Familial Danish Dementia: A Novel Form of Cerebral Amyloidosis Associated with Deposition of Both Amyloid-Dan and Amyloid-Beta

JANICE L. HOLTON, MD, PhD, TAMMARYN LASHLEY, BSC, JORGE GHISO, PhD, HANS BRAENDGAARD, MD, RUBEN VIDAL, PhD, CHRISTOPHER J. GUERIN, PhD, GRAHAM GIBB, PhD, DIANE P. HANGER, PhD, AGUEDA ROSTAGNO, PhD, BRIAN H. ANDERTON, PhD, CATHERINE STRAND, MSc, HILARY AYLING, MSc, GORDON PLANT, MD, BLAS FRANGIONE, MD, PhD, MARIE BOJSEN-MØLLER, MD, and TAMAS REVESZ, MD

Abstract. Familial Danish dementia (FDD) is pathologically characterized by widespread cerebral amyloid angiopathy (CAA), parenchymal protein deposits, and neurofibrillary degeneration. FDD is associated with a mutation of the BRI2 gene located on chromosome 13. In FDD there is a decamer duplication, which abolishes the normal stop codon, resulting in an extended precursor protein and the release of an amyloidogenic fragment, ADan. The aim of this study was to describe the major neuropathological changes in FDD and to assess the distribution of ADan lesions, neurofibrillary pathology, glial, and microglial response using conventional techniques, immunohistochemistry, confocal microscopy, and immunoelectron microscopy. We showed that ADan is widely distributed in the central nervous system (CNS) in the leptomeninges, blood vessels, and parenchyma. A predominance of parenchymal pre-amyloid (non-fibrillary) lesions was found. Aβ was also present in a proportion of both vascular and parenchymal lesions. There was severe neurofibrillary pathology, and tau immunoblotting revealed a triplet electrophoretic migration pattern comparable with PHF-tau. FDD is a novel form of CNS amyloidosis with extensive neurofibrillary degeneration occurring with parenchymal, predominantly pre-amyloid rather than amyloid, deposition. These findings support the notion that parenchymal amyloid fibril formation is not a prerequisite for the development of neurofibrillary tangles. The significance of concurrent ADan and Aβ deposition in FDD is under further investigation.

Key Words: ADan; Amyloid; BRI2 gene; Danish dementia; Neurofibrillary degeneration; Pre-amyloid.

INTRODUCTION

Familial Danish dementia (FDD), described by Strøm-gren et al (1, 2) as heredopathia ophthalmo-oto-encephalica, is a rare, dominantly inherited neurodegenerative disease. It was originally described in 9 members from 3 generations of a family living in the Djursland peninsula, northeast of Århus, Denmark. Cataracts, which may start before the age of 30, appear to be the first abnormality, and other ocular disorders such as hemorrhages may also be present. Impaired hearing in the form of severe or total perceptive loss of hearing tends to appear 10 to 20 yr after the ocular symptoms have started, while cerebellar ataxia develops shortly after the age of 40. Paranoid psychosis usually develops after age 50, followed by dementia in the majority of the cases. Most patients die in their fifties or sixties. The original description provided only scanty neuropathological data based on the examination of a single case. These suggested the presence of a uniform, diffuse atrophy of the brain, thin and demyelinated cranial nerves, as well as “histological involvement,” including a vasculopathy of the cerebellum, cerebral cortex, and white matter (2). Subsequent unpublished observations confirmed that the vascular changes are those of cerebral amyloid angiopathy (CAA) and that hippocampal neurofibrillary tangles (NFTs) are also a feature of this disease (H. Braendgaard, personal communication).

FDD has recently been shown to be associated with a decamer duplication (TTTAATTGT) occurring between codons 265 and 266 of the BRI2 gene, which is 1 codon before the normal stop codon. The 10-nt duplication results in a frame-shift in the gene sequence producing a 277-amino-acid-long extended precursor protein, ADanPP (3). The wild-type precursor protein is a 266-amino-acid-long type II, single-spanning transmembrane protein, which is encoded by the BRI2 gene, located on chromosome 13. A point mutation (T→A) of the stop codon of the BRI2 gene has been described in association with familial British dementia (FBD), which also results in a 277-amino-acid-long mutated precursor protein designated ABriPP (4). Cleavage of 34 amino acids at the C-terminus, by furin-like proteolysis, of both ADanPP in FDD and ABriPP in FBD (5) results in the release of the amyloidogenic peptides, amyloid-Dan (ADan) in FDD, and amyloid-Bri (ABri) in FBD. ADan and ABri share...
common 22-amino-acid-long sequences in their N-terminal regions, but have distinct C-termini (3). The amyloidogenic subunit ADan has been shown to be deposited as amyloid in leptomeningeal and cerebral blood vessels and also as the major component of parenchymal lesions (3).

In this study we investigated the neuropathological features of 3 cases with FDD from the original pedigree. The distribution and patterns of ADan deposition were studied using immunohistochemistry with an antibody recognizing the C-terminus of ADan (Ab 5282) alone and in combination with Thioflavin S. The distribution and relationship of NFTs, neuritophyl threads (NTs), and abnormal neurites (ANs) to ADan deposits was investigated with the modified Bielschowsky silver impregnation method and the anti-tau antibody AT8 recognizing phosphorylated serine 202/threonine 205 epitopes of tau (6). The astrocytic response to ADan deposits was studied using GFAP immunohistochemistry; microglial response was addressed using a rabbit polyclonal antibody (Ab 5282, 1:200) or anti-mouse IgG (DAKO, 1:200), as appropriate, and ABC complex (DAKO). Color was developed with 3,3'-diaminobenzidine/H₂O₂.

Clinical Data

Clinical information and macroscopic descriptions of the brains and spinal cords were collected from the available records.

Tissue Collection

Brain and spinal cord samples from 3 cases of FDD, 5 sporadic Alzheimer disease (AD) cases (diagnosed by using standard criteria [7]), and 3 normal control cases were collected at postmortem. For routine stains and immunohistochemistry, tissue samples were fixed in 10% formalin in PBS. For electron microscopy, selected tissue samples from the hippocampal formation (case 1) were fixed in 3% buffered glutaraldehyde after fixation in 10% formalin. For Western blot analysis of tau, unfixed tissue samples from case 1 were taken at postmortem, frozen, and stored at −70°C.

Antibodies

For these studies a rabbit polyclonal antibody (Ab 5282, 1:2,000), raised to C-terminal amino acids of the ADan protein (CFNLFLNSQEKHY), was used (3). Pre-immune serum from the same animal was also used as a control. To detect microglia/macrophages, an anti-CD68 antibody (monoclonal, PG-M1, DAKO, Ely, UK, 1:150) and one recognizing major histocompatibility complex class II antigens (monoclonal, CR3/43; DAKO, 1:200) were used. An anti-GFAP antibody (polyclonal, DAKO, 1:1,000) was used for demonstrating astrocytes and the AT8 monoclonal antibody (Innogenetics, Ghent, Belgium, 1:600) was used for tau immunohistochemistry. For Aβ immunohistochemistry the 6F/3D antibody (monoclonal, DAKO, 1:100), recognizing residues 8–17 was used. For detecting possible Lewy body pathology a polyclonal anti-α-synuclein antibody (polyclonal, Diane P. Hanger, 1:2,000) and for prion protein immunohistochemistry the 3F4 (monoclonal, DAKO, 1:2,000) and KG9 (monoclonal, TSE Resource Centre, Institute of Animal Health, Compton, UK, 1:150) antibodies were used. For tau immunoblotting, the polyclonal, phosphorylation-independent antiserum TP70 (8), the phosphorylation-dependent mouse monoclonal antibody PHF1 (a gift from Peter Davies, Albert Einstein College of Medicine, New York, NY, 1:2,000), and the AT8, AT180, AT270 antibodies as well as the paired helical filament (PHF)-specific tau specific AT100 antibody (all from Innogenetics, 1:200) were used.

Light Microscopy

For light microscopy, 7-μm-thick paraffin sections of representative areas of the brain, including cerebellum and spinal cord, were cut and stained with hematoxylin and eosin, Luxol fast blue/cresyl violet, and the Bielschowsky’s silver impregnation method. Congo red staining for amyloid was also carried out using a standard protocol, and the preparations were viewed under polarized light. ADan, Aβ, prion protein and α-synuclein immunohistochemistry required pretreatment with 99% formic acid. This procedure was followed by treatment in a pressure cooker in citrate buffer for Aβ immunohistochemistry. Pressure cooker pretreatment was also required for CR3/43 immunohistochemistry, while sections were pretreated in a microwave oven in citrate buffer for AT8 immunohistochemistry. Tissue sections used for GFAP or CD68 immunohistochemistry required pretreatment with trypsin. Incubation with the primary antibodies was followed by biotinylated anti-rabbit IgG (DAKO, 1:200) or anti-mouse IgG (DAKO, 1:200), as appropriate, and ABC complex (DAKO). Color was developed with diaminobenzidine/H₂O₂.

The AD and normal control cases were stained in a similar fashion. For negative controls, primary antibody was replaced with the pre-immune serum.

Fluorescence Labeling for Confocal Microscopy

Twenty-μm-thick tissue sections from the temporal lobe with hippocampal formation, parahippocampus, and neocortical areas were prepared for double staining with Ab 5282 and Thioflavin S in order to determine the relationship between protein deposition and conformational state of ADan. Sections were pre-treated using 70% formic acid, and Ab 5282 (1:2,000) was incubated overnight followed by biotinylated anti-rabbit antibody, and finally ABC reagent. Antibody binding was visualized using the tetramethylrhodamine signal amplification kit (NEN Life Science Products, Boston, MA) and sections were finally counterstained in aqueous Thioflavin S. Twenty-μm-thick tissue sections of the temporal lobe with the hippocampus were also used for double staining with Ab 5282 and the 6F/3D anti-Aβ antibody. Tissue sections were pre-treated using 99% formic acid and a pressure cooker containing citrate buffer. Sections were incubated with Ab 5282 and after washes with...
## TABLE

Distribution of Different ADan and Aβ Lesion Types and Tau Pathology in Familial Danish Dementia

<table>
<thead>
<tr>
<th>Type of Lesion</th>
<th>Amyloid plaques</th>
<th>Diffuse deposits</th>
<th>Amyloid angiopathy</th>
<th>NFT</th>
<th>AN</th>
<th>NT</th>
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<td>ADan Aβ</td>
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<td>Amyloid plaques</td>
<td>Diffuse deposits</td>
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</table>

- Numbers in brackets refer to the number of cases in which this area could be examined.
- NFT = neurofibrillary tangle, AN = abnormal neurite, NT = neuropil thread.
- N/A = not applicable, LC = lateral column, PC = posterior column, VC = ventral column, GM = grey matter.

**Evaluation of Ab 5282 and AT8 Immunohistochemistry**

The presence and frequency of Ab 5282-positive plaques and the severity of CAA were evaluated using a semiquantitative approach as described recently for the assessment of such lesions in FBD (9). In brief, for CAA a four-tiered scoring system was devised such that score “0” represented unaffected areas, while score “+++” represented the most severely affected regions in which the majority of the arterioles and capillaries were Ab 5282-positive. To areas in which only a proportion of the arterioles/small arteries were affected, score “+” was given representing mild involvement, and to those in which the majority of arterioles as well as a minority of the capillaries were immunoreactive, the score “++” was given indicating moderate pathology. Ab 5282-positive parenchymal deposits “plaques” were defined as Ab 5282, Congo red-positive structures with or without an associated blood vessel. “Diffuse deposits” were often ill-defined, Ab 5282-positive but Congo red-negative structures, which were not stained or only weakly stained with Thioflavin S. For semi-quantification of parenchymal lesions, a principle similar to that recommended by CERAD for quantitating neuritic plaques in AD (7) was used. In the absence of either plaques or diffuse deposits, score “0” was given, for sparse or mild lesions score “+”, for moderate score “++”, and to frequent plaques or diffuse deposits score “+++” representing severe disease. Variation between cases was recorded as shown in the Table. The severity of NFT, NT, and AN pathologies was also semi-quantitatively evaluated using a ×10 objective. Score “0” was used if the pathological change was absent, “+” was used if it was sparse, “++” moderate, and “+++” if the change was severe.

**Immunoelectron Microscopy**

Ultrathin sections were mounted on etched nickel grids and treated with sodium metaperiodate solution. After washes, grids were incubated in normal goat serum followed by incubation with Ab 5282 (1:75) for 72 h. After further washes, grids were incubated with goat anti-rabbit IgG gold conjugate (1:20) with a particle size of 20 nm (Sigma, Poole, UK). Grids were post-fixed in 2.5% glutaraldehyde in sodium cacodylate buffer and counterstained with uranyl acetate/lead citrate using a standard protocol. For negative control the primary antibody was omitted.

**Western Blotting**

Insoluble tau was isolated from fresh, frozen samples of the hippocampus of case 1, from an AD case, and from a previously reported FBD case (9) using a published method (10). The heat-stable supernatant from the soluble fraction of control human
brain, which contained soluble tau, was precipitated with ammonium sulphate. This case together with the FBD and AD cases were used as controls. After separation of the constituent proteins by sodium dodecyl sulphate polyacrylamide gel electrophoresis and transfer to polyvinylidene difluoride membrane, the extracts were probed with the following anti-tau antibodies: TP70, PHF1, AT8, AT100, AT180, and AT270 (see above). Blots were developed using either horseradish peroxidase conjugated anti-mouse IgG (1:1,000) or anti-rabbit IgG (1:1,000), as appropriate, followed by ECL detection as described by the manufacturer (Amersham Life Science Ltd, Little Chalfont, UK).

RESULTS

Clinical History and Family (Fig. 1)

Case 1: A male who developed blurred vision, nystagmus, positive Romberg's sign, and mild memory loss at the age of 25. He underwent bilateral vitrectomy some years later. Transient ischemic attacks occurred at the age of 31. Three years before death he had developed double vision, ataxia, and difficulty in walking. EMG and evoked potentials were normal. A CT scan performed 11 yr before death showed multiple small areas of infarction in the middle cerebral artery territory bilaterally. MRI scans carried out 7 and 4 yr before death showed white matter changes. In the final 3 yr of life he developed progressive dementia with threatening behavior and became wheelchair bound. He died at the age of 43 from bronchopneumonia.

Case 2: A female who presented with diminished vision and hearing loss 26 yr before death. In the final 10 yr of life she developed progressive dementia and died from bronchopneumonia.

Case 3: This man developed a progressive neurological disease at the age of 40 yr and died 18 yr later. The cause of death was not recorded.

Macroscopic Description

The brain weights of cases 1 and 2 were 1,438 g and 1,220 g respectively; that of case 3 was not recorded. The leptomeninges were thick and opaque and the degree of atheroma in the vessels at the base of the brain varied from absent in case 3, mild in case 1, to severe in case 2. On slicing, the lateral ventricles were found to be dilated and there was thinning of the cortical ribbon and reduction in the white matter bulk. In addition, case 1 showed focal perivascular gray translucent areas in the white matter. The hippocampi were reduced in bulk. The brainstem and cerebellum were atrophic and the cranial nerve roots of case 3 were noted to be thin. The spinal cord was examined in cases 1 and 2 and found to be narrowed in the antero-posterior dimension and on slicing showed yellow discoloration. The spinal nerve roots were thin and firm.

Histological Findings

All cases showed similar features and will be described together, although there was some variation in severity between cases. Histological examination showed widespread vascular pathology. Many small arteries, arterioles and capillaries in the leptomeninges, and gray and white matter throughout the central nervous system (CNS) had thickened, eosinophilic walls frequently with fine spicules of eosinophilic material radiating into the parenchyma surrounding capillaries (drusige Entartung) (Fig. 2A–
The majority of the parenchymal blood vessels of the hippocampal formation show amyloid deposition (A–C) (A: Case 2, PAS, original magnification ×30; B and C: Case 1, Congo red, original magnification ×120). Numerous neurofibrillary tangles (double arrow) and neurit threads are present throughout the hippocampal formation (arrow) and abnormal neurites (arrowhead) cluster around amyloid laden blood vessels (asterisks) (D, E). Note the absence of argyrophilic plaques (D) (D: Case 1, Bielschowsky’s silver, original magnification ×60; E: Case 1, AT8 immunohistochemistry, original magnification ×60). Ab 5282-positive structures outline the hippocampus, which are either well defined and plaque-like (arrow) or ill-defined (F) (F: Case 1, 5282 immunohistochemistry, original magnification ×5). Activated microglial cells tend to be orientated towards subpial amyloid deposits (G) or blood vessels with amyloid (H), inset showing an activated microglial cell in the subiculum (G and H: Case 2, CR3/43 immunohistochemistry, original magnification ×60 (G), ×30 (H) and ×120 [H, inset]). Reactive astrocytes also tend to cluster around amyloid laden blood vessels (I) (Case 2, GFAP immunohistochemistry, original magnification ×30).

Congophilic material was observed in the parenchyma but the majority of this was found around some of the amyloid-bearing vessels, thus forming perivascular plaques. These were most commonly found in the CA1 region of the hippocampus, subiculum, entorhinal cortex, and neocortex (temporal and occipital lobes) but were relatively uncommon in the cerebellar cortex despite...
severe amyloid angiopathy. Congophilic deposits apparently unrelated to vessels were rare except in the subpial regions.

In addition to vascular pathology, the CA1 sub-region of the hippocampus and the subiculum showed marked abnormalities with neuronal loss, NFTs, extracellular ghost tangles, and hypertrophy of astrocytes. Silver staining revealed the presence of NFTs and fine NTs predominantly in limbic structures (Fig. 2D). Bulbous ANs were found around amyloid-bearing vessels most frequently in the CA1 sub-region of the hippocampus and the subiculum, but neuritic plaques were not a feature (Fig. 2D, E). Extracellular NFTs (ghost tangles) were confirmed in the hippocampal CA1 region and subiculum.

The neocortex showed no spongiosis and only mild neuronal loss. The hemispheric and deep cerebellar white matter was rarified with patchy myelin pallor and axonal loss with occasional axonal retraction balls, and perivascular spaces were frequently enlarged. Several small areas of infarction of varying age were found in the hemispheric white matter in addition to other regions such as the deep gray nuclei, cerebellum, and pons.

The cerebellum was severely affected by amyloid angiopathy and was atrophic with loss of volume in the molecular layer, granule cell depletion, and marked loss of basket and Purkinje cells—the latter frequently displaying axonal torpedoes (Fig. 3A). Bergman glia were increased in number. The deep white matter showed similar changes to those of the hemispheric white matter, although folial white matter was less affected. The dentate nucleus was well preserved although mild neurofibrillary pathology was found in one case.

Examination of the brainstem showed widespread amyloid angiopathy. The substantia nigra and locus ceruleus were well populated by neurons with only small amounts of free pigment indicative of cell loss. Occasional NFTs were seen in the substantia nigra in one case. The pontine base contained small areas of old infarction in 2 cases. The inferior olive in case 2 had a region of cell loss and gliosis, which was most likely to be secondary to the vascular lesion noted in the pons.

The spinal cord was affected by amyloid angiopathy in all areas throughout its length. Silver impregnation demonstrated circumferential peripheral axonal loss. Anterior horn cells were well preserved and the nerve roots available for examination showed no significant pathology.

The retina was detached and showed severe cystic degeneration. Photoreceptor outer and inner segments were virtually absent, with a reduction in photoreceptor cell nuclei. The inner retina was disorganized and hemosiderin-laden macrophages were numerous. Almost all of the retinal vessels contained, and were often occluded by, Thioflavin S-positive material, which was also recognized by Ab 5282. Similar staining deposits were also seen on the inner limiting membrane. There was severe astrocytosis and infiltration by activated microglial cells as demonstrated by GFAP and CR3/43 immunohistochemistry, respectively. The optic nerves were severely affected by deposition of leptomeningeal amyloid and amyloid angiopathy. GFAP immunohistochemistry demonstrated severe fibrous gliosis and there was marked axonal loss in addition to ongoing damage demonstrated by beaded and swollen axons. The optic tract showed gross axonal loss with residual gliosis, and in the occipital lobe the optic radiation was rarified and gliotic.

Immunohistochemical staining for prion protein and α-synuclein was negative.

**ADan Immunohistochemistry, Congo Red, and Thioflavin S Staining of Blood Vessels**

As described above, small arteries, arterioles, veins, and capillaries of the leptomeninges, cerebrum, brainstem, cerebellum, and spinal cord showed features of CAA. Mural amyloid deposition was confirmed by positive staining with Congo red or Thioflavin S and such vessels were invariably recognized by Ab 5282. Preparations double stained with Ab 5282 and Thioflavin S and examined by confocal microscopy confirmed a close overlap between ADan and amyloid deposits (Fig. 4A–F). The severity of CAA varied considerably between different anatomical areas (Table) and was entirely absent only in the globus pallidus in all the cases. CAA was mild in the striatum, nucleus basalis of Meynert, and substantia nigra and was of moderate degree in structures such as the amygdala, entorhinal cortex, different neocortical areas, thalamus, subthalamus, red nucleus, pontine base, spinal cord, and both cerebral and cerebellar white matter. Severe CAA was observed in the hippocampus, cerebellum cortex, and retina.

Small arteries with a diameter of up to 1 mm were labeled by Ab 5282, although the majority of the vessels were smaller than 300 μm. Some of the small arteries and arterioles showed an accentuation of Ab 5282 labeling in the outer perimeter, while in severely affected vessels Ab 5282 was seen throughout the full thickness of the vessel wall (Fig. 3B). The glia limitans and the perivascular parenchyma around some of the affected vessels were also labeled with Ab 5282 (Fig. 3D).

**ADan Immunohistochemistry, Congo Red, and Thioflavin S Staining of Parenchymal Lesions**

ADan-positive parenchymal deposits were severe in the hypothalamus, midbrain tectum and periaqueductal gray, locus ceruleus, retina, the hippocampal formation (Fig. 2F), and other limbic structures. Other regions were less severely affected and there was no involvement of the optic nerve, cingulate gyrus, globus pallidus, nucleus basalis of Meynert, thalamus, sub-thalamic nucleus, substantia nigra, red nucleus, and gray matter of the lumbar.
and sacral cord (Table). Congo red staining of adjacent sections demonstrated that the majority of the parenchymal lesions was negative or weakly positive and therefore largely represents protein deposited in the non-fibrillar or pre-amyloid form, which we have termed diffuse deposits. This finding was confirmed by double labeling with Ab 5282 and Thioflavin S examined by confocal microscopy (Fig. 4D–F). The major exceptions to this observation were the retina in which there was amyloid deposition separate from blood vessels and the subpial regions throughout the CNS in which frequent ADan-positive amyloid deposits were found.

Diffuse deposits were morphologically of 2 types: compact deposits of varying size that showed some similarity with the “cotton wool” plaques of variant AD with spastic paraparesis (11), and ill-defined, cloudy or loose deposits which often had a peri-neuronal disposition. Both types of diffuse deposit were frequent in the hippocampal formation where ghost tangles sometimes appeared to have peptide deposited on their surface. In the

Fig. 3. Cerebellar leptomeningeal and cortical blood vessels containing amyloid (A) (Case 2, PAS, original magnification ×30). Deposition of Ab 5282-positive material in the subpial region (arrowhead) and blood vessels (arrow) of the cerebellar cortex. Double arrow pointing to a blood vessel with perivascular deposition of Ab 5282-positive material (B) (Case 1, Ab 5282 immunohistochemistry, original magnification ×30). The Ab 5282 staining pattern is bilaminar in the entorhinal cortex (asterisk) and becomes united towards the transentorhinal cortex (arrow) (C) (Case 2, Ab 5282 immunohistochemistry, original magnification ×8). Parenchymal Ab 5282-positive deposits around obliterated blood vessels in the temporal neocortex (D) (Case 3, Ab 5282 immunohistochemistry, original magnification ×60). Deposition of Aβ peptide in the temporal neocortex (E) (Case 3, Aβ immunohistochemistry, original magnification ×15). A significant proportion of the capillaries of the hippocampal formation are also affected by deposition of Aβ. The whole circumference of some of the blood vessels is stained (arrow), but only an outer rim is stained in some others (double arrow) (F) (Case 3, Aβ immunohistochemistry, original magnification ×60).
Fig. 4. Confocal images. There is a good overlap between Ab 5282 (A) and Thioflavin S (B) staining of blood vessels in the subiculum (combined image: C) (Case 2, objective ×25). There is a good overlap between Ab 5282 (D) and Thioflavin S (E) staining in small blood vessels (arrow). The ill-defined parenchymal deposits of the CA1 sub-region primarily stain with Ab 5282, but not with Thioflavin S (combined image: F) (Case 2, objective ×10). Neocortical blood vessels showing staining with both Ab 5282 (red) and an antibody to Aβ (green), but the overlap is incomplete (G–I) (Case 2, objective ×63). A neocortical blood vessel with perivascular plaque showing that the blood vessel wall is more strongly stained with Ab 5282 (J), while the perivascular deposit is more strongly stained with the Aβ antibody (K) (combined image: L) (Case 2, objective ×63).
parasubiculum, the paraventricular cell clusters were highlighted by cloudy diffuse lesions. In the entorhinal cortex, ADan was most commonly found as compact diffuse deposits and formed a bilaminar pattern with relative sparing of the middle cortical laminae. The most superficial band of deposits was seen to descend with the Pre-α neurons in the transentorhinal cortex to occupy the deeper cortical laminae where they decreased in frequency as the neocortex was approached (Fig. 3C). Neocortical deposits were of the diffuse type and were only mild to moderate in severity. The cerebellar cortex also contained mild to moderate, loose, diffuse deposits of ADan predominantly in the granular cell layer, and there were similar deposits occasionally found in the molecular layer and white matter. Subpial deposition of ADan was also characteristic in the cerebellum (Fig. 3B). In one case (case 3), compact diffuse ADan deposits were found in the dentate nucleus. The midbrain tectum, periaqueductal gray matter, and IVth nerve nucleus were variably, but often severely, affected by diffuse peptide deposits, although there were no such deposits in the substantia nigra. In the pons, the locus ceruleus contained moderate though there were no such deposits in the substantia nigra. In the hippocampus, the CA1 sub-region was most severely and consistently affected by parenchymal Aβ deposits, which took the form of perivascular lesions, compact diffuse deposits, and loose or cloudy diffuse deposits sometimes surrounding neurons. In the parietal cortex of case 1, several lesions had the appearance of the classical “cored” plaques found in AD, however, these were noted to be unstained by Congo red and did not contain ADan. In the hippocampus the CA1 sub-region was most severely affected with diffuse and perivascular deposits. The subiculum also showed marked perivascular deposition and Aβ peptide, like ADan, was also found to be deposited on extracellular “ghost tangles.” The Pre-α neuronal clusters of the entorhinal cortex were highlighted by diffuse deposition of Aβ. In the neocortex, Aβ deposits were found predominantly in superficial cortical laminae and were often found to be more numerous than those of ADan.

Several areas of the CNS had parenchymal Aβ deposition without ADan deposits; these included the cingulum, striatum, nucleus basalis of Meynert, thalamus, and subthalamus. There were regions, notably the retina, optic nerve and tract, red nucleus, locus ceruleus, dentate nucleus, and the spinal cord, with the exception of the thoracic grey matter, in which ADan was found in the absence of Aβ.

AT8 Immunohistochemistry and its Correlation with ADan and Aβ Deposition

The hippocampus was consistently the region most severely affected by all 3 types of neurofibrillary pathology with abundant NFTs, including ghost tangles, bulbous ANs, and fine NTs (Fig. 2E). Abnormal neurites around amyloid deposits in vessel walls were frequent in the CA1 sub-region and in the subiculum. Neurons showing finely granular cytoplasmic staining representing pre-tangles (12) were also observed. Scanty NFTs were seen in the dentate fascia. Other limbic regions were also affected by these pathological structures but showed greater variation between cases. All neocortical regions examined showed neurofibrillary pathology with NFTs most frequent in the parietal and temporal lobes, while ANs and NTs were most commonly found in the parietal cortex. Neocortical vessel-related neurites were often more commonly seen in the superficial cortical laminae, whereas those related to subpial amyloid were more frequent in sulci than on the gyral crests. The nucleus basalis of Meynert and the locus ceruleus both showed neurofibrillary pathology, but the substantia nigra was inconsistently involved by NFTs and NTs without ANs. Of note was the
Abnormal neurites were only found in relation to AD\textit{an} and/or Aβ deposited as amyloid in vessel walls or in subpial regions, they were not seen in association with diffuse AD\textit{an} or Aβ deposits (Fig. 2E). In contrast, NFTs and NTs were observed in areas with both fibrillar and non-fibrillar peptide deposits. The regions most severely affected by parenchymal AD\textit{an} deposition were also severely affected by neurofibrillary pathology. The neocortex, in which diffuse AD\textit{an} deposits were never more severe than \textit{‘+’}, but showed up to \textit{‘+++’} diffuse Aβ deposits, also had neurofibrillary pathology of all 3 types graded up to \textit{‘+++’}. No CNS regions were observed in which there was neurofibrillary pathology in the absence of either AD\textit{an} or Aβ deposits, although there were areas in which either peptide could be found without accompanying neurofibrillary pathology (Table).

**GFAP, CD68, and CR3/43 Immunohistochemistry**

The astrocytic response demonstrated by GFAP immunohistochemistry showed some variation between cases. The relationship to amyloid deposits was confirmed by double labeling using an anti-GFAP antibody and Congo red. The hippocampus and subiculum displayed severe astrocytosis with reactive astrocytes and numerous processes distributed evenly within the parenchyma and in increased numbers around amyloid-laden blood vessels (Fig. 2I). The neocortex was involved by mild, patchy astrocytosis, which was accentuated around amyloid containing vessels and in the superficial cortex in relation to subpial amyloid deposition. There was moderate to severe fibrillary astrocytosis in the subcortical white matter that was most marked in the posterior frontal and parietal lobes. The cerebellum showed prominent Bergmann gliosis and diffuse astrocytosis of the cortex and white matter with relative sparing of the dentate nucleus.

Microglial distribution was assessed by CD68 immunohistochemistry and the antibody CR3/43 recognizing MHC Class II antigen was used to assess the presence of reactive, activated microglia. Double labeling with each antibody and Congo red in the hippocampus and temporal lobe was used to assess the relationship between microglia and amyloid deposits. Both antibodies showed a diffuse distribution of microglia throughout the hippocampus and subiculum with an increase in density of cells around amyloid-bearing vessels (Fig. 2H). The cortex and white matter showed a pattern of microgliosis similar to that observed for astrocytes (Fig. 2G). In the cerebellum, activated microglia were distributed throughout although they were more frequent in the white matter.

**Electron Microscopy and Immunoelectron Microscopy**

Immunoelectron microscopy was performed on a sample from the hippocampus of case 1 using Ab 5282 to localize AD\textit{an} peptide. Tissue preservation enabled the identification of blood vessels, cell processes, neurons, and glial cells. Several small vessels had deposits of randomly orientated amyloid filaments of approximately 10-nm diameter within the basement membrane and extending into the surrounding parenchyma. These amyloid fibrils were recognized by Ab 5282 (Fig. 5A). In the parenchyma, labeling was found in association with amorphous electron dense material in association with small numbers of 10-nm filaments representing parenchymal pre-amyloid (Fig. 5B). No labeling was found when the primary antibody was omitted. Neurofibrillary tangles localized by Ab 5282 immunoelectron microscopy. Bundles of Ab 5282-labeled amyloid fibrils (A) (original magnification ×10,000). Inset with higher magnification (original magnification ×16,000). Amorphous electron-dense material with sparse fibrils, representing pre-amyloid, is also decorated with Ab 5282 (B) (original magnification ×10,000).
were confirmed to be composed of PHFs with a maximum diameter of approximately 20 to 25 nm and periodic constrictions at about 80 nm (Fig. 6A).

**Tau Immunoblotting**

Soluble tau from the normal control case demonstrated the usual pattern of multiple bands characteristic of post-mortem normal adult human brain. Insoluble tau from the AD and FBD cases showed the triplet T54, T59, and T64 species with a minor T71 band when probed with all of the tau antibodies. The pattern of insoluble tau containing bands in the sample from the FDD case was similar to that found in both AD and FBD (Fig. 6B).

**DISCUSSION**

In this study we showed that FDD, which is an inherited form of CNS amyloidosis, is characterized by widespread CAA, parenchymal lesions, and neurofibrillary tangles. These findings expand the spectrum of neurodegenerative diseases in which the major pathological changes include neurofibrillary pathology and nerve cell death occurring in combination with deposition of extracellular protein aggregates.

The ocular presentation in FDD has been shown to be associated with subcapsular cataract, retinal neovascularization leading to vitreous hemorrhages, and neovascular glaucoma (13). We were able to confirm amyloid deposition in the retinal blood vessels and parenchyma and evidence of previous hemorrhages with consequent severe parenchymal damage. At present no definite answer can be given about the mechanism of hearing loss in FDD as the material that was available to us for neuropathological examination did not include the inner ear and the VIIIth cranial nerve. The severe degeneration of limbic structures and involvement of the cerebral cortices by neurofibrillary degeneration in combination with Binswanger-type white matter changes explain the dementia syndrome, while the cerebellar degeneration accounts for the severe ataxia.

Using the phosphorylation dependent anti-tau antibody AT8 (6) we demonstrated extensive tau pathology, including NFTs, NTs, and ANs. The filaments comprising such lesions appear ultrastructurally as PHFs, and the abnormal tau has a triplet electrophoretic migration pattern thus confirming that, as in FBD, the cytoskeletal pathology in FDD is comparable to that seen in AD (9, 22). In FDD, NFTs and NTs were found to occur in limbic structures, neocortices, and some of the subcortical nuclei, therefore the NFT pathology corresponds to stage V–VI in the Braak and Braak system used for grading the severity neurofibrillary degeneration in AD (14). In all 3 cases, ANs were located around blood vessels with CAA, but not in association with either ADan or Aβ parenchymal pre-amyloid lesions (defined as Congo red-negative, Thioflavin S-negative or weakly positive and ultrastructurally non-fibrillar protein deposits [15, 16]). Perivascular clustering of argyrophilic and tau-immunoreactive abnormal neurites has been described in relation to CAA associated with Aβ (17, 18), prion protein (19), and ABri deposition (9, 20). The presence of ANs around CAA of various origins and their absence in association with pre-amyloid lesions suggests a correlation between the fibrillation of amyloidogenic peptides and formation of ANs (9, 21).

Using Ab 5282 specifically recognizing ADan, we demonstrated that this amyloidogenic peptide, which is associated with the genetic defect in FDD, is a major component of both the vascular and parenchymal lesions throughout the CNS, including the retina. From the pathological data presented here, however, it is not possible to ascertain whether full-length ADan is the only significant species composing the cerebral vascular amyloid and the parenchymal lesions since the residues recognized by Ab 5282 (CFNLFISQESKHY) are not exclusive to ADan, but are also present in ADanPP and potential C-terminal fragments that may be processed from either ADanPP or ADan. Our previous biochemical studies have shown that full-length ADan is the major component of leptomeningeal vascular amyloid (3) and further studies are under way in order to identify all the ADan and other species that are present in the parenchymal lesions.

Although both currently known mutations of the BRI2 gene result in extended, 277-amino-acid-long precursor proteins from which the 34-amino-acid-long amyloidogenic peptides, ADan in FDD and ABri in FBD, with unique C-terminal residues are cleaved (4, 3), there are remarkable differences in both the clinical presentation and CNS pathology between these 2 closely related diseases. In addition to dementia and cerebellar signs, which are prominent features of both FDD and FBD, the presence of cataract and hearing loss are characteristic in affected members of the Danish family, while spastic paraparesis characterizes the British pedigree. The presence of this latter sign makes FBD clinically similar to other syndromes of dementia with spastic paraparesis, such as the Gerstmann-Sträussler-Scheinker syndrome and variant AD with spastic paraparesis first described by van Bogaert et al (22, 23). This study demonstrated that ADan deposition in combination with neurofibrillary degeneration severely affects the limbic structures, similar to ABri deposition and neurofibrillary pathology in FBD. Furthermore, we demonstrated that the neocortical involvement by both parenchymal ADan deposition and neurofibrillary pathology is more extensive in FDD than the neocortical ABri deposition and tau pathology are in FBD (9). The variation in the severity of neocortical deposition between ADan and ABri may be due to the difference in the primary sequence of the 2 amyloidogenic
peptides, which may give rise to differences in the extracellular diffusibility, degradation, and/or clearance of ADan and ABri. The mechanisms by which Aβ is cleared from the CNS have been studied (24, 25), but no data are yet available for ABri and ADan. The relative paucity of ADan deposition in the glia limitans around the cerebellar blood vessels affected by CAA, which is a prominent feature of FBD, was also noted (9, 26). We previously postulated that the severe involvement of both the limbic structures and cerebellum in FBD could support the notion that the amyloidogenic peptide, ABri may be produced locally in the CNS, perhaps by neurons (9), as these 2 anatomical areas are those which show a high level of BriPP mRNA expression in normal human brain (4). We also suggested that alternatively the peptide species deposited could also be of a systemic origin (9) since we observed high levels of circulating ABri and the deposition of ABri in peripheral tissues (27). This study confirmed that the overall regional distribution of ADan is similar to ABri in FBD. This parenchymal pattern of deposition of ADan and ABri in FDD and FBD, respectively, and its similarity to Aβ deposition in AD (28), in which peptide production is thought to be local (29), could lend further support to the hypothesis that production and processing of these 2 novel amyloidogenic peptides may take place within the CNS. However, the elevated levels of ABri in the circulation in FBD (27) raise the possibility that circulating peptide species, which may cross the blood-brain barrier as demonstrated for Aβ (30–32), probably in combination with as yet unidentified CNS tissue factors that could determine the characteristic regional distribution of the disease, could also be relevant in the mechanism of both FBD and FDD. These issues require further investigation.

This study showed that in FDD the majority of the hippocampal and neocortical parenchymal ADan lesions often have ill-defined boundaries and show tinctorial, optical, and ultrastructural features of pre-amyloid rather than amyloid (15, 16). In contrast, in FBD the majority of the hippocampal ABri lesions are well-defined, plaque-like and of amyloid nature, although pre-amyloid lesions are predominant in some of the anatomical areas affected such as the entorhinal and transentorhinal cortices (9, 20). The combination of pre-amyloid lesions and neurofibrillary degeneration in FDD is analogous to that seen in variant AD with spastic paraparesis, which is also characterized by the pre-amyloid Aβ plaques of the cotton wool-type in association with neurofibrillary degeneration (11, 33–35). This observation adds further weight to the evidence that amyloid conformation per se is not a prerequisite for NFT formation. The experimental data currently available are not sufficient to explain the predominance of pre-amyloid lesions in FDD. One possibility is that due to the difference in the primary sequence ADan forms amyloid fibrils less efficiently than ABri does, although conversion of multiconformational proteins into predominantly β-sheet fibrils is not thought to be significantly dependent only on sequence per se (36). It is, however, possible that the difference in the amino acid sequence is sufficient to make ADan less able to attract and/or bind to amyloid associated components such as apolipoprotein E, proteoglycans, serum amyloid P component or α1-antichymotrypsin, which have been shown to modulate aggregation of Aβ in vivo and in vitro (37–43). Another possibility is that the concentration of ADan may not reach a critical level in vivo, which would be required, possibly in combination with other factors, for efficient fibril formation (44).

Surprisingly, in all 3 cases studied, we demonstrated the presence of various degrees of Aβ peptide deposition, either in combination with ADan or alone, in blood vessels, and in brain parenchyma. The Aβ parenchymal deposition, similar to that of ADan, was most severe in the limbic structures, but also occurred in neocortical areas where it was more severe than deposition of ADan. The significance of Aβ deposition cannot be clarified satisfactorily at present. Codeposition of ADan and Aβ, as has been shown for Aβ and cystatin C (45), is a possibility, although the presence of Aβ alone in several anatomical areas makes this possibility less likely. The Aβ deposition is unlikely to represent an aging phenomenon as the youngest patient in our series was 43 yr old at the time of death (46). As the possibility of a second hereditary neurodegenerative disease should be considered, although in the absence of neocortical neuritic plaques the diagnosis of AD could not be made on morphological grounds, further genetic studies are underway.

REFERENCES


