

# Cerebral amyloidosis, amyloid angiopathy, and their relationship to stroke and dementia

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Cerebral amyloid angiopathy (CAA) is the common term used to define the deposition of amyloid in the walls of medium- and small-size leptomeningeal and cortical arteries, arterioles and, less frequently, capillaries and veins. CAA is an important cause of cerebral hemorrhages although it may also lead to ischemic infarction and dementia. It is a feature commonly associated with normal aging, Alzheimer disease (AD), Down syndrome (DS), and Sporadic Cerebral Amyloid Angiopathy. Familial conditions in which amyloid is chiefly deposited as CAA include hereditary cerebral hemorrhage with amyloidosis of Icelandic type (HCHWA-I), familial CAA related to  $A\beta$  variants, including hereditary cerebral hemorrhage with amyloidosis of Dutch origin (HCHWA-D), the transthyretin-related meningocerebrovascular amyloidosis of Hungarian and Ohio kindreds, the gelsolin-related spinal and cerebral amyloid angiopathy, familial PrP-CAA, and the recently described chromosome 13 familial dementia in British and Danish kindreds. This review focuses on the various molecules and genetic variants that target the cerebral vessel walls producing clinical features related to stroke and/or dementia, and discusses the potential role of amyloid in the mechanism of neurodegeneration.

## 1. Introduction

Amyloidosis is a disorder of protein conformation leading to aggregation and fibrillization. A diverse group of proteins normally present in body fluids as soluble precursors can self-assemble into amyloid fibrils and produce insoluble deposits in different tissues which may lead to cell damage, organ dysfunction and death. These fibrils, composed of low molecular weight mass peptides ( $\sim 4$  to 30 kDa), adopt a predominant

$\beta$ -pleated sheet structure, the conformation responsible for their physicochemical properties and tinctoreal characteristics. So far, 20 different proteins have been identified as subunits of amyloid fibrils [56,57,60 (for review and nomenclature)]. Although collectively they are products of normal genes, several amyloid precursors contain abnormal amino acid substitutions that can impose an unusual potential for self-aggregation. Increased levels of amyloid precursors, either in the circulation or locally at sites of deposition, are usually the result of overexpression, defective clearance, or both. Of all the amyloid proteins identified, less than half are known to cause amyloid deposition in the central nervous system (CNS), which in turn results in cognitive decline, dementia, stroke, cerebellar and extrapyramidal signs, or a combination of them.

## 2. $A\beta$ – related cerebral amyloidosis

Alzheimer's disease (AD) is an age-dependent neurodegenerative disorder that causes a chronically progressive decline in cognitive functions. The brains of patients suffering from AD are characterized by the extracellular deposition of amyloid  $A\beta$  ( $A\beta$ ) protein in plaques and cerebral blood vessels, the presence of intraneuronal neurofibrillary tangles (NFT) and reactive gliosis, and the loss of presynaptic terminals and neuronal subpopulations in well defined brain areas. At autopsy, senile plaques and NFT chiefly define the disease whereas other pathological features are often ignored or regarded as coincidental findings. It is now becoming clear that a large majority of the patients diagnosed with AD when examined at autopsy bear stroke-like lesions or infarctions, ranging from CAA, degenerative microangiopathy compromising both the endothelium and smooth muscle cells, cerebral infarcts, microinfarction, white matter changes related to small vessel disease and even hemorrhages [20,22,58]. Although it is accepted that the presence of cerebrovascular disease or strokes may cause rapid cognitive decline and worsen the outcome in AD, it remains debat-

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able whether the vascular lesions are coincidental or causal to the pathological processes. In sporadic AD, wild type A $\beta$  species, 39 to 43 residues in length, are the main constituents of the parenchymal and vascular amyloid lesions and the inheritance of the apolipoprotein E (apoE, chromosome 19) 4 allele is a prevailing risk factor [31,40,49,61].

Mutations in the amyloid precursor protein (A $\beta$ PP) on chromosome 21 or presenilins (PS) 1 (chromosome 14) and 2 (chromosome 1) genes have been linked to autosomal dominant forms of familial AD (FAD) (reviewed in [48]). As indicated in Fig. 1, multiple mutation sites either within or immediately outside the A $\beta$  segment have been identified in the A $\beta$ PP gene. Surprisingly, A $\beta$ PP mutations found outside the A $\beta$  peptide are mainly associated with dementia whereas those found inside the A $\beta$  sequence result in stroke as the main clinical phenotype. These genetic variants are concentrated in the middle of the A $\beta$  sequence (positions 21–22, corresponding to codons 692–693 of A $\beta$ PP) and are invariably associated with extensive cerebrovascular pathology. The Flemish mutation A to G at codon 692), is the only variant so far described at position 21, resulting in an Ala to Gly substitution and a clinical phenotype of presenile dementia and cerebral hemorrhage [19]. At position 22 of A $\beta$ , three variants have been described: i) the Arctic mutation (A to G at codon 693, resulting in the replacement of Glu for Gly), presenting as an early onset AD with prominent vascular symptomatology [23], ii) the Dutch mutation (G to C at codon 693, with the subsequent replacement of Glu for Gln), showing a phenotype predominantly associated to cerebral hemorrhage (see below) [26], and iii) the Italian mutation (G to A at codon 693, bearing Lys for Glu), presenting as a presenile dementia with cerebral hemorrhage [51]. The deposition of these vasculotropic mutants in the cerebral vessel walls suggest alteration of the clearance-uptake mechanisms at the blood-brain barrier.

Of interest is the first mutation described in the A $\beta$ PP gene linked to hereditary cerebral hemorrhage with amyloidosis, Dutch type (HCHWA-D), an autosomal dominant disease clinically defined by recurrent strokes, vascular dementia and fatal cerebral bleeding in the fifth to sixth decades of life [30]. Pedigrees from the villages of Katwijk and Scheveningen have been described in The Netherlands [29]. Around two-thirds of the patients will die as a result of the first acute cerebral hemorrhage, while the rest will have several minor stroke episodes leading to cognitive decline and dementia [18,59]. Histologically, there is a mas-

sive amyloid deposition in the walls of leptomeningeal and cortical arteries and arterioles as well as in vessels in the brainstem and cerebellum. In addition to the vascular involvement, there is a moderate number of parenchymal amyloid deposits resembling the diffuse plaques seen in Alzheimer disease. Dense plaque cores and neurofibrillary tangles are absent; cortical and hippocampal neurons outside the infarcts appear to be well preserved. Cerebral and cerebellar white matter show varying degrees of edema and myelin loss. The amyloid subunit in HCHWA-D is homologous to A $\beta$ ; amyloid deposits are composed of a mixture of wild-type A $\beta$  peptide and the A $\beta$ -Q22 variant [41,54]. A single nucleotide change (G for C) at codon 693 of A $\beta$ PP [26] results in a single amino acid substitution (glutamine for glutamic acid) at position 22 of the A $\beta$  peptide. This was the first of a large series of mutations in the A $\beta$ PP gene described in several kindreds with early onset familial Alzheimer disease [48] (Fig. 1). Although the mechanism of fibril formation is not known, *in vitro* studies [3,5,62] have shown that the A $\beta$ -Q22 variant forms fibrils at a more accelerated rate than wild-type A $\beta$ . This increased amyloidogenic tendency is probably due to a higher content of  $\beta$ -pleated sheet structure and a conformational change in the middle part of the molecule induced by the disappearance of the negatively charged Glu side-chain in the mutant peptide [9,45]. The presence of both allele products in the amyloid deposits suggest that the mutant peptide may induce a conformational change in the structure of the normal A $\beta$  peptide. Treatments to inhibit amyloidogenesis have been proposed [44,46].

### 3. Cystatin C – related cerebral amyloid angiopathy

Hereditary cerebral hemorrhage with amyloidosis, Icelandic type (HCHWA-I) is an autosomal dominant disorder characterized by massive amyloid deposition within small arteries and arterioles of leptomeninges, cerebral cortex, basal ganglia, brainstem and cerebellum [16]. Although brain involvement is the main clinicopathological feature, silent amyloid deposits are also present in peripheral tissues such as skin, lymph nodes, spleen, salivary glands, and seminal vesicles [2].

Including information on progenitors born up to 200 years ago, seven pedigrees have been described in small rural communities of West Iceland [21]. The main clinical hallmark of the disease is cerebral hemorrhage with fatal outcome in the third to fourth decade of life in

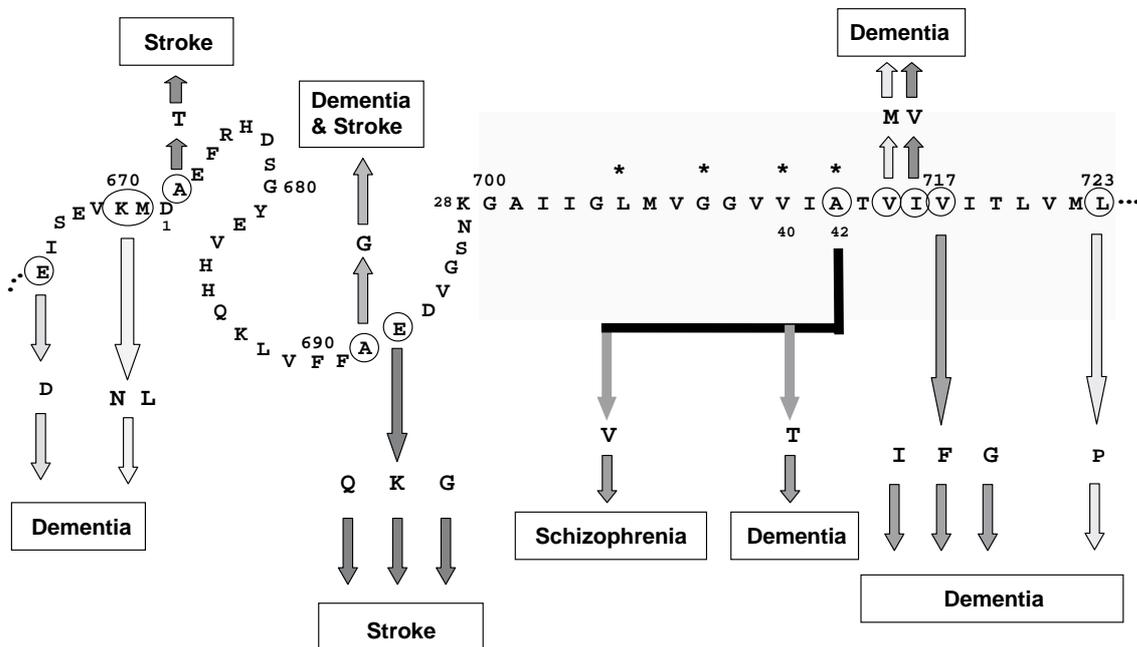


Fig. 1. AβPP mutants associated to different phenotypes in familial AD.

approximately 50% of the cases. Strokes are rare after the age of 50, and cognitive decline followed by dementia may occur in those cases that survive the hemorrhagic episodes. In addition to the amyloid deposition, patients have low levels of cystatin C in their cerebrospinal fluid.

The constituent protein of the amyloid deposits in HCHWA-I was the first amyloid purified and characterized from the CNS (1983) [6]. It is a genetic variant of cystatin C (ACys-Q68), a ubiquitously expressed inhibitor of cysteine-proteases codified by a single gene located on chromosome 20 and normally present in biological fluids [1]. The 110-residues-long ACys-Q68 amyloid subunit is degraded at the N-terminus, starting at position 11 of the normal cystatin C and bearing an amino acid substitution (glutamine for leucine) [13] as a result of a single nucleotide change, A for T at codon 68 [28] (Fig. 2).

#### 4. Transthyretin – related cerebral amyloid angiopathy

Familial transthyretin (TTR) amyloidosis is usually associated with peripheral neuropathy and involvement of visceral organs, whereas signs of central nervous system involvement are exceptional. Meningocerebrovascular and oculoleptomeningeal amyloid deposits con-

sisting of TTR variants ATTR-G18 [55] and ATTR-G30 [37] have been reported in two families carrying different point mutations in the *TTR* gene mapped to chromosome 18. A kindred of Hungarian origin containing 56 members spanning 4 generations was recently described. The major clinical symptoms include short-term memory decline, hearing loss, a cerebellar dysfunction with ataxia and bilateral pyramidal dysfunction with progressive spasticity. The onset of symptoms varied from ages 36 to 53, with death occurring between ages 51 and 60. Extensive amyloid deposition is present in meningeal vessels and subpial areas; although not associated to the clinical symptoms, small systemic deposits are present in kidney, skin, ovaries and peripheral nerves. All amyloid lesions are immunoreactive with antibodies against TTR. A single nucleotide change (A for G) at codon 18 results in the presence of glycine instead of aspartic acid (Fig. 2). A second kindred comprising 59 members spanning 4 generations has been described in a large Ohio family of German ancestry. It is clinically characterized by the presence of slowly progressive dementia, seizures, ataxia, hemiparesis, decreased vision and mutism. The age of onset is 46–56 years and the duration of the disease varies between 3 and 26 years. Amyloid deposits are present in the arachnoid and arachnoid blood vessels in the brain and spinal cord, with small and medium size vessels being the most severely affected.

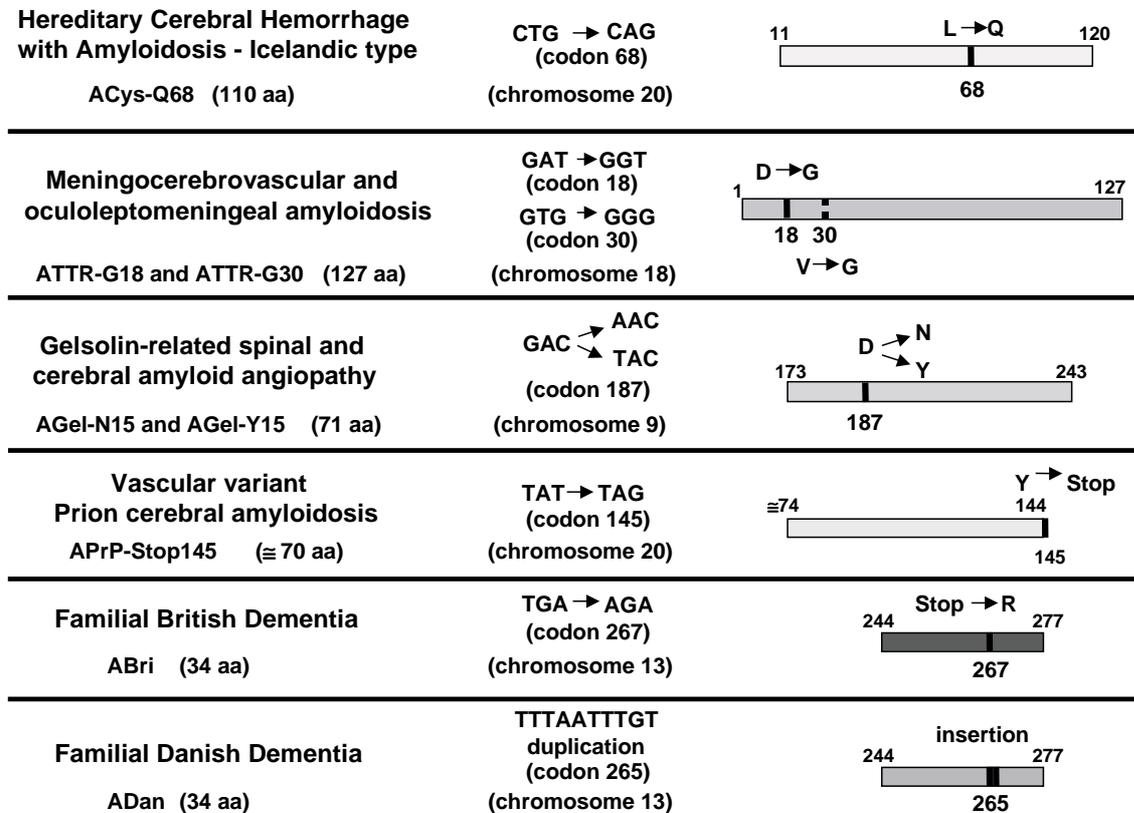


Fig. 2. Diagram summarizing the amyloid diseases and the corresponding amyloid subunits associated with familial cerebral amyloid angiopathies related to dementia.

All these lesions are Congo red positive and immunoreactive with anti-TTR antibodies. Interestingly, vascular amyloid is not longer detectable after the vessels penetrate into the brain parenchyma. Amyloid deposits are also detectable in choroid plexus, ventricular regions and, although infrequently, in vessels of virtually all visceral organs, skin, and skeletal muscle. In this family, a T for G substitution at codon 30 results in the substitution of valine for glycine in the TTR molecule.

### 5. Gelsolin – related spinal and cerebral amyloid angiopathy

Familial amyloidosis, Finnish type (FAF), is an autosomal dominant systemic form of amyloidosis characterized by slowly progressing cranial and peripheral neuropathy, dry and itchy skin, intermittent proteinuria and cardiac abnormalities [34]. The patients gradually develop a typical facies with droopy eyelids and protruding lips. Corneal lattice dystrophy, lace-like deposition of amyloid within the stroma, is the earliest

clinical finding of the syndrome. It is more common in southeastern Finland, but it is encountered elsewhere in Europe, the United States and Japan [24]. The genetic defect underlying FAF has been revealed and the amyloid subunit (AGel) identified. The amyloid fibrils correspond to a 7 kDa internal degradation product of human gelsolin, a widely abundant regulatory protein involved in actin severing and gel – sol transformation [12,32]. AGel spans from position 173 up to residue 243 of the gelsolin protein and bears an amino acid substitution at position 187 (aspartic acid for asparagine) due to a single G to A transition at position 654, the first nucleotide at codon 187 [27] (Fig. 2). FAF cosegregates with the mutation, and the disease is particularly severe in those homozygously affected. The mutation has been detected in Finnish, Dutch, American and Japanese families. A different amino acid substitution at the same codon 187 has been described in patients of Danish and Czech origin suffering the same disorder. In these cases, a transition of G to T at the same codon results in the presence of a tyrosine instead of the normally occurring aspartic acid [8]

(Fig. 2). Although facial palsy, mild peripheral neuropathy and corneal lattice dystrophy are characteristic of both gelsolin-related amyloidoses, atrophic bulbar palsy, ataxia of gait and minor cognitive impairment also occur. Recent immunohistochemical studies in Finnish cases carrying the 187 Asn for Asp mutation have demonstrated a widespread spinal, cerebral and meningeal amyloid angiopathy [25]. The study showed that deposition of AGel in the spinal and cerebral blood vessel walls, meninges as well as spinal nerve roots and sensory ganglia is an essential feature of this form of systemic amyloidosis that contribute to the CNS symptoms. In addition, anti-FAF antibodies stained Lewy bodies in the cytoplasm of nigral neurons and occasionally in other brainstem nuclei, including locus ceruleus and substantia innominata [10]. The relationship between intracellular gelsolin deposits and synuclein, the main component of Lewy bodies [47], remains to be determined.

## 6. Prion – related cerebral amyloidosis

A unique category in the conformational disorders are the prion – related diseases (or prionoses), where the etiology is thought to be related to the conversion of the normal prion protein PrP<sup>C</sup> into an infectious and pathogenic form PrP<sup>SC</sup>. Prionoses include Creutzfeldt-Jakob disease (CJD), kuru, Gerstmann-Sträussler-Scheinker disease (GSS) and fatal familial insomnia in humans as well as scrapie and bovine spongiform encephalopathy in animals. Extensive cortical spongiform change, gliosis and neuronal loss are common although not invariable features of prionoses. The parenchymal amyloid load, characteristic of the autosomal dominant GSS, is only present in about 10% of the CJD cases, whereas amyloid angiopathy is virtually absent in all of them. Interestingly, an early onset form of dementia characterized by the deposition of PrP amyloid in leptomeningeal and parenchymal blood vessels in conjunction with neurofibrillary lesions in the cerebral gray matter has been recently described. Although a single case of the so-called PrP cerebral amyloid angiopathy (PrP-CAA) has been reported, it constitutes the first example of PrP amyloid angiopathy in humans [11]. The patient, with clinical diagnosis of AD, presented with memory disturbance and disorientation at age 38, developing a progressive severe dementia. Neuroimaging at the terminal stage (age 58) indicated a severe atrophy of the cerebrum with dilation of the lateral ventricles.

Neuropathologically, extensive amyloid deposition was observed in parenchymal and leptomeningeal vessels as well as in the perivascular neuropil. Cerebral and cerebellar gray matter were the most affected areas whereas amyloid was absent in the white matter. In addition, classical neurofibrillary tangles, neuropil threads and dystrophic neurites were abundant in the cerebral gray matter, particularly in the hippocampus.

The amyloid subunit composing the vascular deposits was identified as a 7.5 kDa fragment of PrP. In this patient, a point mutation TAT to TAG was found at codon 145 of the *PRNP* gene, replacing the normally occurring tyrosine for a newly created stop codon. Based on molecular size, immunoreactivity and molecular genetic analysis, it was deduced that the PrP amyloid subunit in the Y145Stop variant comprises around 70 amino acids and is an N- and C-terminal truncated form of the normal PrP (Fig. 2). In PrP-CAA, the association of amyloid and neurofibrillar lesions does not appear to be casual, since abnormal neurites immunolabeled with anti-phosphorylated tau antibodies co-localize with the PrP amyloid in the neuropil surrounding blood vessels, suggesting that the dyschoric angiopathy may affect the neuronal cytoskeleton [11].

## 7. ABri – related cerebral amyloidosis

Familial British dementia (FBD), is an autosomal dominant form of CAA clinically characterized by progressive dementia, spastic tetraparesis and cerebellar ataxia, with an age of onset in the fourth to fifth decade. A single extensive pedigree with the disease occurring in multiple generations has been reported [33,39]. Neuropathologically, there is severe and widespread amyloid angiopathy of the brain and spinal cord with perivascular amyloid plaque formation, periventricular white matter changes resembling Binswanger's leukoencephalopathy, neuritic and non-neuritic amyloid plaques affecting cerebellum, hippocampus, amygdala and occasionally cerebral cortex, and neurofibrillary degeneration of hippocampal neurons. In spite of the extensive amyloid deposition of the CNS vasculature, large intracerebral hemorrhage is a rare feature of the disease. The disease was originally described as a familial presenile dementia with spastic paralysis [63]; however, due to the extensive cerebrovascular involvement, the disorder was later on designated familial cerebral amyloid angiopathy, British type [39] and cerebrovascular amyloidosis, British type [42].

The biochemical nature of the amyloid fibrils extracted from leptomeningeal deposits in FBD was recently uncovered [56]. The amyloid subunit, named ABri, is composed of 34 amino acids (EASNC-FAIRHFENKFAVETLICSRVKKNIIEEN) with certain degree of N- and C-terminal heterogeneity and no sequence identity to any known amyloid protein. ABri is devoid of glycine, methionine, proline, aspartic acid, tryptophane, tyrosine and glutamine, featuring pyroglutamate at its N-terminus. The post-translational modification observed at position 1 (pyroglutamate) has been previously found in other brain amyloids, i.e. peptides derived from Alzheimer A $\beta$  [35,43,52]. The N-terminal pyroglutamate may offer protection against in vivo proteolysis as well as increase the  $\beta$ -sheet content of the ABri peptide and its tendency to aggregate and polymerize. The two cysteine residues at positions 5 and 22 may be of importance for polymerization and fibrillization, and the predicted isoelectric point (7.0) suggests low solubility at physiologic pH, a property mimicked by synthetic peptides homologous to the full length ABri that spontaneously polymerize and aggregate in solution. This new amyloid protein is a degradation product of a 277 amino acids precursor molecule with a primary structure that resembles a type II single-spanning transmembrane protein. The precursor protein is codified by a single gene *BRI* (also known as *ITM2B* [38]) located on the long arm of chromosome 13. In patients with FBD, a single nucleotide substitution (TGA to AGA at codon 267) results in the presence of an arginine residue in place of the stop codon normally occurring in the wild type precursor molecule and a longer open-reading frame of 277 amino acids instead of 266. The ABri amyloid peptide is formed by the 34 C-terminal amino acids of the mutated precursor protein (Fig. 2).

## 8. ADan – related cerebral amyloidosis

Familial Danish dementia, also known as hereditary ophthalmic-oto-encephalica, is an early-onset autosomal dominant disorder originating in the Djursland peninsula, Denmark. The disease, identified in nine cases spanning three generations of a single family, is clinically characterized by the development of cataracts, deafness, progressive ataxia and dementia [50]. Cataracts seem to be the early manifestation of the disease, starting before the age of 30, whereas impaired hearing usually develops 10–20 years later. Cerebellar ataxia occurs shortly after the age of 40,

followed by paranoid psychosis and dementia 10 years later. Most patients die in their fifth to sixth decade of life. Neuropathologically, the disease is characterized by diffuse atrophy of all parts of all parts of the brain with a particularly severe involvement of the cerebellum, cerebral cortex and white matter, as well as very thin and almost demyelinated cranial nerves. There is a widespread amyloid angiopathy in the blood vessels of the cerebrum, choroid plexus, cerebellum, spinal cord, and retina. The presence of parenchymal plaques and neurofibrillary tangles is the major histological finding in the hippocampus, whereas the cerebral white matter also shows some ischemic lesions.

Biochemical analysis of the vascular lesions unveiled a 34 amino acids amyloid subunit, ADan (EASNC-FAIRHFENKFAVETLICFNLFLNSQEKHY), with N-terminal homology to the ABri, the peptide originated by a point mutation at the stop codon of gene *BRI* in familial British dementia. The ADan molecule has an isoelectric point of 6.1 and is devoid of glycine, proline, aspartic acid, methionine and tryptophane residues. As in ABri, the isolated ADan amyloid features N-terminus pyroglutamate and two cysteine residues which may be important for the formation and perpetuation of fibrillar deposits. Molecular genetic analysis of the *BRI* gene in the Danish kindred showed a different defect, namely the presence of a 10 nucleotides duplication (795–796insTTTAATTTGT) between codons 265 and 266, one codon before the normal stop codon 267. The decamer duplication mutation produces a frame-shift in the *BRI* sequence generating a larger-than-normal precursor protein, of which the amyloid subunit ADan comprises the last 34 C-terminal amino acids [57] (Fig. 2).

## 9. Conclusion

There is now extensive data indicating that the neuropathology of AD extends beyond amyloid plaques and neurofibrillary tangles. A wide range of vascular lesions (CAA, microvascular degeneration and periventricular white matter lesions) are evident in almost all cases of AD. Single lobar hemorrhages due to amyloid angiopathy, usually associated with focal neurological symptoms, are not a common clinical feature of AD; however, multiple micro-hemorrhages can cause (or contribute to) cognitive decline and dementia. Whether these vascular lesions are coincidental or causal in the pathogenetic processes of AD constitutes an issue of interest to investigate in the next coming years. The poor

correlation between the severity of amyloid angiopathy and the magnitude of parenchymal changes together with the failure to reproduce neuronal loss in transgenic animal models of AD suggest that other still undiscovered factors may modulate the generation of amyloid and neurofibrillary tangles. New clues regarding the relationship between amyloid and neuronal dysfunction may well arise from future research involving other mutant peptides different from A $\beta$ . Particularly interesting will be the study of the novel molecules ABri and ADan, originated from different defects in a gene not previously known to be related to human neurological disorders. Immunohistochemical and electron microscopical studies have demonstrated that the cytoskeletal pathology in FBD and FDD patients is identical to that seen in patients with other neurodegenerative conditions, including Alzheimer disease, Prion disorders, brain trauma, or mutations in chromosome 17 [4,7,14,42,53]. Therefore, different amyloid peptides at distinct cerebral areas could trigger similar neuropathological changes leading to the same scenario: neuronal loss and dementia, supporting the notion that amyloid peptides may be of primary importance in the initiation of neurodegeneration.

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