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Review

Chromosome 13 dementias

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Abstract. The importance of cerebral amyloid deposition in the mechanism of neurodegeneration is still debatable. Classic arguments are usually centered on amyloid β (Aβ) and its role in the neuronal loss characteristic of Alzheimer's disease, the most common form of human cerebral amyloidosis. Two non-Aβ cerebral amyloidoses, familial British and Danish dementias (FBD and FDD), share many aspects of Alzheimer's disease, including the presence of neurofibrillary tangles, parenchymal preamyloid and amyloid deposits, cerebral amyloid angiopathy and a variety of amyloid-associated proteins and inflammatory components. Both early-onset conditions are linked to specific mutations at or near the stop codon of the chromosome 13 gene *BRI2* that cause generation of longer-than-normal protein products. Furin-like processing of these longer precursors releases two de novo-created peptides, ABri and ADan, which deposit as amyloid fibrils in FBD and FDD, respectively. Due to the similar pathology generated by completely unrelated amyloid subunits, FBD and FDD, collectively referred to as chromosome 13 dementias, constitute alternative models for studying the role of amyloid deposition in the mechanism of neuronal cell death.

Key words. Familial British dementia; familial Danish dementia; congophilic amyloid angiopathy; ABri; ADan; cerebral amyloidosis; amyloid β; Alzheimer's disease.

Introduction

Amyloid diseases are part of an emerging heterogeneous group of clinical conditions collectively known as disorders of protein folding (reviewed in [1]). In all of these diseases, and for reasons poorly understood, normal soluble and, often, circulating proteins change their conformation and accumulate in selected tissues in the form of intracellular or extracellular insoluble aggregates. In amyloidoses, these aggregates are found in bundles of 6–8 nm twisted fibrils that are birefringent under polarized light after Congo red staining and that show yellow-green fluorescence after exposure to thioflavin S. The most frequent forms of amyloidosis are those localized to the central nervous system (CNS). In spite of the long list of proteins forming systemic and localized amyloid deposits in humans (25 different proteins and more than 100 genetic variants described so far (reviewed in [2]), only about one-third are known to produce fibrillar deposits in the CNS, resulting in cognitive deficits, dementia, stroke, cerebellar and extrapyramidal signs or a combination of these.

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Classical amyloid lesions in the CNS are usually found in the form of (i) parenchymal preamyloid lesions that are immunoreactive with specific anti-amyloid antibodies, negative to Congo red or thioflavin S staining and amorphous nonfibrillar in structure under electron microscopy (EM); (ii) parenchymal amyloid deposits, usually in the form of plaques or perivascular plaques, that are Congo red- and thioflavin S-positive, immunoreactive with specific anti-amyloid antibodies and typically fibrillar in appearance under EM; and (iii) cerebral amyloid angiopathy (CAA), characterized by Congo red- and thioflavin S positive, anti-amyloid reactive fibrillar deposits compromising the media and adventitia of medium and small cerebral arteries and arterioles as well as many cerebral capillaries. Although, depending on the particular clinical condition, either only some or all of the above-described lesions may coexist, their individual contribution to development of a clinical phenotype is still debatable. The amyloid β (Aβ) peptide found associated with sporadic and familial Alzheimer's disease (AD), Down syndrome, sporadic CAA and normal aging is by far the most common form of amyloid in humans and the most extensively studied among the proteins forming fibrillar deposits in the CNS. Nevertheless, non-Aβ cerebral amyloidoses constitute attractive alternative models for studying the relationship between amyloid formation and neurodegeneration. Particularly significant are the newly identified amyloid subunits ABri and ADan, which are completely unrelated to the Alzheimer's Aβ but are associated with the dementia linked to two hereditary conditions identified in separate kindreds from England and Denmark.

The *BRI* gene family

The *BRI* gene belongs to a multigene family comprising at least three homologues in both mice and humans, *BRI1*, *BRI2* and *BRI3* (also commonly referred to as *ITM2A*, *ITM2B* and *ITM2C*, or *E25A*, *E25B* and *E25C*, respectively) [3–6]. In addition to the human and murine isoforms, homology searches of expressed sequence tag (EST) data banks indicate the existence of BRI-like genes in rat, monkey, chicken, rabbit, pig and horse and in non-vertebrate animals, including flies and worms [3, 6, 7]. These findings, together with the similarities in genomic organization of the human and mouse isoforms described below, are suggestive of an evolutionarily conserved gene family. The genomic organization in both human and mouse consists of six exons and five introns. The first intron is remarkably longer in comparison with the other four and may contain regulatory sequences [6, 8]. Each of the family members was mapped to a different chromosome by fluorescence in situ hybridization: the human *BRI1* gene is located in chromosome X [8], *BRI2* in the long arm of chromosome 13 [5] and *BRI3* in chromosome 2 [6]. The gene expression pattern of the human isoforms is strikingly dissimilar. Whereas *BRI2* is highly ubiquitous, *BRI1* and *BRI3* show a limited expression profile. *BRI1* is particularly restricted to the mouse osteogenic and chondrogenic tissues [4], being mostly expressed in postnatal mandibular condyle, bone trabecula of mature bone, tooth germs and hair follicles, with strong expression in osteogenic tissues, such as neonatal calvaria, paws, tail and skin. In addition to being developmentally regulated in chondrogenesis and osteogenesis, *BRI1* is expressed in CD34(+) hematopoietic progenitor cells [9] and upregulated in T-cell development, with enhanced expression during thymocyte selection and T-cell activation causing CD8 downregulation when overexpressed in CD4/CD8 double-positive thymocytes [10]. *BRI3*, in contast, is localized predominantly to the brain [6, 11], with the highest levels of expression in cerebral cortex, medulla, amygdala, hippocampus, thalamus, caudate nucleus and spinal cord. Recently, *BRI3* was also demonstrated to be expressed in plasmacytoid dendritic cells, granulocytes [12], bone marrow, fetal liver and, to a lesser extent, in spleen, lymph nodes and thymus.

The *BRI2* gene locus (fig. 1A) is in the long arm of chromosome 13, specifically at 13q14, as demonstrated by fluorescence in situ hybridization analysis [5]. It is broadly expressed in peripheral organs, with high levels of expression in heart, kidney, pancreas, liver and placenta, as well as brain, where it is ubiquitously present in white and gray matter but shows more abundant distribution in the hippocampus and cerebellum compared with the cerebral cortex [5]. In situ hybridization studies of human cells in culture showed that within the brain, *BRI2* messenger RNA (mRNA) is widely distributed in different cerebral cell populations (fig. 1A) and its presence can be demonstrated in neurons, astrocytes and microglial cells, as well as in smooth muscle and cerebral endothelial cells.

The BRI2 protein

At the protein level, the human and mouse *BRI* isoforms share over 90% homology with the same isoform in the other species, but with a less pronounced amino acid similarity among the different isoforms within each species. So far, very little is known about the physiological functions of BRI proteins. Database scans for possible functional roles of these proteins revealed an ~100-amino acid BRICHOS domain, a conserved motif common to members of the BRI, ChM-I, SP-C and CA11 protein families [7]. The conserved BRICHOS domain is located within the pro-peptide region, which is removed after proteolytic processing by pro-protein convertases and which includes a pair of conserved cysteine residues that most likely form a disulfide bridge, with the blocks of sequence conservation located predominantly around these cysteines. It

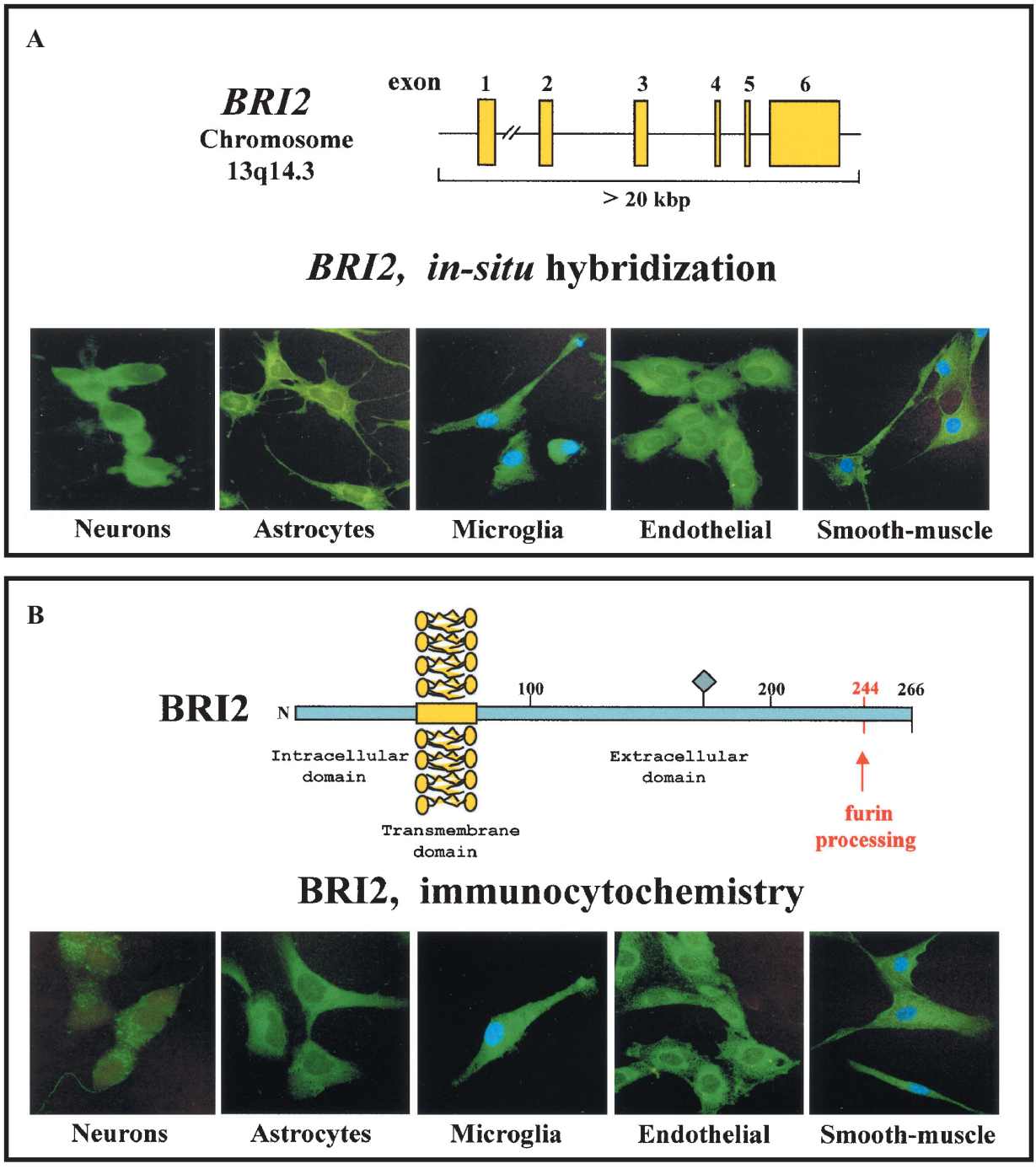

Figure 1. *BRI2* gene, mRNA and BRI2 protein product. (*A*) Top: Schematic representation of the *BRI2* genomic organization. It is composed of six exons and five introns spanning >20 kb. Intron 1 is extremely long, suggesting the presence of regulatory components. Bottom: Fluorescence in situ hybridization analysis of different human brain cells using FITC-labeled probes followed by Alexa-Fluor 488 signal amplification indicated the presence of *BRI2* mRNA in neurons, astrocytes and microglia, as well as in cerebral endothelial and smooth muscle cells (magnification, × 400). (*B*) Top: Schematic representation of the 266-amino acid type II transmembrane protein BRI2. The single transmembrane segment spans positions 52–74; the diamond at position 170 denotes a single N-glycosylation site; post-translational proteolytic processing at peptide bond 243–244, carried out by furin-like pro-protein convertases, is highlighted in red. Bottom: The same brain cells used for in situ hybridization in *A* were probed with anti-BRI2 antibody directed to residues 222–233 and the immunoreactivity was detected by an Alexa-Fluor 488-labeled secondary antibody. Consistent with the in situ hybridization data, BRI2 was detected in neurons, astrocytes and microglia, as well as in cerebral endothelial and smooth-muscle cells. In microglia and smooth-muscle cells, nuclear DNA is shown counterstained with 4′-6-diamidino-2-phenylindole (DAPI).

has been postulated that this domain may be involved in the targeting of the respective proteins to the secretory pathway or in intracellular processing. Although all of the proteins described as possessing the BRICHOS motif are dissimilar and associate with a diverse range of phenotypes, varying from dementia to cancer and respiratory distress, they share two common features: (i) all are type II transmembrane proteins and (ii) all are processed by furin-like endoproteases.

The protein product of the *BRI2* gene, referred to here as protein BRI2, is 266 amino acids long and contains a single transmembrane domain, a single BRICHOS domain and an N-glycosylation site at position 170. It also has the predicted structure of a type II transmembrane glycoprotein, in which the extracellular portion is the C-terminal region, and the N-terminus is intracytoplasmic [5]. The pro-protein convertase furin processes BRI2 at the sequence …KGIQKR^{243}↓E^{244}A…, resulting in the release of a short C-terminal peptide [13, 14]. Members of the pro-protein convertase family that includes furin exhibit a subtilisin-like activity and cleave most pro-hormones and neurotransmitter precursors; numerous other cellular proteins, including growth factors, proteases of the blood clotting and complement systems; and some viral and bacterial toxins [15]. Comparison of the activities of several pro-protein convertases revealed that furin is the most efficient in endoproteolysis of BRI2, whereas PACE4, PC6A, PC6B and LPC, all ubiquitously expressed in brain and peripheral tissues and active in the constitutive secretory pathways, show a much lower level of activity [14]. Recently, it was shown that furin, and to a lesser extent two other pro-protein convertases, PC7 and PC5A, was able to cleave the BRI3 isoform [16], which also contains the recognition sequence for furin [6]. Interestingly, BRI3 colocalizes in neurons with the membrane-associated β-amyloid converting enzyme 1 (BACE 1), which participates in the amyloidogenic pathway that generates the Alzheimer's Aβ peptide. Although BACE 1, which is also a substrate for furin [17–19], is capable of binding BRI3 through its cytosolic tail, it does not mediate BRI3 endoproteolytic processing. Whether this colocalization has any implications in disease pathogenesis remains unknown.

Studies aimed at determining the brain's topographical distribution of BRI2 in humans demonstrated its presence in normal pyramidal neurons in the CA3 and CA4 of the hippocampus as well as in Purkinje cells in the cerebellar cortex [20]. In pathological conditions, BRI2 was detected in dystrophic neurites in senile plaques and around ischemic lesions derived from axons and dendrites in AD tissues, and it was highly positive within Lewy neurites in dementia with Lewy bodies and Parkinson's disease cases [20].

Immunocytochemical analysis of cerebral cells in culture employing polyclonal antibodies raised against residues 222–233 of BRI2 demonstrated that, consistent with mRNA data, the BRI2 protein is present in neurons (both in the cellular body and in axons), in cerebral smooth muscle and endothelial cells and in astrocytes and microglia (fig. 1B).

Although the function of BRI2 remains unknown, its cellular distribution throughout the brain and its presence in dystrophic neurites in various neurodegenerative conditions [20], along with its morphological distribution within neuronal cell bodies and axons (fig. 1B) and its axonal localization in *BRI2* transfected neurons [21], suggest a role in transport along neuronal processes and a putative role in nerve terminals. The immunocytochemical demonstration of BRI2 in other cerebral cell types (fig. 1B) and the broadly systemic distribution of *BRI2* in peripheral organs suggest a wide-ranging function for the protein; however, its potential participation in more general mechanisms remains to be elucidated. It is interesting to note that the chromosome segment 13q14, to which *BRI2* maps, is one of the distinct chromosomal regions most frequently associated with tumor-related mutations in primary prostate cancer. Given the identification of *BRI2* as one of only six genes on that locus that show significantly reduced levels in prostate tumors compared with normal tissues, together with its low expression in prostate tumor cell lines [22] as well as evidence of allelic loss at the same locus in other cancers (including ovarian carcinoma, head and neck squamous cell carcinoma and pituitary tumors [22, 23]), *BRI2* has been proposed as a tumor suppressor gene with an important role in the initiation and progression of prostate carcinoma [23].

A short form of BRI2, generated by alternative gene splicing and lacking exon 1, has been demonstrated to possess pro-apoptotic functions that correlate with caspase-9 and caspase-3 activation and to participate in cell death in a p53-independent mechanism [24, 25]. BRI2 has a Bcl-2 homology-3 (BH-3) domain encoded in exon 2 that is known to be important for dimerization and regulation of Bcl-2 apoptotic activities. Mutagenesis of critical amino acids within the BH-3 domain of the BRI2 short form inhibits apoptosis, suggesting the importance of this domain in BRI2 apoptotic regulation [24]. Interestingly, parallel roles in induction of apoptosis have been claimed to be accountable for the detrimental effect of other amyloid proteins, of which Aβ and its genetic variants have been the most studied [26, 27].

Familial British and Danish dementias

Two newly described hereditary conditions linked to genetic defects in the *BRI2* gene, familial British dementia (FBD) and familial Danish dementia (FDD), collectively known as 'chromosome 13 dementias', are associated with

neurodegeneration and extensive amyloid deposition in the CNS, particularly with vascular and perivascular localization.

Clinical features

FBD is an early-onset autosomal dominant disorder clinically characterized by progressive dementia, spastic tetraparesis and cerebellar ataxia. The disease affects males and females in approximately equal proportion, with median ages of onset and death at 48 and 56 years, respectively [28]. FBD was first reported in 1933 by Worster-Drought et al. in two siblings who had familial presenile dementia with spastic paralysis [29, 30]. Patients with the disease have marked memory impairment progressing to global dementia. Personality changes are the earliest clinical manifestation, followed by cerebellar ataxia and spastic paralysis more severe than that seen in atypical AD or in Gerstmann-Sträussler-Scheinker disease. Brainstem signs, pseudobulbar palsy and dysarthria are common, and strokelike episodes may be present, although in contrast to other familial cerebrovascular amyloidoses, cerebral hemorrhage is relatively rare. Patients progress to a chronic vegetative state, becoming mute, unresponsive, paraplegic and incontinent. So far, FBD has been identified in three pedigrees, two of which may be related. The original Worster-Drought pedigree has been expanded since its original description, and common ancestors have been identified in separate family case reports. At present, it comprises 343 individuals over nine generations dating back to ca. 1780 [31–34]. Magnetic resonance imaging (MRI) scans of individuals affected with FBD typically show a moderate ventricular dilatation and a relatively preserved cortex with extensive periventricular white matter changes compatible with leukoaraiosis from early stages of the disease. These are most pronounced around the frontal and occipital horns of the lateral ventricles. Histopathological examination of FBD brain tissue using classical stainings such as silver, H&E or PAS reveals the characteristic lesions of this disorder: (i) severe amyloid angiopathy of the brain and spinal cord with perivascular amyloid plaque formation, (ii) parenchymal plaques affecting the limbic areas, cerebellum and occasionally, cerebral cortex, (iii) neurofibrillary degeneration of hippocampal neurons and (iv) periventricular white matter changes [31, 33].

FDD, also known as heredopathia ophthalmo-otoencephalica [35], is an early-onset autosomal dominant disorder originating on the Djürsland peninsula, Denmark. The disease, identified in 13 cases spanning 5 generations of a single family, is clinically characterized by the development of cataracts, deafness, progressive ataxia and dementia [35–38]. Cataracts seem to be the earliest manifestation of the disease, starting before the age of 30, whereas impaired hearing usually develops about 10 years later. Cerebellar ataxia occurs shortly after the age of 40, followed by paranoid psychosis and dementia about 10 years later. In the FDD cases for which information is available, males and females are equally affected: the median age of onset of visual symptoms is 27 years, while the median age at death is 58 years. As described above for FBD, 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's subcortical arteriosclerotic encephalopathy. Routine histopathological examination of FDD cases usually unveils diffuse brain atrophy with a particularly severe involvement of the cerebellum, cerebral cortex and white matter, as well as the presence of very thin and almost demyelinated cranial nerves. There is widespread amyloid angiopathy in the 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.

Amyloid subunits ABri and ADan

Biochemical analysis of amyloid deposits in FBD and FDD unveiled two new molecules not previously known to be associated with disease (fig. 2). The deposited amyloid proteins, ABri in FBD [5] and ADan in FDD [39], are C-terminal fragments of the above-described type II transmembrane precursor molecule BRI2 that bear distinct genetic alterations. ABri and ADan originate as a result of two different genetic defects in the *BRI2* gene, namely, a stop-to-Arg mutation in FBD and a 10-nucleotide duplication insertion occurring immediately before the stop codon in FDD (fig. 2). Although the nucleotide changes are completely different, the final outcome is common to both diseases: the ordinarily occurring stop codon is not operational (either nonexistent or out of frame), causing genesis of the extended precursors ABriPP and ADanPP which feature C-terminal segments that do not exist in normal conditions. The nucleotide substitution in FBD creates a restriction site for the *XbaI* endonuclease, useful in detecting carriers of the disease [5]. Although this restriction site is not present in FDD, the duplication insertion can be detected in agarose gels under particular experimental conditions [39].

The de novo-created ABri and ADan amyloid subunits, with no sequence identity to any other known amyloid protein, are released from their precursors via the above-described furin-like proteolytic processing at peptide-bond 243–244 [13, 14]. The resulting amyloid subunits are both 34 residues long, sharing 100% homology in their first 22 residues and having a completely different 12-amino acid C-terminal segment (fig. 2). Within the amyloid deposits, both ABri and ADan feature pyroglutamate instead of

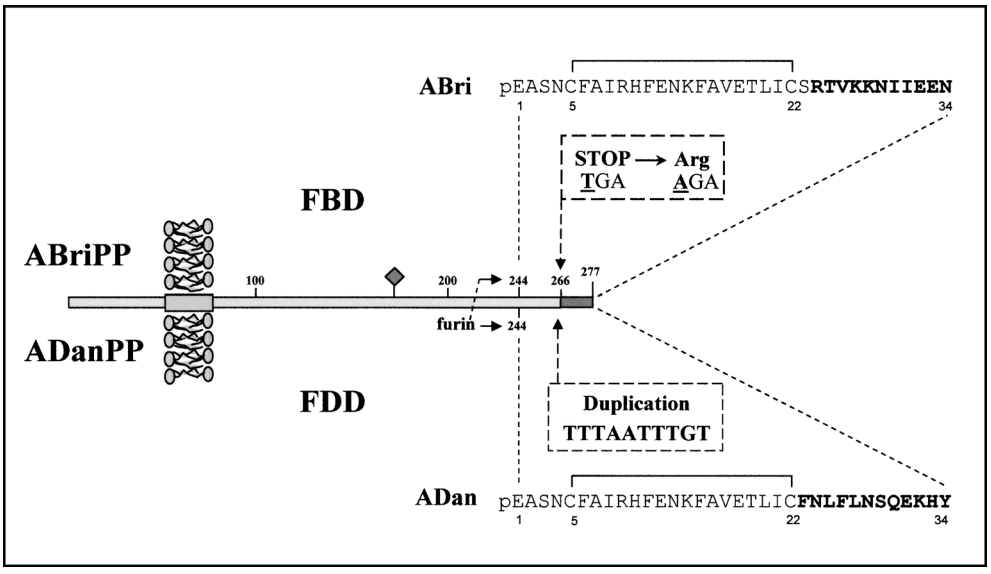

Figure 2. Genetic defects in *BRI2*. Genesis of the ABri and ADan precursors in patients with FBD and FDD. Schematic representation of genetic defects in the *BRI2* gene: stop-to-Arg at codon 267 in FBD (top box) and a 10-nucleotide duplication insertion after codon 265 (bottom box) generate longer-than-normal precursors ABriPP and ADanPP (gray rectangle, positions 266–277). Furin-like pro-convertase activity proteolytically processes both extended precursors at peptide bond 243–244, generating ABri and ADan C-terminal fragments composing amyloid deposits in FBD and FDD. ABri and ADan are both 34 amino acids long, identical in their first 22 residues and completely different in their 12 C-terminal amino acids (bold type). They contain a single disulphide bond between cysteine residues 5 and 22.

glutamate at their N-terminus, a post-translational modification previously observed in other brain amyloids, i.e. in truncated forms of the Alzheimer's Aβ peptide as well as in some hormones and neuropeptides, including neurotensin and thyrotropin- and gonadotropin-releasing hormones in which the biological activities largely depend on the cyclization of amino-terminal glutaminyl residues [40–44]. The presence of the N-terminal pyroglutamate suppresses one negative charge, increases the β-sheet content of the ABri and ADan peptides as well as their tendency to aggregate and polymerize and may offer protection against in vivo proteolysis by amino peptidases. Both peptides contain two cysteine residues that, in patients with FBD and FDD, are oxidized, forming a single intrachain disulphide bond between residues 5 and 22 [45].

As previously described for other amyloid subunits, soluble forms of ABri and ADan are present in biological fluids of affected and nonaffected carriers of the respective genetic defects [45]. Interestingly, FBD- and FDD-circulating peptides differ from the deposited homologues in that they lack the N-terminal post-translational modification, featuring glutamate exclusively. Since the formation of pyroglutamate from glutamate is not chemically reversible, the presence of glutamate-only species in plasma is a clear indication that the circulating species do not represent a clearance mechanism for the cerebral deposits, but rather the immediate precursors of the deposited amyloids. Supporting this view, systemic ABri amyloid deposits were identified in peripheral organs (i.e. pancreas, myocardium) in two FBD cases for which full autopsy was available [45]. Amyloid deposits were restricted mainly to the vessels, although the presence of pre-amyloid lesions was evident in the surrounding parenchyma. Biochemical analysis indicated the presence of the same species found in the brain, including those with N-terminal pyroglutamate, suggesting that similar mechanisms of amyloid formation and deposition occur inside and outside the CNS. Although no peripheral tissue is so far available from FDD cases, it is likely that similar systemic deposits may exist in this disorder.

Neuropathological findings

The unique C-terminal differences between ABri and ADan were used to raise polyclonal antibodies 338 (anti-ABri [5]) and 5282 (anti-ADan [39]), specific for FBD and FDD, respectively. Using these antibodies, neuropathological studies were able to evaluate the extent of ABri and ADan deposits in FBD and FDD. Blood vessels and parenchyma are both severely affected throughout the CNS (fig. 3) with the limbic structures and cerebellum being the most compromised areas. Neurofibrillary tangles, mostly in the hippocampus are characteristic of both diseases (fig. 3E, F). CAA has been found to be much more widespread in both FBD and FDD than in the common sporadic form of CAA due to Aβ deposition, as it is present in nearly all CNS areas, including the cerebrum, cerebellum, brainstem and spinal cord, and is absent in only a few areas, such as the striatum and substantia

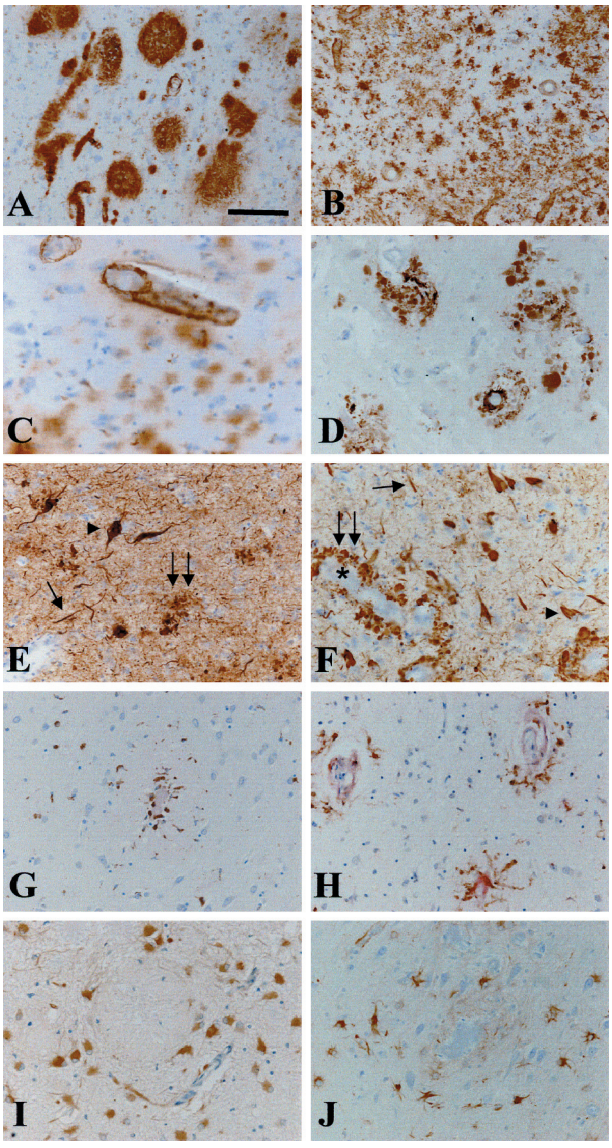

Figure 3. Immunohistochemical features of FBD and FDD. ABri amyloid plaques in the hippocampus (*A*) and preamyloid diffuse plaques in the entorhinal cortex (*C*) in a case of FBD, highlighted by anti-ABri antibody 338. In addition to the overwhelming vascular ADan pathology, ADan hippocampal diffuse (preamyloid) plaque (*B*), highlighted by anti-ADan antibody 5282, is the characteristic parenchymal lesion in FDD. In a proportion of blood vessels affected by ADan deposition, Aβ can also be seen (*D*; antibody 6E10, Signet). There is striking neurofibrillary tangle pathology in limbic structures in both FBD (*E*) and FDD (*F*), highlighted by anti-tau AT8 antibody (Innogenetics). Both diseases show neurofibrillary tangles (arrowhead) and neuropil threads (arrow), although abnormal neurites (double arrow), which are seen around amyloid plaques in FBD, are found only in association with amyloid-laden blood vessels (asterisk) in FDD. Microglia expressing MHC (major histocompatibility class) class II (CR3/43 antibody, Dako) is seen around all types of amyloid lesions in FBD (*G*: amyloid-laden vessel with a perivascular amyloid plaque), and mainly in relation to blood vessels with amyloid deposition in FDD (*H*). Reactive astrocytes are also found to cluster in a manner similar to microglia (*I*: FBD; *J*: FDD). Bar on *A* represents 150 µm on *A*, *B*, *C*, *D*, *G* and *H* and 75 µm on panels *E*, *F*, *I* and *J*.

nigra. Neuropathological investigations using confocal and immunoelectron microscopy showed that the parenchymal ABri deposits are of a predominantly amyloid (fibrillar) nature in FBD, while the ADan parenchymal deposits are characteristically of the preamyloid (nonfibrillar) type in FDD (fig. 3, 4). An interesting aspect of all the FDD cases analyzed so far is the codeposition of Aβ in a proportion of blood vessels affected by ADan deposition (fig. 3D, 4; see below). In both FBD and FDD, the neurofibrillary tangle pathology is immunohistochemically, ultrastructurally and biochemically identical to that seen in AD; tangles are immunoreactive with several anti-hyperphosphorylated tau antibodies, ultrastructurally composed of paired helical filaments and display an electrophoretic migration pattern that is indistinguishable from that seen in Alzheimer's paired helical filament tau [38, 46, 47]. Amyloid deposits and neurofibrillary tangles coexist primarily in the limbic structures, although amyloid burden is also evident in other areas not affected by tangle pathology. As in AD, there is no complete overlap between these two pathologies, and neurons from some structures with significant parenchymal amyloid load (e.g. the cerebellum) remain resistant to neurofibrillary tangle formation. This lack of complete overlap of tau and amyloid pathologies in FBD and FDD is puzzling and indicates that, although amyloid and preamyloid deposits are of paramount importance, the mechanism of neurodegeneration is complex and likely involves additional factors and interrelationships among different cellular pathways.

A striking astrocytic and activated microglia response was found to be associated with vascular and parenchymal amyloid deposition in FBD and with vascular amyloid in FDD (fig. 3G–J). Interestingly, furin colocalizes with ABri in compact plaques and amyloid angiopathy in FBD. Neurons in the hypothalamus and locus coeruleus show intense furin immunoreactivity coinciding with high density ABri deposits [48]. This colocalization, together with the demonstrated in vitro capability of furin to cleave ABri peptides, strongly indicates that this protease may be relevant in the in vivo mechanism of BRI2 processing. Another element contributing to FBD and FDD's close similarity to AD is the presence of a group of so-called amyloid-associated proteins in the lesions, which are unrelated components that colocalize with the amyloid deposits, although they are not structural parts of the final fibril [1, 49, 50]. Serum amyloid P component (SAP), α1-antichymotrypsin, apolipoprotein E, apolipoprotein J, complement components, vitronectin, extracellular matrix proteins and glycosaminoglycans are among the many amyloid-associated proteins described [1, 51–56]. To date, it is not clear whether they are innocent bystanders or whether their presence is related to the mechanism of amyloidogenesis, although several lines of investigation favor the latter notion, at least for some of them (e.g. SAP

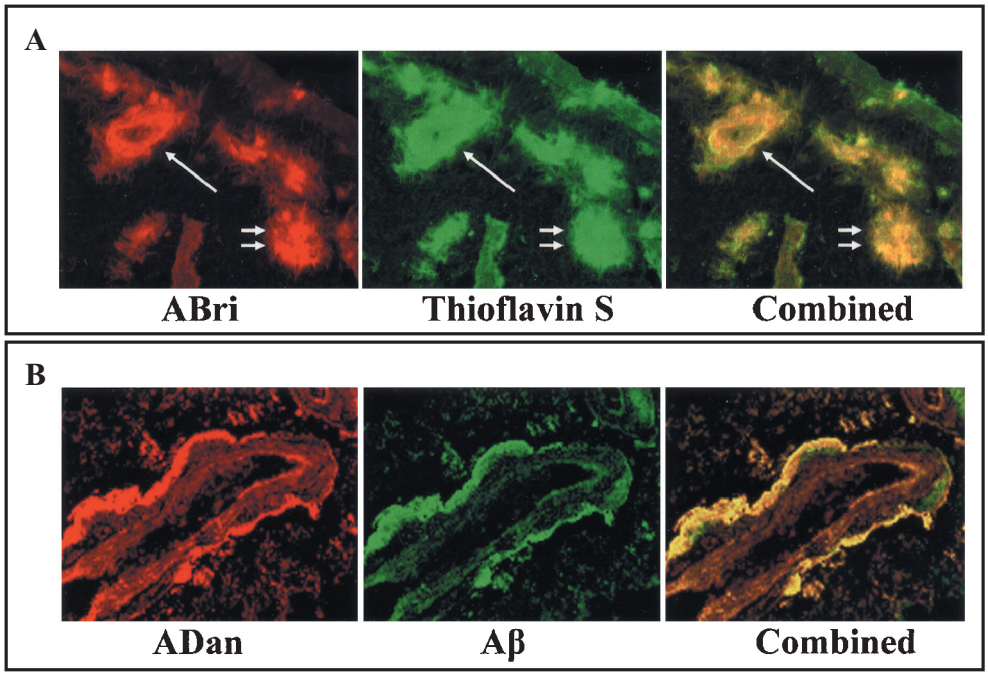

Figure 4. Confocal microscopy of FBD and FDD lesions. (*A*) There is a good overlap between ABri deposition (red) and thioflavin S staining (green) in the hippocampus in both amyloid-laden vessels (arrow) and plaques (double arrow) in FBD (objective ×25). (*B*) In FDD, both ADan (red) and Aβ (green) are present in a proportion of amyloid-laden blood vessels, although the deposition of the two proteins is not entirely overlapping (objective ×63).

is invariably present in several types of fibrillar deposits but absent in nonfibrillar lesions) [57].

Biochemical properties of amyloid subunits ABri and ADan

ABri and ADan peptides show a high tendency to oligomerize and aggregate both in vivo (fig. 5) and in vitro (fig. 6). As observed in every known amyloid, the subunits isolated from tissue deposits showed a variable degree of oligomerization. For ABri and ADan, the process of oligomerization seems to be directly related to the degree of peptide insolubility. From FBD and FDD cerebral homogenates, water-based buffers extracted only monomeric ABri and ADan species together with a small percentage (<10%) of dimeric and trimeric forms. Detergent (SDS) solubilized fractions, representing mainly preamyloid deposits, consisted of a similar composition but with some enrichment (<40%) in dimeric and trimeric forms that, in some cases, appeared in an equivalent proportion to the monomeric species. Lastly, the most insoluble fractions, representing fibrillar deposits, were solubilized in formic acid and showed a pattern of heavy oligomerization and the simultaneous appearance of a broad spectrum of species ranging from monomeric forms to high-molecular-mass aggregates (>70 kDa) [44] (fig. 5). Although ABri was the sole amyloid subunit identified by immunohistochemical analysis of FBD cases [47], and full-length as well as N-terminal degradation products of ABri were biochemically retrieved from parenchymal and vascular lesions [5], similar studies in FDD amyloid deposits revealed unexpected features. Detailed immunohistochemical studies with a variety of anti-amyloid antibodies revealed the presence of Aβ in a

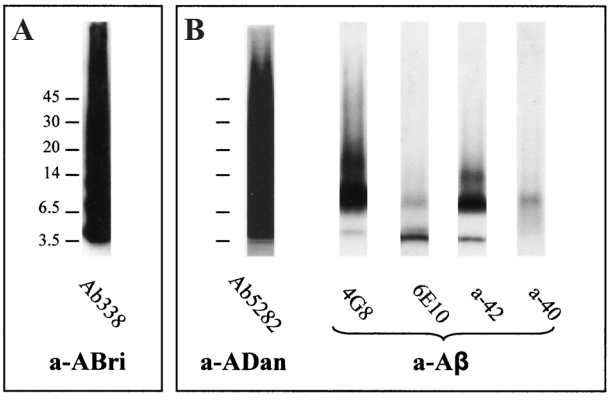

Figure 5. Amyloid deposits in FBD and FDD: oligomers of ABri and ADan/Aβ. Western blot analysis of formic acid extracts from amyloid deposits in FBD (*A*) and FDD (*B*). Amyloid deposits in FBD are composed of highly oligomerized ABri that is immunoreactive with anti-ABri (Ab338) antibodies. In FDD, deposits are mainly composed of oligomerized ADan (Ab5282), but in many instances, Aβ partially colocalizes with ADan, particularly in vascular lesions (fig. 4). Biochemical analysis of these vascular deposits identified Aβ ending at position 42 as the main Aβ component.

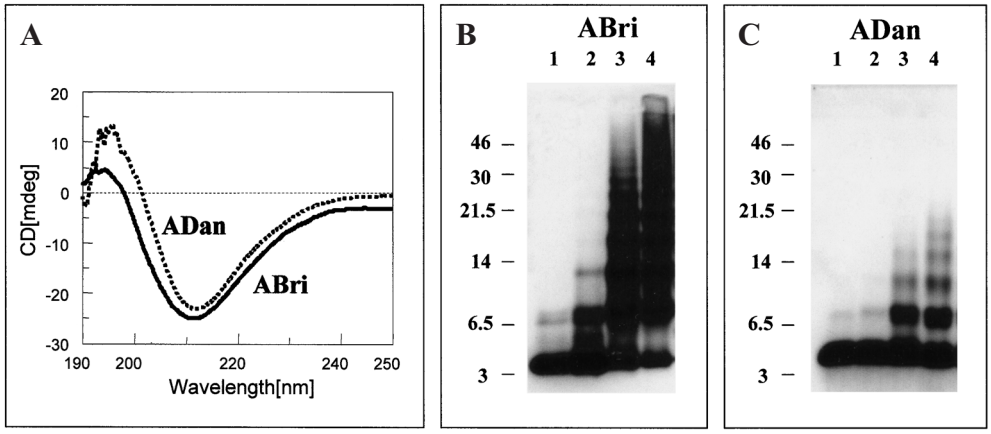

Figure 6. Synthetic ABri and ADan oligomerize as the tissue-extracted counterparts. (*A*) Synthetic ABri and ADan peptides exhibit predominantly β-pleated-sheet secondary structure, as indicated by the typical CD spectra. Western blot analysis of ABri (*B*) and ADan (*C*) indicates fast oligomerization kinetics in PBS at 37°C (lanes 1, zero time; lanes 2, 2-h incubation; lanes 3, 12-h incubation; lanes 4, 24-h incubation).

proportion of both parenchymal and vascular ADan lesions [38] (fig. 3D, 4B). Biochemical analysis indicated that, opposite of any prediction and in striking difference with AD [1], the Aβ peptides isolated from vessels and pre-amyloid deposits were primarily ending at position 42 (fig. 5), with the vast majority N-terminally degraded at peptide bond 3–4 whereas Aβ40 species were negligible. Whether the codeposition of two different amyloid subunits is important for the pathogenic mechanism of the disease or simply reflects specific conformational mimicry interaction in currently being investigated.

In vitro, synthetic homologues of full-length ABri and ADan are able to recapitulate the oligomerization propensity observed in the amyloid deposits by forming spontaneous β-sheet-rich structures with the characteristic circular dichroism spectra, shown in figure 6A, and by exhibiting fast aggregation kinetics, and they have an even higher tendency than Alzheimer's Aβ42 to form high-ordered oligomers. In an extremely short time (<24 h), under physiological conditions (phosphate buffer saline, 37°C] both peptides achieve a high degree of oligomerization, with ABri exhibiting faster kinetics (fig. 6B, C). Interestingly, under these conditions fibrillization seems to proceed very slowly (from weeks to months). In fact, enhanced aggregation kinetics and protofibril formation by ABri is favored by slightly acidic pH [58], consistent with the behavior of other amyloid-forming proteins [59] and supporting the premise that a common mechanism may be involved in protein misfolding and amyloidosis. ABri formed protofibrils as intermediate structures during fibril maturation at pH 4.9 and, similar to previous findings with Aβ, fibril seeds are required to initiate amyloid formation at neutral pH [58]. As previously observed with Aβ, synthetic peptides homologous to ABri and ADan [60, 61] exhibited cytotoxic properties to neuronal cell lines. Oligomeric forms of the peptides seem to be the species relevant for in vitro neurotoxicity [60, 62], in agreement with current views indicating that nonfibrillar aggregates of the amyloid peptides constitute the significant pathogenic elements [63–65]. The striking abundance of parenchymal preamyloid deposits of a nonfibrillar nature in FDD coexisting with neuronal damage and dementia [66] could very well be explained based on the detrimental effect of these oligomeric assemblies.

Inflammatory process in FBD and FDD

Compelling evidence continues to accumulate for a significant role of local inflammatory processes in the progression of neurodegenerative disorders, these mechanisms having been most studied in AD [67]. In particular, complement activation and its proinflammatory consequences have been demonstrated to contribute extensively to disease pathogenesis [68], and inflammation-related cytokines are today considered a driving force in the neuropathological cascade associated with AD (for reviews see [67, 69]). The complement system is a highly regulated, powerful effector mechanism of the immune system that destroys and clears deleterious substances. It consists of more than 20 proteins that become sequentially activated in a proteolytic cascade. Originally, activation of the complement system was thought to occur only by binding of immune complexes to C1q, the recognition component of the classical pathway. Later, it became evident that the complement system can be directly activated, in the absence of antibody, by the interaction of certain foreign molecules with C3 (alternative activation pathway) or C1q (antibody-independent classical activation pathway), or by specific lectins on the surface of certain microorganisms (lectin activation pathway) [68, 70, 71]. Activation by any of these pathways results in the

sequential cascade of enzymatic processes that finally lead to the formation of the membrane attack complex (MAC) C5b-9, a transmembrane channel capable of producing cell lysis [72]. In addition, activation of the complement system results in concomitant generation of opsonins and anaphylotoxins, which drive numerous inflammatory mechanisms, including scavenger cell activation, chemotaxis and frustrated phagocytosis and in secretion of cytokines, chemokines and reactive oxygen and nitrogen species, which contribute to the inflammatory picture seen in neurodegenerative disorders.

Inflammation-related components are abundant in chromosome 13 dementias. Activated microglia, expressing the major histocompatibility class II antigens characteristic of inflammatory processes, are seen around all types of amyloid lesions in FBD (fig. 3G) and mainly in relation to blood vessels with amyloid deposition in FDD (fig. 3H) [38, 47]. Reactive astrocytes are also found to cluster in a manner similar to that of microglia (fig. 3I, J). Complement activation products from both the classical and the alternative pathways colocalize with FBD and FDD parenchymal plaques and cerebrovascular amyloid deposits, mainly associated with Congo red- and thioflavin-positive amyloid deposits rather than with Congo red- and thioflavin-negative parenchymal preamyloid lesions [73]. The activation-derived fragments of the classical pathway present within the vascular and parenchymal lesions in both diseases include the recognition component C1q, intermediate components such as C4d and C3d and the MAC C5b-9. In vitro both ABri and ADan peptides are able to fully activate the complement cascade at levels comparable to those generated by the Alzheimer's Aβ peptides, as determined by hemolytic experiments and quantitation of the activation products iC3b, C4d, Bb and C5b-9 by enzymo-immuno assays. Based on these quantitative assays, it was established that complement activation in chromosome 13 dementias proceeds ~70–75% through the classical pathway while only ~25–30% activation seems to occur through the alternative pathway, in a manner very similar to that reported for AD [74]. Thus, the chronic inflammatory response seen in FBD and FDD, most likely initiated by the amyloid deposits, is probably a contributing element to disease progression and pathogenesis as it has been proposed to be for AD.

Concluding remarks

FBD and FDD, two non-Aβ cerebral amyloidoses collectively referred to as chromosome 13 dementias, share important neuropathological features with AD, including widespread amyloid angiopathy, perivascular plaques, neuritic and non-neuritic parenchymal plaques and neurofibrillary tangles. The amyloid proteins ABri, in FBD, and ADan, in FDD, share no structural identity with Aβ. Neurofibrillary tangles containing the classical paired-helical filaments as well as neuritic components in many instances colocalize with the amyloid deposits. In both disorders, the pattern of hyperphosphorylated tau immunoreactivity is almost indistinguishable from that seen in AD. Moreover, the same amyloid-associated proteins and inflammatory components seen in Alzheimer's lesions are also featured in FBD and FDD. All of the above findings indicate that different amyloid peptides, irrespective of their primary structure, can trigger similar pathological pathways that result in neuronal loss and clinical dementia, which emphasizes the importance of structural conformational changes and common additional factors (e.g. chaperones) in the process of amyloidogenesis and neurodegeneration. We propose FBD and FDD as alternative models for the study of the mechanisms of amyloid formation, neurofibrillary degeneration and cell death in the brain.

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