presumably is the result of altered proteolytic cleavage of APP. Aβ is made

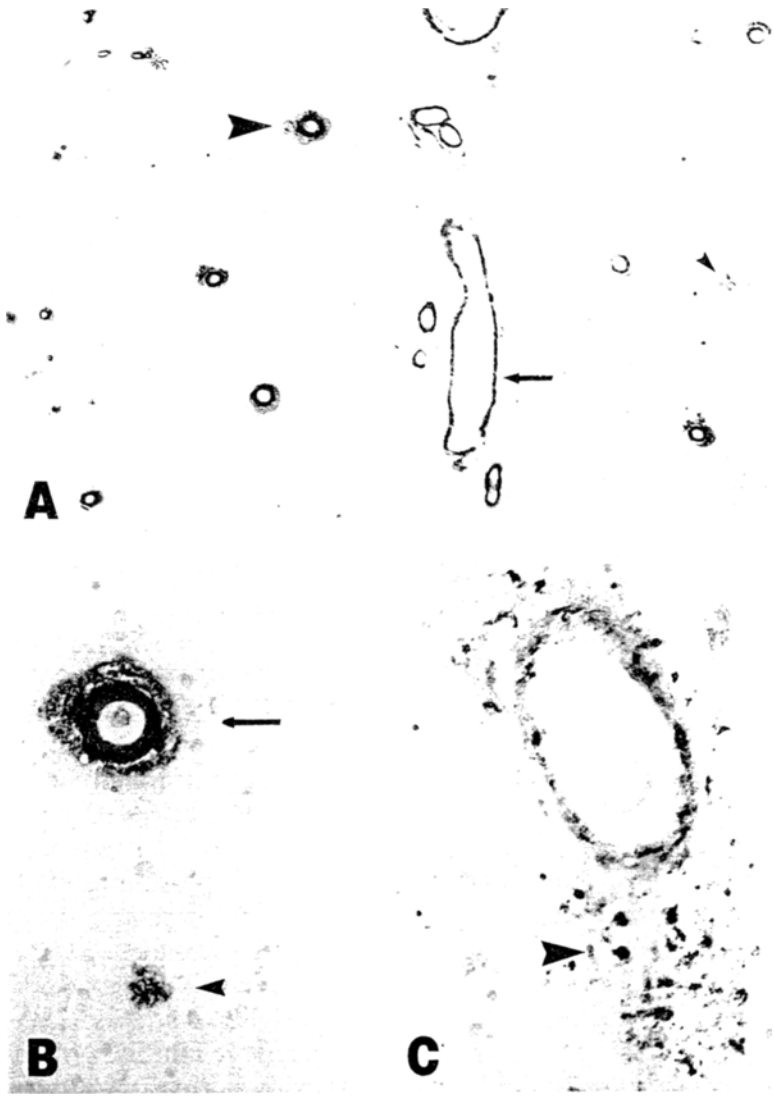

Fig. 1. Sections of frontal cortex from a HCHWA-D patient. **A:** The section was immunostained with polyclonal antibodies raised against the first 28 residues of Aβ (anti-SP28). Aβ can be seen in leptomeningeal vessels (arrow), parenchymal vessel walls, parenchyma surrounding amyloid-laden vessels (large arrowhead), and preamyloid or diffuse plaques (small arrowhead). **B:** Higher magnification of amyloid-laden vessel wall surrounded by amyloid extending into the parenchyma (arrow) and a diffuse plaque (arrowhead), immunostained with anti-SP28. C: Immunostaining with polyclonal antibodies to ubiquitin of amyloid-laden vessel. Ubiquitin-reactive profiles (arrowhead) suggestive of dystrophic neurite processes surround the vessel.

up of a membrane-spanning domain (11–14 residues) and part of the predicted extracellular domain region adjacent to the membrane (28 residues) (Kang et al., 1987; Dyrks et al., 1988; Fig. 2).

Mutation in HCHWA-D and FAD

Recently, HCHWA-D has been found to segregate with a mutation in the APP gene at nucleotide 1852 in exon 15 (APP695 numbering), where

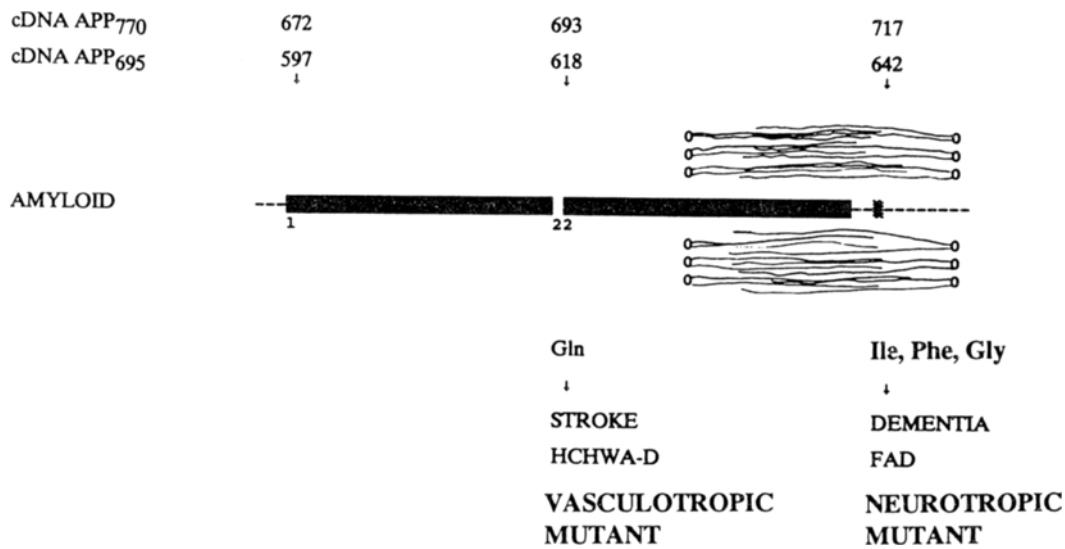

Fig. 2. Illustration of the Aβ sequence of APP with its relationship to the cell membrane. Codon numbering according to both APP770 and APP695 is given on the top. At codon 22, within the ectodomain of Aβ ( ), the glutamic acid to glutamine substitution found in HCHWA-D is shown. At codon 642 of APP695, the FAD mutations of ( ) valine to either isoleucine, phenylalanine, or glycine is shown. Mutations in the APP at these two sites are either neurotropic with predominantly senile plaque and neurofibrillary tangle formation or vascultropic with mainly congophilic angiopathy.

guanine is replaced by cytosine (Levy et al., 1990). The latter causes a single amino acid substitution of glutamine instead of glutamic acid at codon 618 of APP 695 and corresponding to residue 22 of the Aβ (Fig. 2). The significance of this mutation is corroborated by close linkage, with a Lod score of 7.59, between the APP gene and the disease (Van Broeckhoven et al., 1990).

Furthermore, this mutation has been found in all Dutch amyloidosis-affected patients tested so far (Bakker et al., 1991; Fernandez-Madrid et al., 1991). The finding of a mutation in the APP gene in HCHWA-D sparked off a renewed search for a mutation in AD. Analysis of numerous familial AD (FAD) pedigrees over the last 4 yr had shown them to be genetically heterogenous. Some families with early onset symptoms had linkage to chromosome 21 (Pericak-Vance et al., 1988; St George-Hyslop and Members of the FAD Collaborative Study Group, 1990), whereas others with late onset or early onset did not (Goate et al., 1989; St. George-Hyslop et al., 1990; Schellenberg et al., 1991).

Recently, a mutation was found in some early onset FAD families (Goate et al., 1991), where there is a C to T transition at nucleotide 1924 in exon 15 (APP_{695} numbering), causing a valine to isoleucine change at codon 642 (Fig. 2). This is three to four residues away from the known carboxyl terminus of the Aβ, obtained from senile plaques. This mutation was originally found in two out of 16 families with early onset FAD and was not detected in 100 normal unrelated individuals or from nine different families with late onset FAD (Goate et al., 1991). This mutation has also been found in several Japanese early onset FAD families (Yoshioka et al., 1991; Naruse et al., 1991) and one French FAD family (Lucotte et al., 1991), indicating segregation of the mutation with FAD in racially different populations. However, this mutation is relatively rare even among early onset FAD families (Schellenberg et al., 1991). Two further mutations at codon 642 have been reported in different families, where the valine is replaced by either phenylalanine (Murrell et al., 1991) or glycine (Chartier-Harlin et al., 1991).

These reports of mutations in the APP gene show that mutations at different sites can result in a clinical picture that is qualitatively and quantitatively distinctive. It appears that some mutations in the APP gene are neurotropic, with prominent senile plaque and neurofibrillary tangle formation, and others are vasculotropic, with deposition of amyloid predominantly in blood vessels, as in HCHWA-D. This segregation of APP mutations with two related syndromes implies that APP mutations are likely to be responsible for different β-amyloid–related disease phenotypes.

AD and HCHWA-D, Two Sides of the Same Coin

The finding of a mutation in the APP gene in both HCHWA-D (Levy et al., 1990) and at least some FAD cases (Goate et al., 1991; Lucotte et al., 1991; Naruse et al., 1991; Murrell et al., 1991; Chartier-Harlin et al., 1991) raises the question of whether these are really two separate disorders or different faces of a similar pathological process. The neuropathological features of AD (Probst et al., 1991) include senile plaques (Simchowicz, 1911) and neurofibrillary tangles (NFTs) (Alzheimer, 1907), as well as in more than 90% of cases, amyloid deposits in cerebral blood vessels (Glenner et al., 1981; Joachim et al., 1986), similar to that seen in HCHWA-D. In AD, NFTs are thought by many investigators to be a secondary change (Wisniewski K., et al., 1979; Wisniewski H. M., et al., 1989), indicating a pathological response of neurons to Aβ deposition. This is suggested by data from DS patients, where AD changes are noted very early in life. When DS brains are examined at various ages, it is the senile plaques that are the first change (Giaccone et al., 1989; Mann, 1989; Motte and Williams, 1989; Rumble et al., 1989). Neurofibrillary tangles occur in diverse neuropathological conditions, such as subacute sclerosising panencephalitis, postencephalitic Parkinson's disease, dementia pugalistica, and supranuclear palsy (Wisniewski et al., 1979); thus, they appear to be part of the neuron's limited repertoire of responses to injury. HCHWA-D patients often die early in life as a result of their first stroke (Wattendorf et al., 1982; Luyendijk et al., 1988; Haan et al., 1991). A majority of those that survive also become demented (Haan et al., 1990). It is not known whether these individuals would develop NFTs if they lived long enough. Indeed, immunohistochemical studies with ubiquitin antibodies suggest the presence of dystrophic neuritic processes, which accompany NFTs in AD, evident in the vicinity of the preamyloid and diffuse plaques of HCHWA-D patients (Fig. 1C).

Effect of the Dutch Mutation on Fibrillogenesis

The finding of a point mutation within the APP in HCHWA-D raises the question of how a substitution of glutamic acid for glutamine at codon 618 could promote fibrillogenesis. Significantly, in HCHWA-Icelandic type, another autosomal dominant form of cerebral amyloidosis (Hann et al., 1989), there is also a point mutation in the precursor protein's gene (Levy et al., 1989; Palsdottir et al., 1988) resulting in the substitution of an amino acid for glutamine (Ghiso et al., 1986). The neuropathological picture in the Icelandic amyloidosis is similar to the Dutch (Hann et al., 1989), although the precursor molecule in the former is a variant of cystatin C (Ghiso et al., 1986; Levy et al., 1989; Palsdottir et al., 1988), an inhibitor of cyteine proteinase. Recent evidence indicates that the single amino acid substitution at residue 22 of Aβ alters its fibrillogenic properties. Prior studies have shown that synthetic peptides corresponding to residues 1–28 of Aβ, form Congo Red-positive fibrils in vitro under physiological conditions (Castaño et al., 1986; Kirschner et al., 1987).

The presence of the glutamine at residue 22 accelerates this fibril formation (Wisniewski et al.,

1991). Furthermore, it was shown that a synthetic peptide corresponding to residues 21–28 of Aβ (SP8), has fibrillogenic qualities only when glutamine is present as the second residue (SP8Q) (Wisniewski et al., 1991) (Fig. 3). Isolated amyloid from Dutch patients contains both the mutated and nonmutated forms (Prelli et al., 1990); hence, it is possible that these can exist as a dimer in amyloid fibrils and their precursors. Such studies (Barrow and Zagorski, 1991; Hilbich et al., 1991; Wisniewski et al., 1991) suggest that when Aβ or smaller fragments of Aβ are released from APP, they can directly assemble into amyloid fibrils. The presence of the Dutch mutation seems to accelerate this process (Wisniewski et al., 1991).

It remains unknown what processing steps release these fragments of APP. It is possible that the presence of an amino acid substitution at position 22 of Aβ could cause the APP to be processed abnormally, but this is unlikely as no such mutation is found in the amyloid of leptomeningeal vessels in AD or cerebral congophilic angiopathy. It is likely that the type of amino acid substitution and localization of the mutation in the APP gene is more important in determining the rate, site, and age at which fibrils are deposited. Normal processing of the APP, releasing soluble derivatives, occurs by cleavage within the Aβ sequence, as first shown in CHO cells transiently transfected with a series of expression constructs where varying regions of the APP were missing (Sisodia et al., 1990). Direct sequencing of the soluble derivative released by this intra Aβ cleavage shows that it occurs at Aβ 16 (Oltersdorf et al., 1989; Esch et al., 1990; Wang et al., 1991).

Postcleavage, the lysine at residue Aβ 16 is removed from the secreted amino terminal fragment by a basic carboxyl peptidase (Wang et al., 1991). The cleavage enzyme has been named *APP secretase* (Esch et al., 1990). This pathway is presumably the major one in normal brains. Evidence of different pathways is now emerging. A 16–19 kDa immunoreactive protein containing Aβ and the carboxyl end of the APP has been isolated from microvessels purified from leptomeninges of normal and AD brains (Ghiso and Frangione, 1992; Norstedt et al., 1991; Gandy et al., 1992; Estus et al., 1992; Golde et al., 1992). The concentration of different species of the APP holoprotein and carboxyl terminal fragments in human brains from young individuals, nondemented aged, and AD patients has been measured. A 19 kDa fragment that presumably contains the Aβ and the carboxyl terminus of the APP was found to increase two to threefold with age and in AD individuals (Nordstedt et al., 1991) (Fig. 4). The identification of potentially amyloidogenic carboxyl-terminal fragments of APP provides a useful system to study the conversion of APP to Aβ.

HCHWA-D and Implications for the Origin of Aβ in AD

A debate has raged the last few years over whether Aβ originates from a vascular (Castaño and Frangione, 1991) or neuronal (Beyreuther and Masters, 1991) source in AD. In HCHWA-D, it is likely that either cellular elements of the blood or cells intrinsic to blood vessels (van Duinen et al., 1987) are the source of Aβ. The senile plaques of the Dutch disease also seem related to blood vessels. Penetrating amyloid-laden vessels often have a halo of Aβ immunoreactivity extending into the surrounding neuropil. Immunohistochemical studies using antibodies to Aβ and various regions of the APP suggest that the APP and amyloid fibrils coexist in the same vessel in AD and HCHWA-D and that APP immunoreactivity is present in a large number of senile plaques in AD (Tagliavini et al., 1990). Such information indicates that the vascular system is the source of the APP and that processing into Aβ occurs *in situ*.

In HCHWA-D, despite the presumed vascular source of Aβ, there is no evidence thus far of extracranial Aβ deposition. However, in Icelandic amyloidosis, there is clearly evidence for extracranial amyloid, although in this disease, the amyloid precursor is cystatin C, which is a serum protein (Benedikz et al., 1990). Given the many similarities between Dutch amyloidosis and AD,

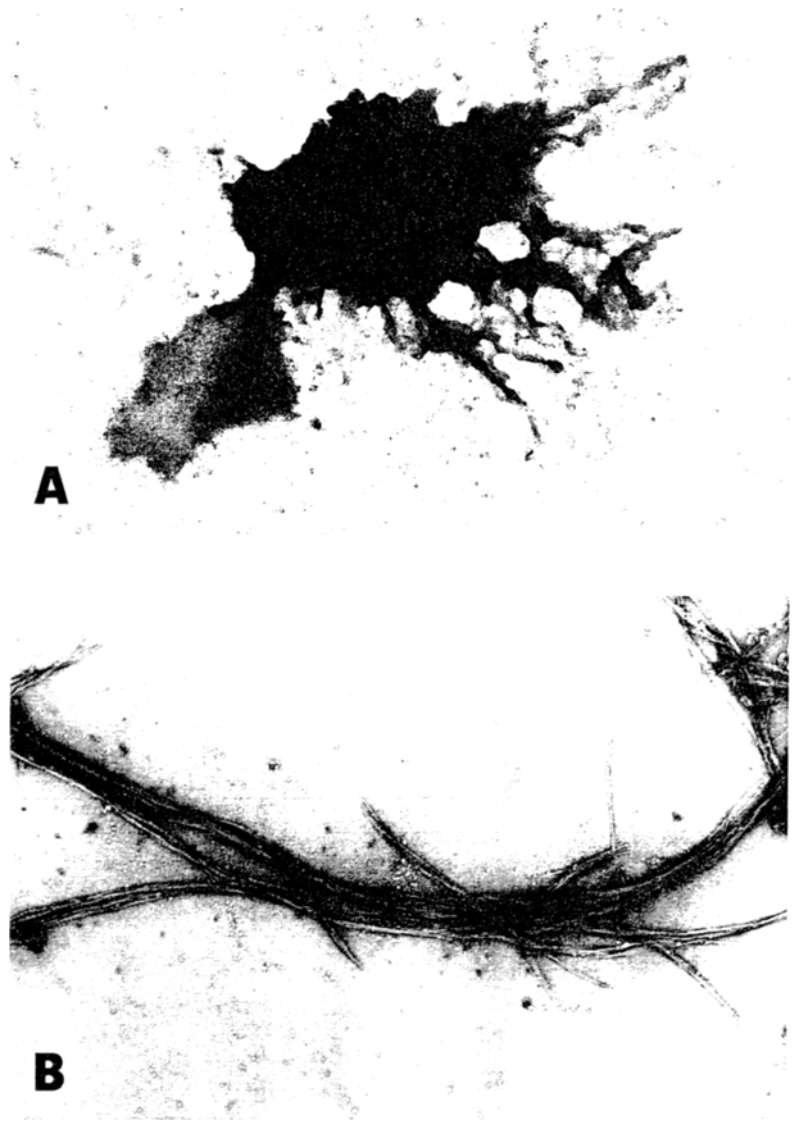

Fig. 3. **A**: Electron micrograph of a synthetic peptide preparation corresponding to amino acids 21–28 (SP8) of Aβ. No fibril formation is present. **B**: Electron micrograph of fibril formation by a synthetic peptide the same as SP8, expect for the presence of the Dutch amyloidosis mutation of glutamine for glutamic acid at position 22 of Aβ.

it is possible to extrapolate a vascular origin for Aβ in the latter. A vascular source for amyloid in these cerebral diseases would mirror a similar process in other systemic amyloidoses, where local deposition of amyloid is known to be derived from circulating precursors. Examples include immunoglobulin amyloid in multiple myeloma and secondary systemic amyloidosis, AA amyloid in the secondary amyloidosis of chronic inflammatory disease, and transthyretin in familial amyloidotic polyneuropathy. However, an intra-cranial origin for Aβ cannot be ruled out given the brain-restricted topography of the lesions in AD. The issue of a vascular or intracranial source for Aβ remains unresolved in AD and awaits further evidence.

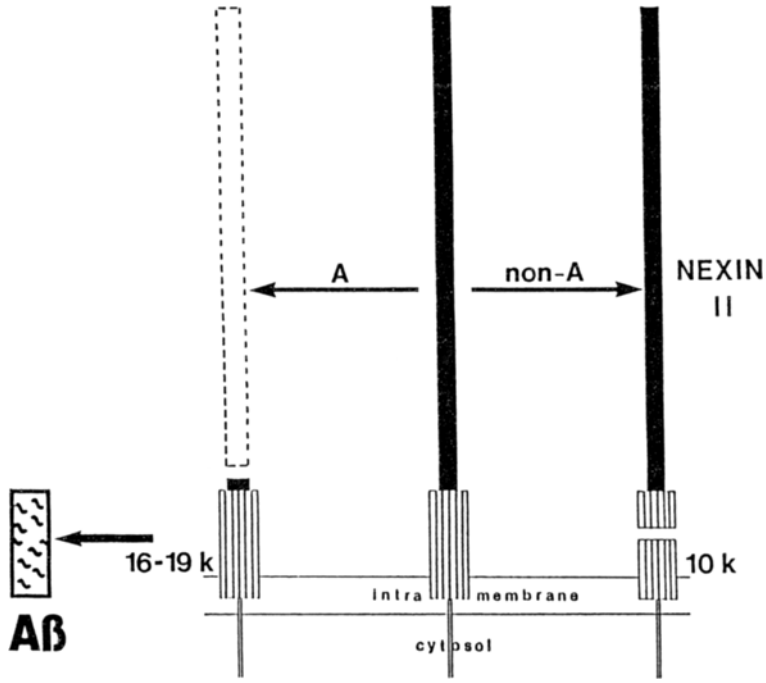

Fig. 4. Alternative processing of AD APP in vessel walls. The APP (center of figure) can be processed in at least two ways. The nonamyloidogenic pathway (non-A) is presumably the major normal route, with cleavage occurring within the Aβ sequence. This normal cleavage of APP releases soluble derivatives (Nexin II) and leaves a small 10 kDa membrane fragment. This pathway cannot produce Aβ. The amyloidogenic pathway (A) is presumably very minor under normal conditions. Here cleavage occurs closer to the amino terminal of Aβ, resulting in a larger 16–19 kDa membrane fragment that contains the entire Aβ sequence. Alternation of the normal processing of this fragment can release the fibrillogenic Aβ.

Lessons from HCHWA-D

HCHWA-D provides a powerful example of how the study of a rare disorder provides lessons about more common disorders. The finding of a point mutation in Dutch amyloidosis (Levy et al., 1990) and in a subset of early onset FAD patients (Goate et al., 1991; Naruse et al., 1991; Yoshioka et al., 1991; Murrell et al., 1991; Chartier-Harlin et al., 1991) indicate that mutations at different sites of the APP can produce distinctive pathological presentations. However, the Aβ fibril can also be deposited in leptomeningeal vessels without the presence of the Dutch mutation, albeit at a later age, in AD and sporadic cerebral amyloid angiopathy (CAA). It is likely that multiple factors can influence Aβ deposition, including a mutation in the APP gene, posttranslational modification and/or other still-unknown extrinsic factors.

The recent finding of different processing pathways for APP in AD and aged brains raises the interesting question of how the Dutch mutation influences channeling of APP metabolism down each pathway. It can be predicted that Dutch amyloid mutation containing APP will be catabolized more rapidly, as occurs in Icelandic amyloid, where levels in cerebrospinal fluid (CSF) of cystatin C are reduced by approx 1/2 in affected individuals (Grubb and Lofberg, 1985). Further studies are under way in transgenic mice and cells lines transfected with various transcripts containing the Dutch mutation to evaluate their amyloid fibril potential. The clear delineating of the causative mutation in HCHWA-D can illuminate the origin of amyloid in AD and its influence in the aging process (Coria et al., 1987). Interventions inhibiting amyloid deposition can then be tested, with resultant therapeutic benefits.

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