Variant Alzheimer Disease With Spastic Paraparesis: Neuropathological Phenotype

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Abstract. Variant Alzheimer disease (varAD) is clinically characterized by the combination of presenile dementia with spastic paraparesis and is caused by certain mutations of the presenilin 1 (PS-1) gene. We now present the unusual neuropathological phenotype of varAD as seen in 5 affected members of the original Finnish family with a genomic deletion encompassing exon 9 of the PS-1 gene. Their primary and association cortices and hippocampus showed a profusion of eosinophilic, roundish structures termed “cotton wool” plaques (CWPs). The CWPs were immunoreactive for Aβ42/43 but weakly or not at all for Aβ40 isoforms of the amyloid β-peptide (Aβ). They were devoid of a congophilic core, and fibrillar amyloid could not be identified within them by electron microscopy. Confocal microscopy showed reduced density of axons within individual CWPs and only few CWP-related PHF-tau-positive dystrophic neurites. CWPs were particularly numerous in the medial motor cortex representing the lower extremities, and degeneration of the lateral corticospinal tracts was observed at the level of the medulla oblongata and the spinal cord. In addition to the predominant CWPs, variable numbers of diffuse and cored plaques were found in the cerebral cortex. Diffuse and non-neuritic cored amyloid plaques but no CWPs occurred in the cerebellum. In conclusion, varAD in this Finnish family is distinct from classic AD because of the degeneration of lateral corticospinal tracts, predominance of CWPs devoid of fibrillar amyloid cores in the cerebral cortex, and presence of non-neuritic amyloid plaques in the cerebellum.

Key Words: Corticospinal tract degeneration; Cotton wool plaque; Presenilin 1 deletion; Spastic paraparesis; Variant Alzheimer disease.

INTRODUCTION

Autosomal dominant Alzheimer disease (AD) is caused by numerous mutations in at least 3 genes, the β-amyloid precursor gene (APP) on chromosome 21, and the presenilin 1 (PS-1) and 2 (PS-2) genes on chromosomes 14 and 1 (1–5). Despite this remarkable molecular genetic heterogeneity, there is little reported variability in the clinical and pathological phenotype of AD.

Familial AD (FAD) patients with mutations in the APP gene at codon 717 have more severe pathology than patients with sporadic AD, but share the same pattern and distribution of pathologic features (6–8). However, 2 exceptional APP gene mutations are associated with extensive angiopathy. A mutation at codon 693 of the APP gene, causing E693Q substitution leads to hereditary cerebral hemorrhage with amyloidosis of Dutch type (HCHWA-D) without mature neuritic plaques (9). A mutation at the neighboring codon 692, causing A692G substitution, leads to HCHWA of the Flemish type (10) in which patients also develop dementia and senile plaques with large amyloid β-peptide (Aβ) cores surrounded by a meshwork of dystrophic neurites (11).

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FAD caused by PS-1 gene mutations is, in general, pathologically indistinguishable from sporadic AD, apart from exceptionally florid amyloid and neurofibrillary pathology (8, 12–15). However, 3 mutations of the PS-1 gene have been associated with unusual features. Patients with mutation M146V showed mild cortical vacuolar changes (3, 16), mutation E280A caused prominent vacuolation of the cortical neuropil and severe gliosis (17), while mutation A260V was associated with silver- and ubiquitin-positive inclusions resembling Pick bodies in the neurons of the dentate gyrus (18).

Kindreds with specific mutations and unusual neuropathologic features may provide valuable information on the underlying pathogenetic mechanisms. We now present the distinctive neuropathological phenotype of variant AD with spastic paraparesis (19, 20) in a large Finnish family with a unique genomic deletion encompassing exon 9 of the PS1 gene (21).

MATERIALS AND METHODS

Patients

Autopsies of the nervous system were performed on 5 affected individuals of a Finnish family with varAD (pedigree numbers III:7, III:14, III:15, III:18, III:21 according to ref. 20). There were 3 males and 2 females. The age at death ranged from 54 to 69 yr and disease duration from 5 to 12 yr (Table 1). Four of these patients had had spastic paraparesis with presenile dementia while 1 patient (III:21) had presenile dementia only without definite evidence of paraparesis (Table 1). Their clinical features have been described in detail in a previous article (20). Molecular genetic analysis performed on 2 (III:14, III:15) of these 5 patients showed a deletion encompassing exon
9 of the PS-1 gene (19, 21), both patients being homozygous for the ε3 allele of the apolipoprotein E gene (20).

**Histology, Immunocytochemistry, and Confocal Microscopy**

The brains and spinal cords of all 5 patients were fixed in 4% phosphate-buffered formaldehyde. Representative tissue samples from frontal, temporal, parietal, and occipital association cortices, precentral motor cortex (both lateral and interhemispheric aspects), hippocampus, amygdala, basal ganglia, cerebellum, midbrain,pons, medulla, and spinal cord at different levels were embedded in paraffin. Sections were stained with the hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Luxol Fast Blue-cresyl violet (LFB-CV), and modified Bielschowsky stains. Amyloid was identified by red-green dichroism in polarized light after alkaline Congo red staining with the hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), Luxol Fast Blue-cresyl violet (LFB-CV), and modified Bielschowsky stains. Amyloid was identified by red-green dichroism in polarized light after alkaline Congo red staining. Sections were viewed after thioflavin S staining (Fig. 1B). In silver impregnation, the predominant plaques had a characteristic appearance (temporal, frontal, parietal, and occipital) cortices. The bound primary antibodies were visualized by using an appropriate peroxidase-labeled secondary antibody (Vector Laboratories, Burlingame, CA), diaminobenzidine as the chromogen, and hematoxylin as the counterstain.

For confocal microscopy, paraffin sections of various thickness (5 to 20 μm) were double immunostained with polyclonal rabbit antisera raised against Aβ1–40, Aβ1–42, or Aβ1–43, and mouse monoclonal antibody to reaper ifolament or PHF-tau (Table 2). FITC-conjugated goat anti-mouse and TRITC-conjugated swine anti-rabbit secondary antibodies (Table 2) were used to label the bound primary antibodies. The sections were viewed using a Leica TCS SP confocal microscope with version 1.6.587 software, and the pictures were edited using Adobe Photoshop 5.5 program.

**Electron Microscopy**

For electron microscopy, samples were taken from the cerebral cortex of brains routinely fixed in 4% phosphate-buffered formaldehyde. After postfixation with buffered osmium tetroxide the samples were dehydrated and embedded in epon. Semithin sections were stained with toluidine blue. Regions of interest were selected for cutting thin sections, which were contrasted with uranyl acetate and lead citrate and examined in a JEOL JEM 1200 electron microscope.

**Biochemical Analyses**

Aβ40 and Aβ42 were extracted from fresh-frozen brain tissue (middle frontal gyrus) of 2 patients (III:14, III:15) autopsied 14 and 5 h postmortem, as described previously (22). The peptides were captured using BNT77 antibody, and detected using the BA27 HRP (for Aβ40) and BC05 HRP (for Aβ42) antibodies with peroxidase substrate/solution (22).

**RESULTS**

**Macroscopic Findings**

The brain weights ranged from 1,075 g to 1,470 g (Table 1), the highest weight being due to marked brain edema in patient III:18. There was moderate to severe generalized cerebral gyral atrophy and pronounced shrinkage of the medial temporal lobes, including the hippocampi. At most, slight atherosclerosis of the intracranial arteries was observed. Patient III:15 showed an old left frontal intracerebral hemorrhage of the lobar type.

**Histological, Immunocytochemical and Confocal Microscopic Findings**

On histological examination, the cerebral isocortex revealed abundant plaques in both the primary and association (temporal, frontal, parietal, and occipital) cortices. The predominant plaques had a characteristic appearance on the basis of which they were termed “cotton wool” plaques (CWP). CWPs were readily detectable in H&E-stained sections (Fig. 1A) as roundish homogenous structures with distinct borders, often with diameters exceeding 100 μm. The CWPs showed neither congophilia/red-green dichroism nor bright fluorescent cores after thioflavin S staining (Fig. 1B). In silver impregnation, only minor plaque-related neuritic pathology was visible.

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<td>12</td>
<td>10</td>
<td>6</td>
<td>5</td>
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<tr>
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TABLE 2
Antibodies Used in the Present Study

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<th>Pretreatment</th>
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Abbreviations: mm = mouse monoclonal, rp = rabbit polyclonal.

(Fig. 1C). The CWPs did not stain for the amyloid P-component or protease resistant prion protein and only inconsistently and minimally for apoE and complement components C1q, C3d, and C9. Their immunoreactivity for synaptophysin was accentuated in comparison with the surrounding neuropil. The CWPs avidly bound antibodies raised against Aβ1–16, Aβ42, and Aβ43; however, in contrast to plaques with amyloid cores, were weakly or not at all visualized by antibodies raised against Aβ40 (Fig. 1D–F). The CWPs were usually separate from each other but sometimes, especially in layer III–IV, they coalesced and formed conglomerates. Particularly large conglomerates of CWPs occurred in the interhemispheric precentral cortex corresponding to the motor representation of the lower extremities, while the lateral motor cortex showed fewer CWPs (Fig. 2A, B). Occasional CWPs were also detected in the subcortical white matter and a few were found deep in the white matter. CWPs were also frequent in the entorhinal cortex (Fig. 2C) and hippocampus. They were very abundant in the subiculum, and displaced neurons and even interrupted and distorted the granular cell layer of the dentate gyrus (Fig. 3A, B) and the pyramidal cell band of the hippocampus. In addition to the iso- and allocortex, CWPs were present in the amygdala, putamen, globus pallidus, and claustrum.

Individual CWPs were frequently composed of homogeneous granular material only, although some embraced neuronal perikarya. In most neocortical samples, only few PHF-tau-positive dystrophic neurites were associated with CWPs (Fig. 3C). Also the density of neuropil threads was low in the isocortex (Fig. 3C) while it was much higher in the hippocampus. However, neurons bearing neurofibrillary tangles (NFTs) were common even in the isocortex (Fig. 1C), while they comprised the majority in the entorhinal cortex and hippocampus. This
Fig. 1. A: In H&E-stained sections, “cotton wool” plaques (CWPs) appear as roundish, eosinophilic structures with distinct borders. Occasional nuclei are seen within some plaques. B: In thioflavin S staining, weakly fluorescent CWPs barely emerge from the background contrasting with a bright fluorescent blood vessel affected by amyloid angiopathy. C: In silver impregnation only minor neuritic pathology without thick dystrophic neurites is visible within the CWPs. Most neurons between the CWPs harbor silver-positive NFTs. D–F: In 3 consecutive sections, only plaques with amyloid cores and a small blood vessel (arrow) stain positively for Aβ40, whereas CWPs are hardly detectable (D). The CWPs strongly bind antibody to Aβ42 (E) with less intense staining for Aβ43 (F). Note the subpial band of Aβ immunoreactivity. Paraffin sections from frontal cortex. Magnifications: A–C, ×160; D–F, ×36.

The density of axons within individual CWPs was often markedly reduced when compared to the surrounding neuropil, as demonstrated by silver impregnation, LFB-CV, and double immunostaining for Aβ peptide and neurofilament proteins, including analysis with the confocal microscope (Fig. 3D, E). The axons seemed to either wind around the CWP or degenerate on entering into the CWP. The number of plaque-related macro- and microglial cells was small in comparison with other forms of AD. The typical CWPs were not associated with any significant astroglial reaction (Fig. 3F, G).

In addition to the CWPs, there were diffuse plaques and cored or non-cored plaques with dystrophic or PHF type neurites in the cerebral cortex. The relative proportions of these plaques varied between different cortical regions and individual patients. The relative number of cored plaques was highest in patient III:15. However,
Fig. 2. In the medial precentral cortex (corresponding to the motor representation of the lower extremities) (A), the CWPs are much more numerous than in the lateral motor cortex (B). Note the coalescence of the plaques in layers III–IV and the particularly thick subpial layer of Aβ immunoreactivity in the medial precentral cortex. C: Numerous CWPs are present in the entorhinal cortex. A, B: Anti-Aβ42 antibody and hematoxylin. C: H&E. Magnifications: A, B, ×58; C ×120.

even in this patient, CWPs were predominant in the deeper cortical layers. There were no CWPs in the cerebellum. Instead, scattered amyloid plaques with compact cores strongly immunoreactive for both Aβ40 and Aβ42/43 and surrounded by Aβ42/43 positivity occurred mainly in the molecular layer (Fig. 4A). These cored plaques were associated with clusters of microglial cells (Fig. 4B). In addition, in all 3 layers of the cerebellar cortex there were irregular diffuse plaques, which were immunopositive for Aβ42/43 but not for Aβ40. No obvious difference was observed between the vermis and the hemispheres. PHF-tau-positive material was not present around the cerebellar plaques.

Congophilic Aβ deposits were common in the walls of leptomeningeal and cortical penetrating blood vessels. Amyloid angiopathy was particularly extensive in the precentral cortex and in the cerebellum.

In the medulla oblongata and spinal cord, myelin and neurofilament staining disclosed marked degeneration of the corticospinal tracts (Fig. 4C). Immunoreactivity for Aβ was observed only as small diffuse plaques in the central gray matter of the cord, whereas the tracts in the white matter were Aβ-negative. PHF-tau-positive perikarya or neurites were not encountered in the spinal cord.

Electron Microscopy

In toluidine blue-stained semithin sections, neocortical CWPs appeared as areas of granular tissue texture with reduced density of axons. At the ultrastructural level (Fig. 5A), these areas were composed of degenerating myelinated axons and membrane-bound vesicular structures within which mitochondria and smaller vesicles were identifiable. Granular osmiophilic material was present between the vesicles, possibly in the extracellular space. Only an occasional filament to suggest presence of fibrillar amyloid was detected among the granular osmiophilic material within CWPs. In the markedly thickened blood vessel walls, abundant bundles of straight nonbranching filaments with a diameter of approximately 10 nm, corresponding to amyloid fibrils, were present in the lamina elastica interna and media where smooth muscle cells were destroyed (Fig. 5B).

Biochemical Findings

Biochemical analyses of the brain tissue of 2 patients (III:14, III:15) disclosed exceptionally high Aβ concentrations. In the frontal lobes, the concentrations of Aβ40 were 3.88 and 22.53 μg/g and those of Aβ42 were 9.55 and 14.90 μg/g, i.e. markedly higher than the reported concentrations of Aβ40 (1.66 μg/g) and Aβ42 (3.14 μg/g) in sporadic AD (24).

DISCUSSION

VarAD is a syndrome of presenile dementia and spastic paraparesis caused by several different mutations of the PS-1 gene (19, 24–28). In our family with a deletion...
Fig. 3. A, B (consecutive sections): In the hippocampal dentate gyrus CWPs displace neurons and even interrupt the granular cell layer. C: The number of PHF-tau-positive dystrophic neurites (green fluorescence for hyperphosphorylated tau) associated with a CWP (red fluorescence for Aβ42) is minimal. D: The number of axons (green fluorescence for neurofilament) within a CWP (red fluorescence for Aβ42) is markedly diminished. Axons are either interrupted within the CWP or they wind around it. E: In deep frontal cortex only few myelinated axons traverse the CWPs, suggesting axonal damage. F, G: CWPs induce only minimal reactive gliosis as depicted in consecutive sections from the temporal cortex. A: H&E; B: Anti-Aβ42 antibody and
encompassing exon 9 of this gene, the clinical syndrome of varAD is associated with a neuropathological phenotype, characterized by the predominance of CWPs and degeneration of the corticospinal tracts. CWPs are distinct from the various types of plaques previously described in association with AD (29). In contrast to classic neuritic plaques, CWPs lack dense congophilic cores, and definite fibrillar amyloid deposits are not found within them by electron microscopy. Furthermore, they are associated with only minor neuritic pathology. CWPs differ from diffuse plaques because CWPs are readily distinguishable in H&E preparations by their distinct borders, roundish configuration, hypocellularity, and homogenous eosinophilic staining. In addition, CWPs are composed of full-length Aβ as evidenced by positive immunostaining also for the domain Aβ1–16, which is often lacking in diffuse plaques (29). CWPs are not specific for our 4.5 kb ∆exon9 varAD but also occur in certain other PS-1 mutations (P436Q, DelIM, 5.9 kb ∆exon9) associated with varAD (24, 28). Furthermore, scattered CWPs were recently reported to occur in occasional sporadic AD cases (30). The structurally somewhat similar plaques are found mainly in the lateral entorhinal areas (31).

Familial British Dementia (FBD), characterized by progressive spastic tetraparesis, cerebellar ataxia, and progressive dementia with onset in the sixth decade (32–34) may be difficult to clinically distinguish from varAD. The main pathological features of FBD include severe, extensive amyloid angiopathy of small cerebral and spinal arteries and arterioles, characteristic cerebellar degeneration with perivascular plaques, white matter degeneration similar to that seen in Binswanger disease, and 3 different types of plaques (32, 35–36). None of the FBD plaques is structurally similar to CWPs, and the amyloid in FBD is composed of a unique 4K protein subunit amyloid-Bri (ABri) created by a stop-codon mutation in the BRI gene on chromosome 13 (37).

In all 5 patients the CWPs showed strong immunoreactivity for Aβ42/43, but only weak or no immunoreactivity for Aβ40. Biochemical analysis of the cortical brain tissue of 2 patients demonstrated markedly higher concentrations of Aβ42 than in sporadic AD (24). Interestingly, exceptionally large increases in secreted Aβ42 levels were recently observed in cells transfected with mutant PS-1 genes causing variant AD with spastic paraparesis (24). In addition to Aβ42 concentrations, the levels of Aβ40 were also high in our patients. This most likely reflects the contribution of vascular amyloid composed mainly of Aβ40 (38). Despite the high levels of fibrillogenic Aβ42 in our patients, definite fibrillar amyloid could not be identified in their predominant CWPs. This interesting situation may be associated with the observed paucity of macro- and microglial cells and the lack of fibrillogenic Aβ42.
of complement activation and amyloid P-component in CWPs.

All of our varAD patients had a memory impairment progressing to dementia (20). The relative significance of Aβ and tau pathology for the development of dementia in AD is still unsettled (39). In addition to the cortical intraneuronal NFTs (Braak stage V–VI), the abundant CWPs may have contributed to the development of dementia in our varAD patients, despite the absence of congophilic cores and lack of marked plaque associated PHF-tau pathology. It has been demonstrated that in sporadic AD, neurites are disrupted within Aβ deposits (40). Neurites that pass through Aβ deposits lose their normal straight configuration, and this change in geometry has marked consequences in terms of signal transduction properties of dendrites (41). The apparent paucity of axons traversing CWPs may indicate axonal damage within the CWPs. Furthermore, numerous CWPs in the entorhinal cortex and hippocampus of our varAD patients caused striking distortion of the normal cytoarchitecture of the dentate gyrus and the pyramidal cell layer of hippocampus. It is likely that the above structural indicators of neuritic damage and cytoarchitectural disorganization are associated with functional disturbances and may well have contributed to dementia.

Four of our patients had clinically verified spastic paraparesis. Neuropathological analysis showed degeneration of the corticospinal tracts at the level of medulla oblongata and spinal cord. Plaques and NFTs were virtually absent in the brainstem and spinal cord, and significant spinal amyloid angiopathy was not present. This indicates that the upper motor neurons are damaged above the brainstem, and the primary lesion may well occur at the cerebral level. In our patients, remarkably large conglomerates of CWPs were present in the interhemispheric precentral cortex (paracentral lobule) corresponding to the motor representation of the lower extremities, while the more lateral motor area was less affected. In contrast to previous notions, extensive Aβ deposition was recently shown to occur even in the primary motor cortex of AD patients and was proposed to cause motor dysfunction in late stages of AD (42). The interhemispheric motor cortex of our patients also showed abundant NFTs and a particularly pronounced cortical amyloid angiopathy, which may have further aggravated motor dysfunction.

There is clinical evidence of cerebellar involvement in varAD, such as clumsiness of hands and dysarthria (20). Concordantly, the neuropathological analysis of our patients’ cerebella showed abundant deposition of amyloid as cored Aβ40-positive plaques, more diffuse Aβ42/43-positive deposits, and as severe angiopathy in both the vermis and the hemispheres. Amyloid plaques have previously been described in the cerebellar cortex of patients with the PS-1 gene mutations E280A (17) and L250S (43).

Our patients had a pronounced leptomeningeal and cortical amyloid angiopathy, a known risk factor for intracerebral hemorrhage (ICH) (9, 10). One of our patients had had acute ICH in his left frontal lobe in addition to asymptomatic brain infarcts. These lesions may have
been caused by the amyloid angiopathy, the most common cause of lobar ICH (44).

According to the amyloid cascade hypothesis, Aβ generated and deposited in the brain in the form of amyloid fibrils is toxic to nerve cells and the main cause of neurodegeneration in AD (45). The marked increase in the generation of Aβ, especially its 42 amino acid-long isoform in FAD, is regarded as a strong argument for this hypothesis. Those advocating other pathogeneses have regarded amyloid deposits as a secondary phenomenon. The presence of dementia in patients with CWP without amyloid cores and definitive fibrillary amyloid supports the view that the extracellular, congophilic amyloid plaques are not the only initiators of neurodegeneration in AD.


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