# Genetic Alterations of the *BRI2* gene: Familial British and Danish Dementias

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**Classic arguments sustaining the importance of amyloid in the pathogenesis of dementia are usually centered on amyloid β (Aβ) and its role in neuronal loss characteristic of Alzheimer disease, the most common form of human cerebral amyloidosis. Two non-Aβ cerebral amyloidoses, familial British and Danish dementias, share many aspects of Alzheimer disease, including the presence of neurofibrillary tangles, parenchymal pre-amyloid and amyloid deposits, cerebral amyloid angiopathy, and a widespread inflammatory response. Both early-onset conditions are linked to specific mutations in the *BRI2* gene, causing the generation of longer-than-normal protein products and the release of 2 de novo created peptides ABri and ADan, the main components of amyloid fibrils in these inherited dementias. Although the molecular mechanisms and signal transduction pathways elicited by the amyloid deposits and their relation to cognitive impairment remain to be clarified, new evidence indicates that, independent of the differences in their primary structures, Aβ, ABri, and ADan subunits are able to form morphologically compatible ion-channel-like structures and elicit single ion-channel currents in reconstituted lipid membranes. These findings reaffirm the notion that non-Aβ amyloidosis constitute suitable alternative models to study the role of amyloid deposition in the mechanism of neuronal cell death.**

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## INTRODUCTION

Cerebral amyloid disorders are commonly associated with neurodegeneration and ischemia and, less frequently, with hemorrhagic strokes. They are part of an expanding group of diseases collectively known as "disorders of protein folding," in which normal soluble proteins improperly folded accumulate intra- and extra-cellularly in the form of aggregates and/or amyloid fibrils. Misfolded proteins, in turn, trigger apoptotic/necrotic mechanisms, assemble into ion channel-like structures producing Ca2+ imbalance, induce inflammatory responses and the release of cytokines, elicit complement activation and stimulate the production of undesirable reactive oxygen species, all relevant pathways that contribute to the complex mechanism of cell toxicity. Eight of the 26 amyloid subunits known to produce amyloidosis in humans have been found to be associated with cerebral forms of the disease, being Alzheimer Aβ the most studied and so far the only one exclusively restricted to the brain. The reasons for tissue selectivity are not known and may reflect exacerbated local synthesis, protein-protein interactions with specific tissue factors, resistance to enzymatic degradation, differential blood-brain barrier permeability, disregulated brain clearance mechanisms or a combination of these factors. The latest addition to the list of cerebral amyloid subunits, ABri and ADan, are linked to 2 hereditary conditions known as familial British dementia (FBD) and familial Danish dementia (FDD), respectively, arising from distinct genetic defects affecting the stop codon in the *BRI2* gene located on the long arm of chromosome 13. Although structurally unrelated, ABri, ADan and Aβ are all associated with cerebral amyloid deposition, similar neurofibrillary pathology and clinical dementia. The availability of these alternative models may help elucidate the relevance of amyloid in the molecular mechanisms leading to neurodegeneration and dementia.

## THE *BRI2* GENE AND THE BRI2 PROTEIN

***The BRI gene family.*** The evolutionarily conserved *BRI* gene family comprises 3 isoforms *BRI1* to *BRI3* in both humans and mice, which are also referred to as *ITM2A*, *B*, and *C*, or *E25A*, *B*, and *C*, respectively (11, 55, 81, 82). *BRI*-like genes exist in many vertebrate animals (eg, rat, monkey, chicken, rabbit, pig, horse) and were also identified in non-vertebrate species including flies and worms (11, 70, 81). Each one of the *BRI* family members was mapped to a different chromosome; in humans, the *BRI1* gene is located in chromosome X (56), *BRI2* in the long arm of chromosome 13 (82), and *BRI3* in chromosome 2 (81). All of them have a remarkably similar 6 exons-5 introns genomic organization, the first intron being considerably longer in comparison with the other 4, a feature that suggests the existence of regulatory sequences (56, 81). In contrast to the similar genomic organization, the gene expression pattern of the isoforms is strikingly different; whereas *BRI2* is highly ubiquitous, *BRI1* and *BRI3* show limited expression. *BRI1* is particularly restricted to the mouse osteo- and chondrogenic tissues during development (55); it is also expressed in CD34 (+) hematopoietic progenitor cells (44) and is up-regulated in T-cell devel-

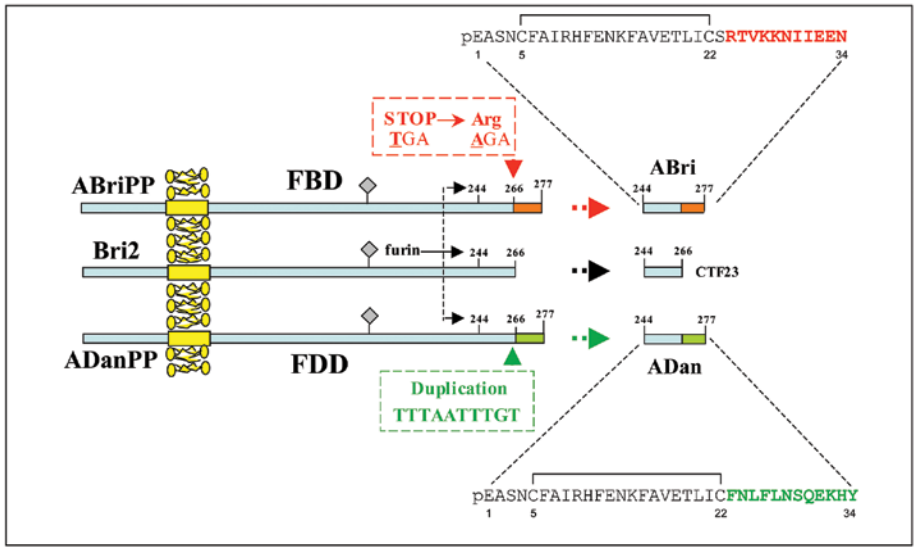


**Figure 1.** *Normal processing and genetic variants of protein BRI2.* Schematic representation of the 266-amino acid long type II transmembrane protein BRI2 and the genetic defects associated with FBD (top) and FDD (bottom). The single transmembrane segment is highlighted in yellow; the diamond symbol at position 170 denotes the single N-glycosylation site. A stop-to-Arg point mutation at codon 267 in FBD (top diagram, red box) and a 10-nucleotide duplication insertion after codon 265 in FDD (bottom diagram, green box) generate longer-than-normal precursors ABriPP and ADanPP, which are 277 amino acids long. Post-translational proteolytic processing at peptide bond 243-244, carried out by furin or furin-like pro-protein convertases, generates a C-terminal fragment of 23 amino acids (CTF23) from wild type BRI2. The same proteolytic process of both extended precursors (red path for FBD, green path for FDD) generate ABri and ADan C-terminal fragments, both 34 amino acids long, identical in their first 22 residues and completely different in their 12 C-terminal amino acids (bold type), and containing a single disulphide bond between residues 5 and 22. Biochemical analysis of deposited ABri and ADan shows pyroglutamate modification at the N-terminus.

opment, with enhanced expression during thymocyte selection and T-cell activation, causing CD8 downregulation when overexpressed in $CD4^+/CD8^+$ thymocytes (30). *BRI3* was predominantly localized to the brain (7, 81) with the highest levels of expression in cerebral cortex, amygdala, hippocampus, thalamus, caudate nucleus and spinal cord. Recently, *BRI3* was also demonstrated in plasmacytoid dendritic cells, granulocytes (63), bone marrow, fetal liver, and to a lesser extent in spleen, lymph nodes, and thymus. The *BRI2* gene, on the contrary, is broadly expressed in peripheral organs with high levels of expression in brain, heart, kidney, pancreas, liver, and placenta. Within the brain, it is ubiquitously present in white and gray matter, showing more abundant distribution in the hippocampus and cerebellum compared with cerebral cortex (82). In situ hybridization studies of human brain cells in culture showed that *BRI2* mRNA is widely distributed in the different cerebral cell populations and its presence can be demonstrated in neurons, astrocytes and microglial cells as well as in smooth muscle and cerebral endothelial cells (67).

***The BRI protein isoforms.*** All the *BRI* mRNAs translate into BRI protein isoforms which share over 90% homology with the corresponding counterparts in other species, although homology among the different isoforms within each species is less pronounced. Data base scans for possible functional roles of these highly conserved BRI isoforms revealed the existence of a ~100 amino acid BRICHOS domain, a common motif among members of the BRI, ChM-I, SP-C and CA11 protein families which may be involved in the targeting of these proteins either to the secretory pathway or to an intracellular processing pathway (70). Although all these proteins are dissimilar and associate to a diverse range of phenotypes varying from dementia to cancer and respiratory distress, they share 2 common features: *i)* they all are type-II transmembrane proteins and *ii)* they all are proteolytically processed by furin. Figure 1 illustrates the protein product of the *BRI2* gene, referred here as protein BRI2. It is a 266-amino acids long protein containing a single membrane-spanning domain (positions 52-75), a single BRICHOS domain (residues 137-231), an N-glycosylation site at asparagine 170 and the predicted structure of a type II transmembrane glycoprotein featuring an intra-cytoplasmic N-terminus and an extracellular C-terminus (82). BRI2 is proteolytically processed at peptide bond 243-244 by furin and other members of the pro-protein convertase family (50) using the cleavage consensus ...$KGIQKR^{243}$ β $E^{244}A$... and resulting in the release of a short C-terminal fragment of 23 amino acids (CTF23) of unknown fate and biological function (28, 29, 67). In the human brain, BRI2 was topographically demonstrated in normal pyramidal neurons in the CA3 and CA4 of the hippocampus, as well as in Purkinje cells in the cerebellar cortex (2). In cultures of human brain cells, protein BRI2 was present in neurons, both in the cellular body and axons, in smooth muscle and endothelial cells as well as in astrocytes and microglia, consistent with the mRNA expression pattern (67). In pathologic conditions, BRI2 was detected in dystrophic neurites in senile plaques and around ischemic lesions derived from axons and dendrites in Alzheimer disease tissues, and it was highly positive within Lewy neurites in dementia with Lewy Bodies and Parkinson disease cases (2).

***Biological function of protein BRI2.*** The biological function of BRI2 remains largely unknown. Modulation of APP processing, tumor suppression activity and pro-apoptotic properties are some of the diverse suggested functions for this molecule. Its cellular distribution throughout the brain and its presence in dystrophic neurites in various neurodegenerative conditions (2) together with its morphological distribution within neuronal cell bodies and axons (67) and its axonal localization in *BRI2* transfected neurons (8) suggests transport along neuronal processes and a putative role in nerve terminals. Consistent with this concept, BRI2 was recently identified as a major ligand of APP, the Alzheimer Aβ precursor protein, which is also known to be anterograde axon transported (32). Short deletion mutants inclusive of the full transmembrane domains of BRI2 and APP are sufficient for the interaction to occur, implying that both proteins must be expressed on the same cell membrane (cis) rather than on adjacent cells (trans) (17, 45). Notably, BRI2 expression in transfected cells was shown to modulate the processing of APP and result in reduced levels of Aβ and

APP C-terminal fragments. The biological relevance of the BRI2-APP interaction is puzzling and further studies are required to assess its relevance in disease pathogenesis.

A short form of BRI2 lacking exon 1 and generated by alternative splicing has been demonstrated to possess pro-apoptotic functions correlating with caspase-9 and caspase-3 activation, and to participate in the process of cell death in a p53 independent mechanism (15, 16). Of note, BRI2 has a Bcl-2 homology-3 (BH-3) domain encoded in exon 2; the BH3 domain in Bcl2 is required for protein dimerization and the regulation of its apoptotic activities (24).

An unrelated function as a tumor suppressor gene has also been proposed for *BRI2*. The chromosome segment 13q14 to which *BRI2* maps, is the region most frequently associated with tumor-related mutations in primary prostate cancer and BRI2 is one of the 6 genes on that loci that show significantly reduced levels in prostate tumors compared to normal tissues. Evidence of allelic loss at the same locus has been reported in other types of cancer, including ovarian carcinoma, head and neck squamous cell carcinoma, and pituitary tumors (38, 53).

## FAMILIAL BRITISH AND DANISH DEMENTIAS

Two hereditary conditions have been recently linked to genetic defects in the *BRI2* gene. These disorders, FBD and FDD, are both associated with neurodegeneration and extensive amyloid deposition in the CNS, although they show some differences in their clinical presentation and target tissues. The clinical and neuropathological features of both disorders, their genetic defects, and the biochemical composition of the corresponding amyloid deposits are described below.

***Clinical and histopathological aspects of FBD and FDD.*** FBD is an early onset autosomal dominant disorder clinically characterized by progressive dementia, cerebellar ataxia, and spastic tetraparesis, causing death at the median age of 56 years (57). FBD was first reported in 1933 by Worster-Drought et al in 2 siblings with familial presenile dementia with spastic paralysis (86, 87). Since then, it has been identified in 3 pedigrees, 2 of which may be related. At present, the original Worster-Drought pedigree comprises 343 individuals over 9 generations dating back to ~1780 (21, 42, 48, 58). Patients with the disease have early personality changes followed by memory impairment progressing to global dementia, cerebellar ataxia and spastic paralysis. Brainstem signs, pseudo-bulbar palsy, and dysarthria are common features of the disease and although cerebral hemorrhage is relatively rare, stroke-like episodes may be present. Magnetic resonance image (MRI) scans typically show a moderate ventricular dilation and a relatively preserved cortex with extensive periventricular white matter changes compatible with leukoaraiosis from early stages of the disease. Hyperintensities due to white matter changes are most pronounced around the frontal and occipital horns of the lateral ventricles. Histopathologic examination of FBD brain tissue using classical staining methods (eg, silver, H&E, PAS, Congo red) reveals severe amyloid angiopathy of the brain and spinal cord with perivascular amyloid plaque formation, parenchymal plaques affecting limbic areas, cerebellum, and occasionally cerebral cortex, neurofibrillary degeneration of hippocampal neurons and periventricular white matter changes (48, 58).

FDD, previously known as heredopathia ophthalmo-oto-encephalica (72), is also an early-onset autosomal dominant disorder similar to FBD although presenting with unique clinical manifestations. The disease, identified in thirteen cases spanning five generations of a single family originating in the Djürsland peninsula, Denmark, is clinically characterized by the early development of cataracts and deafness, followed by progressive ataxia and dementia (5, 26, 72, 73). Cataracts seem to be the earliest manifestation, starting before the age of 30, whereas impaired hearing usually develops ~10 years later. Shortly after the age of 40, patients develop cerebellar ataxia followed by paranoid psychosis and dementia about 10 years later; the median age at death is 58 years. MRI scans of individuals affected with FDD also show ventricular dilatation and a relatively preserved cortex with periventricular hyperintensities due to white matter changes similar to those found in Binswanger subcortical arteriosclerotic leukoencephalopathy. Histopathologic examination of FDD cases reveals widespread amyloid angiopathy in small blood vessels and capillaries of the cerebrum, choroid plexus, cerebellum, spinal cord, and retina. Parenchymal compact plaques are consistently absent, whereas neurofibrillary tangles are the major histological finding in the hippocampus. In addition, cranial nerves are consistently thin and almost demyelinated (60).

***Genetic abnormalities in the BRI2 gene generate ABri and ADan amyloid subunits.*** As illustrated in Figure 1, FBD and FDD are linked to 2 different genetic defects in the *BRI2* gene. The Stop-to-arginine mutation in FBD (82) and a 10-nucleotide duplication-insertion occurring immediately before the stop codon in FDD (83) produce a common outcome in both diseases: the normally occurring stop codon at position 267 is not operational (either non existent in FBD or out of frame in FDD), longer coding DNA sequences are created at the expense of intronic nucleotides and the extended precursors ABriPP and ADanPP are created, both 277 amino acids in length featuring C-terminal segments that do not exist in normal conditions (Figure 1). Since the consensus sequence for furin cleavage is not affected by any of the 2 genetic abnormalities, regular proteolytic processing of ABriPP and ADanPP at peptide bond 243-244 generates the C-terminal fragments ABri and ADan (28, 29) which are found deposited as fibrils in FBD and FDD amyloid lesions. These de novo created ABri and ADan amyloid subunits, with no homology to any other known amyloid protein, are both 34-residues long, share 100% homology in their first 22 residues although bearing a completely different 12- amino acid C-terminus (18). Taking advantage of this distinct feature, antibodies anti-ABri (82) and anti-ADan (83), specific for each disorder, have been developed and used for immunohistochemical and biochemical analysis of tissue samples and biological fluids, as described below.

***Neuropathology of FBD and FDD.*** In addition of highlighting Congo red positive amyloid deposits in FBD and FDD (Figure 2A-D), polyclonal antibodies 338 (anti-ABri) and 5282 (anti-ADan) revealed the existence of extensive parenchymal non-fibrillar, Congo red negative, pre-amyloid lesions in both diseases. These pre-amyloid deposits were more prominent in FDD

in which limbic structures, neocortex and cerebellum were particularly affected (24-26). In addition to the pre-amyloid and amyloid deposits, neurofibrillary tangles (NFTs) mostly affecting hippocampus and entorhinal cortex are constantly present in both diseases (Figure 2E, F). The tangle pathology is ultrastructurally and biochemically similar (if not identical) to that seen in AD (61); NFTs are composed of classic PHF and their hyperphosphorylated tau electrophoretic migration patterns are indistinguishable from that seen in AD (25, 26, 62). Confocal and immunoelectron microscopy revealed that the parenchymal ABri deposits are predominantly of amyloid (fibrillar) nature whereas ADan parenchymal deposits are characteristically of preamyloid (non-fibrillar) type (24, 26). Notably, co-deposition of Aβ in a proportion of blood vessels affected by ADan has been a constant finding in FDD cases (26, 60), whereas Aβ immunostaining was never observed in any of the FBD specimens analyzed so far (24, 25). Immunohistochemical studies also identified activated microglia expressing the major histocompatibility class II antigens characteristic of inflammatory processes as well as reactive astrocytes clustered around all types of amyloid lesions in both diseases (25, 26, 67). In addition, an array of amyloid associated proteins (eg, among them serum amyloid P component, β1-antichymotrypsin, ApoE, ApoJ, several activated complement components, vitronectin, extracellular matrix proteins as well as many proteoglycans and glycosaminoglycans) are associated with the lesions (36a, 67). Although the role played by these molecules in the process of amyloid formation and deposition is not certain, their presence in AD, FBD and FDD suggest mechanistic commonalities, as discussed below.

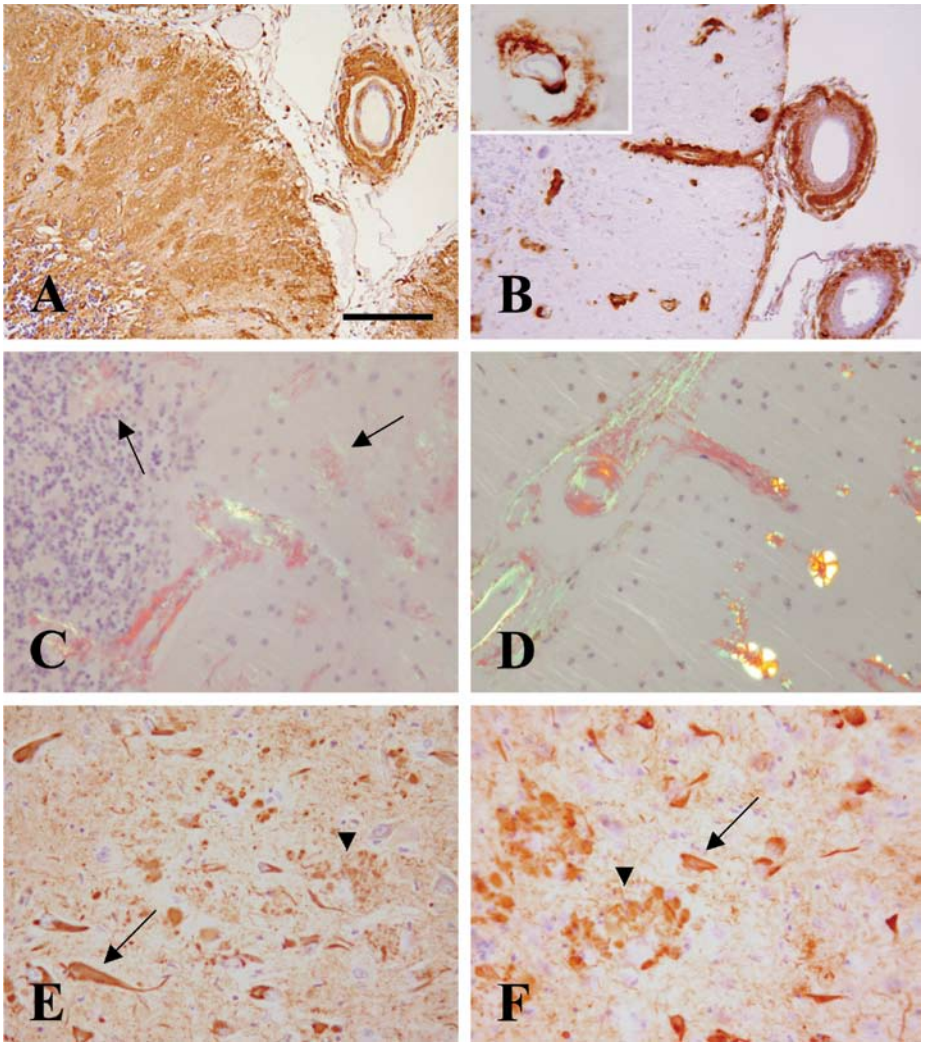


**Figure 2.** *Neuropathology of FBD and FDD.* **A.** In FBD there is both vascular and parenchymal ABri deposition in the cerebellum. **B.** ADan deposition in FDD is mainly seen in blood vessels. In a proportion of the hippocampal and cerebral cortical, but not cerebellar blood vessels, there is also Aβ deposition (insert). **C.** Congo red-positive deposits in both blood vessels and parenchyma (arrow) in FBD. **D.** Congo red positivity is mostly found in blood vessels in FDD. **E.** Hyperphosphorylated tau deposition in hippocampal neurons (arrow) and plaque-associated abnormal neurites (arrowhead) in FBD. **F.** Neurofibrillary tangles are also numerous in FDD (arrow), although abnormal neurites mainly occur around amyloid-laden blood vessels (arrowhead). **A.** ABri immunohistochemistry, antibody 338; **B.** ADan immunohistochemistry, antibody 5282; **C, D.** Congo red staining; **E** and **F**: Tau immunohistochemistry, antibody AT8. Bar on A represents 130 μm on A and B, 65 μm on C-F

***Biochemical analysis of ABri and ADan in biological fluids and amyloid deposits.*** Using anti-ABri and anti-ADan antibodies, soluble forms of ABri and ADan have been identified in biological fluids of affected and non-affected carriers of FBD and FDD, respectively (18, 79). Immunoprecipitation in combination with mass spectrometry analysis illustrate that both circulating peptides are monomeric, 34-residues in length, and clearly the result of furin processing, starting at glutamic acid 244 of the parent molecule and containing a single intra-chain disulphide bond between positions 5 and 22 (Figure 3A, E). Since soluble ABri and ADan do not exist in the normal population, their presence in biological fluids constitutes a biological marker for the disease that can be used for diagnostic purposes.

The complexity and heterogeneity of FBD and FDD tissue deposits was assessed using a similar biochemical approach (immunoprecipitation and mass spectrometry) in combination with a differential solubility strategy: water-based buffers for the extraction of soluble peptides, detergent for the removal of non-fibrillar pre-amyloid deposits, and strong acid to solubilize fibrillar amyloid deposits. In general terms, both disorders rendered similar findings, as illustrated in Figure 3. The complexity of the extracted material increased as the solubility of the deposits diminished, as reflected in the degree of peptide oligomerization, predominant amyloid subunits, extent of post-translational modifications, and magnitude of N- and C-terminal proteolytic degradations. In general terms, non-fibrillar deposits appeared less complex than the fibrillar counterparts, perhaps reflecting the presence of early intermediate states of the fibrillization process. The degree of oligomerization in the material solubilized by

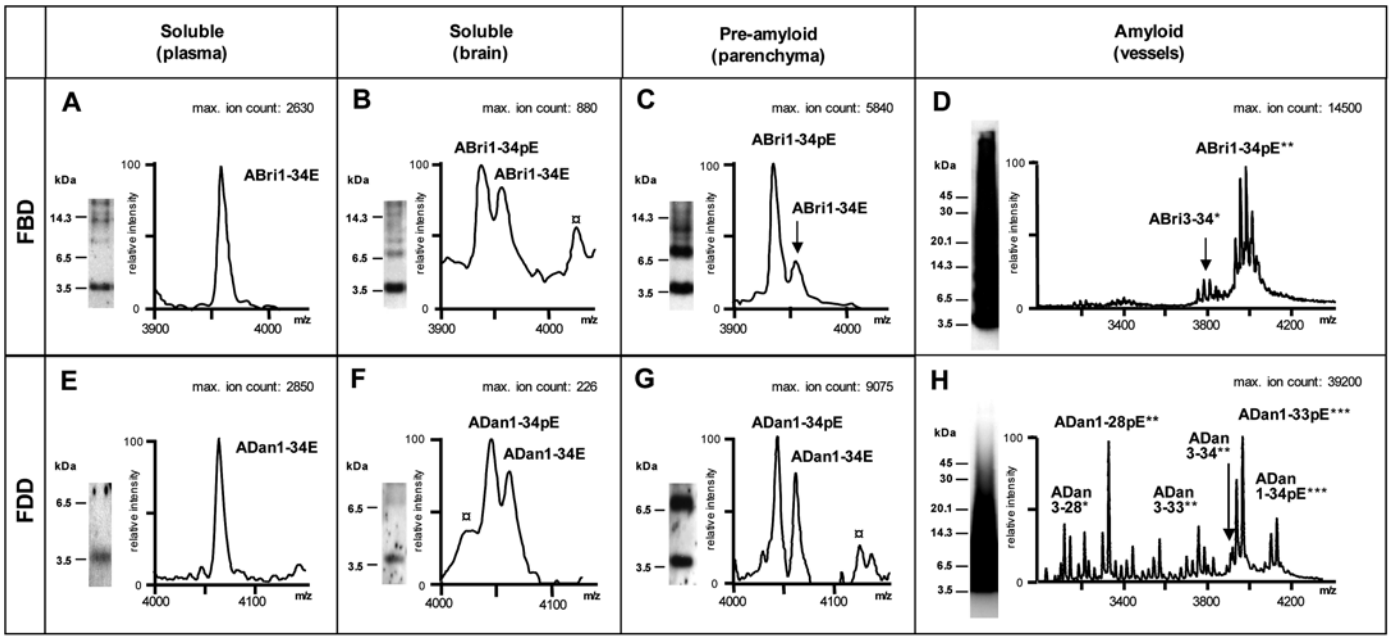


**Figure 3.** *Biochemical characterization of ABri and ADan species.* Western blot and mass spectrometry analysis of ABri (**A-D**) and ADan (**E-H**). **A**, **E.** Soluble plasma ABri and ADan. **B**, **F.** Water-soluble extracts from FBD and FDD; **C**, **G.** Parenchymal pre-amyloid lesions extracted with 2% SDS. **D**, **H.** Formic acid extracts from FBD and FDD.

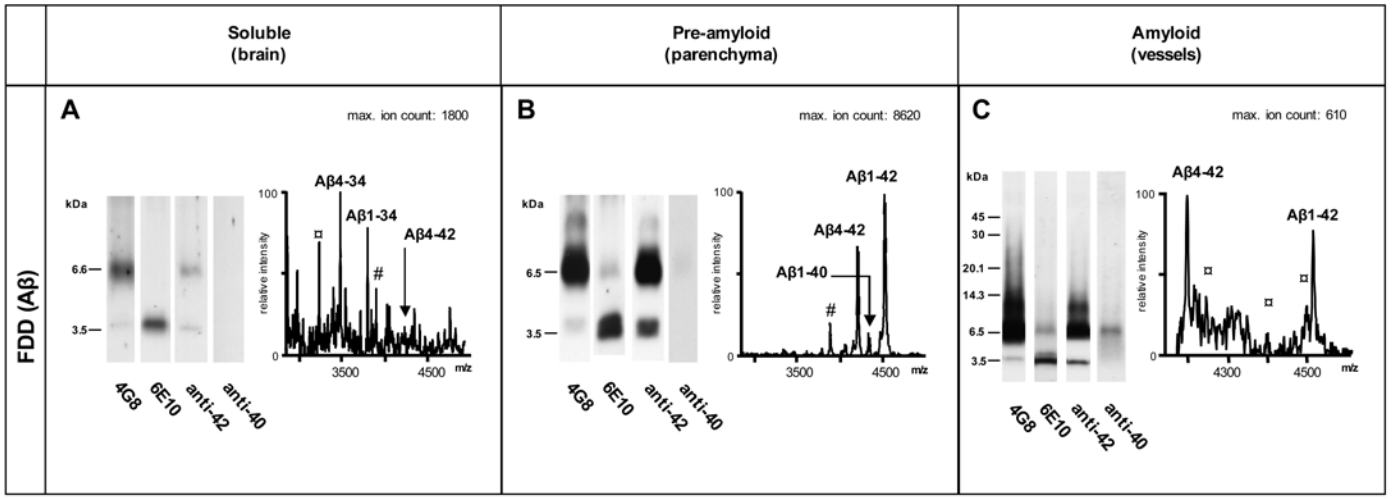


**Figure 4.** *Biochemical characterization of Aβ in FDD lesions.* Western blot and mass spectrometry analysis of deposited Aβ sequentially recovered from water-based buffers (**A**), detergent (**B**) and formic acid (**C**) extracts in FDD

water-based buffers was limited to dimeric forms of intact amyloid subunits partially post-translationally modified at their N-terminus, featuring pyroglutamate instead of glutamate in about 60% of the molecules (Figure 3B, F). Detergent solutions recovered more material with a similar degree of oligomerization than water-based buffers, although with a higher proportion (about 60%-80%) of species bearing a post-translationally modified N-terminus (Figure 3C, G). Amyloid species extracted with formic acid from both FBD and FDD were more heterogeneous. ABri and ADan were fully post-translationally modified to pyroglutamate at the N terminus, partially N- and C-terminally degraded and heavily oligomerized (Figure 3D, H). The N- and C-terminal degradation was more evident in FDD than in FBD. Interestingly, Aβ species with a more restricted pattern of heterogeneity were also retrieved in all the above referred extracts from FDD but not from FBD tissues (Figure 4). Against the dogma that Aβ40 is always the principal component of Aβ vascular deposits whereas Aβ42 is the main component of parenchymal lesions, intact Aβ42 and its N-terminal degradation derivative Aβ4-42 were the main components of vascular Aβ deposits in FDD whereas Aβ40 was negligible (80). Aβ degradation fragments ending at position 34 were found only in water-soluble extracts and not in the amyloid deposits, suggesting their relation with clearance mechanisms rather than amyloid formation. Whether the co-deposition of 2 different amyloid subunits is important for the pathogenic mechanism of the disease or simply reflects a specific conformational mimicry interaction is currently being investigated.

The post-translational modification of the N-terminus from glutamate to the cyclic pyroglutamate observed in both ABri and ADan is particularly interesting and mechanistically informative. Since the formation of pyroglutamate is chemically

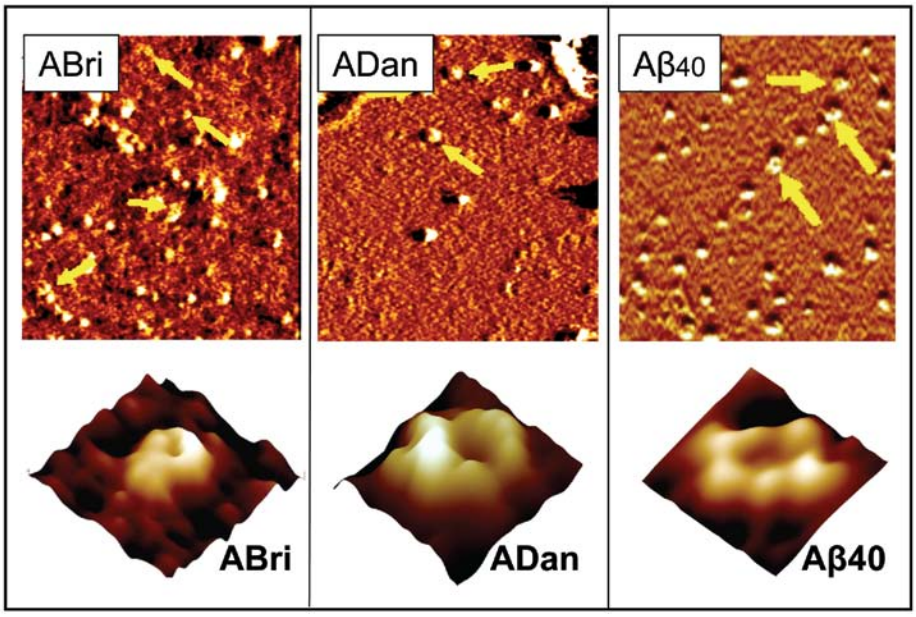


**Figure 5.** *Atomic force microscopy images of ion channels.* Top panels: Atomic force microscopy image of ABri, ADan and Aβ1-40 reconstituted in membrane bilayers showing channel-like structures with a central pore at low resolution (yellow arrows). Bottom panels: Individual channel-like structures formed by ABri, ADan,and Aβ1-40 at high resolution (image size: 35 nm for ABri, 20 nm for ADan and 25 nm for Aβ1-40).

stable and poorly reversible, the presence of glutamate-only species in plasma is a clear indication that the circulating species do not represent a clearance mechanism from the cerebral deposits but rather the immediate precursors of the deposited amyloids. In addition, the existence of pyroglutamate-only species in the amyloid lesions strongly suggests that the conversion glutamate to pyroglutamate occurs in situ at the place of deposition. The presence of N-terminal pyroglutamate was previously reported in truncated forms of the Alzheimer Aβ peptide (51, 68, 69, 77) as well as in some hormones and neuropeptides (75). Although the final product is the same, neuropeptides and amyloids differ in the amino acids that serve as a substrate for the post-translational modification. In the neuropeptides the N-terminal post-translational modification occurs at a glutamine residue. Cyclization involves the nucleophilic attack of the β-amino group on the amidated carboxyl group and the release of NH3 catalyzed by glutaminyl cyclase at neutral pH (6, 14). In the case of ABri, ADan and truncated forms of Aβ, the post-translational modification arises from glutamic acid as a substrate and involves the loss of a molecule of water instead of deamidation. Whether or not the same glutaminyl cyclase is responsible for the dehydration process remains unknown.

## POTENTIAL MECHANISMS OF DISEASE PATHOGENESIS IN FBD AND FDD

In general terms, the detrimental effect of amyloidogenic peptides is believed to result from a change in their native conformations into more insoluble structures which accumulate in the form of either intra- and extra-cellular aggregates or fibrillar lesions. In turn, these deposits trigger a complex set of interlinking pathways usually associated with the local release of inflammatory mediators, oxidative stress, complement activation, cell toxicity, and alterations in the membrane permeability which separately or synergistically result in cell damage, organ dysfunction and eventually death. ABri and ADan peptides although not as well studied as Alzheimer Aβ, appear to share at least some of these mechanisms of action.

The exact nature of the toxic species involved in neurodegeneration has been the subject of intense debate for many years. Although the Aβ fibrillar deposits were originally claimed responsible for the neurotoxic effect of amyloid, the poor correlation between plaque number, time of appearance or morphological distribution with neurodegeneration or clinical dementia, raised questions as to the precise structure of the toxic elements (22, 31, 36). Recently, soluble oligomers have been implicated as the primary pathological species and are believed to ultimately be the cause of the synaptic loss and dementia associated with AD (22, 31, 36, 71). Stable oligomers of Aβ42 have been identified in vivo and isolated from brain, plasma, and CSF (33, 65) and their presence seems to better correlate with the severity of neurodegeneration (43, 47). Furthermore, experiments with non-fibrillar structures including oligomers, amyloid-derived diffusible ligands (*ADDLs*) (34, 35, 54, 85) and protofibrils (84) demonstrated that these peptide assemblies are also neurotoxic. A constant finding in cerebral amyloid disorders and of particular magnitude in FBD and specially FDD is the presence of non-fibrillar Congo red negative parenchymal pre-amyloid lesions composed of similar species as those identified in fibrillar lesions although less polymerized, circumventing the need of formic acid for their tissue extraction (Figures 3, 4). Notably, in FBD and FDD these lesions co-exist with neurofibrillary tangles identical to those found in AD even with limited or practically absent mature cored plaques. Whether these pre-amyloid lesions represent the oligomeric assemblies responsible for toxicity remains to be determined, but it is clear from the FBD and FDD examples that the development of dementia is neither exclusive of Aβ nor dependent on the presence of compact plaques.

Studies using neuronal cells in culture demonstrated that ABri and ADan are able to induce toxicity in vitro through the triggering of apoptotic pathways. These mechanisms, although originally assumed to participate mainly in physiological processes occurring during brain development, seem to play a role in many neurodegenerative disorders and have been demonstrated in AD both in vitro and in vivo (4, 10, 41, 49, 74, 89). The process of apoptosis involves an increasing number of proteins, a regulatory balance between pro- and anti-apoptotic pathways, as well as various receptors (for review, see 12, 88). The major pathways converge on the sequential activation of the caspase family of cysteine proteases (for review, see 9, 76). Activation of the effector caspases leads to DNAse activation with subsequent DNA cleavage and nuclear fragmentation as well as to the enzymatic degradation of a number of proteins that coordinate the cell death process (for review, see 46). Panactivation of caspases induced by ABri and ADan oligomeric pep-

tides, have been demonstrated to occur in vitro and translate into enhanced neurotoxicity (19), pointing out to a potential role of apoptosis in the pathogenesis of FBD and FDD. Whether caspase-independent apoptotic pathways or necrotic processes contribute to the ABri- or ADan-induced cell death mechanisms, as demonstrated for Aβ peptides (4, 52), remains to be elucidated.

The contribution of inflammation driven mechanisms to disease pathogenesis has been widely documented in neurodegenerative disorders, particularly in AD. Complement activation, with its pro-inflammatory consequences as well as the action of inflammation-related cytokines, are today considered a driving force in the cascade of events leading to cell damage (1, 13, 20, 64, 78). As indicated above, inflammation-related components are also abundant in FBD and FDD. Complement activation products of both the classical and the alternative pathways co-localize with FBD and FDD parenchymal plaques and cerebrovascular deposits mainly associated with Congo red- and thioflavin-positive deposits (66). The activation derived components present in the lesions in both diseases include elements of the classical pathway, such as the recognition component C1q, the intermediate proteins C4d and C3d, and the terminal complex C5b-9, as well as the activated form of factor B (Bb) of the alternative pathway. In vitro, ABri and ADan peptides are able to fully activate the classical and alternative complement cascades at levels comparable to those generated by the Alzheimer Aβ peptides (66), thereby indicating that the inflammatory response originated by the deposits is likely to contribute to disease progression and pathogenesis as in AD.

There is increasing data indicating that the cytotoxic action of amyloidogenic peptides might be initiated by their interaction with plasma membranes and the subsequent formation of ion channels-like assemblies (for review, see 3). Atomic force microscopy revealed the formation of doughnut-shaped structures protruding out of the membrane surface with a centralized pore-like depression representing individual channels (40, 59). The formation of ion channel structures was reproduced with many amyloid subunits (27, 37, 39, 40, 59) and was accompanied by a process of protein oligomerization (59). The formation of ion channels for ABri, ADan and Aβ40 peptides, evaluated by atomic force microscopy, is illustrated in Figure 5. Electrophysiological data corroborated the formation of ion permeable channels and demonstrated their dependence on the aggregation state of the amyloid subunits by a shift to larger single-channel conductances concomitant with an increase in peptide oligomerization (23, 59). Although the contribution of channel formation to disease pathogenesis remains to be further studied, it is important to note that many of the effects of amyloid in vivo, including Ca2+ dysregulation, membrane depolarization, mitochondrial dysfunction, inhibition of long-term potentiation, and cytotoxicity may be attributed to channel formation in both plasma and intracellular membranes.

## CONCLUDING REMARKS

The process of neurodegeneration associated with cerebral amyloidosis is extremely complex and interlinks a diversity of mechanistic pathways leading to cell toxicity and death. Two hereditary conditions, FBD and FDD, share striking neuropathologic features with Alzheimer disease, including the presence of NFTs in limbic structures. The amyloid subunits ABri and ADan are unrelated to the Alzheimer Aβ peptide, offering a different paradigm to examine the role of amyloid in the mechanism of neurodegeneration. Although the exact nature of the toxic pathways triggered by the amyloid peptides remains elusive, Aβ, ABri and ADan seem to elicit similar apoptotic mechanisms and assemble onto lipid bilayers forming comparable ion channel structures likely inducing cytotoxicity through the modulation of Ca2+ homeostasis and membrane depolarization. ABri and ADan support the concept that different amyloid peptides irrespective of their primary structure are able to trigger similar pathologic pathways resulting in neuronal loss and clinical dementia. The lessons learned from the study of non-Aβ cerebral amyloid disorders may bring insight into the field of neurodegeneration by providing alternative models to study the molecular basis of neuronal cell death.

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