Vascular variant of prion protein cerebral amyloidosis with τ-positive neurofibrillary tangles: The phenotype of the stop codon 145 mutation in PRNP

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ABSTRACT Deposition of PrP amyloid in cerebral vessels in conjunction with neurofibrillary lesions is the neuropathologic hallmark of the dementia associated with a stop mutation at codon 145 of PRNP, the gene encoding the prion protein (PrP). In this disorder, the vascular amyloid in tissue sections and the ~7.5-kDa fragment extracted from amyloid are labeled by antibodies to epitopes located in the PrP sequence including amino acids 90–147. Amyloid-laden vessels are also labeled by antibodies against the C terminus, suggesting that PrP from the normal allele is involved in the pathologic process. Abundant neurofibrillary lesions are present in the cerebral gray matter. They are composed of paired helical filaments, are labeled with antibodies that recognize multiple phosphorylation sites in τ protein, and are similar to those observed in Alzheimer disease. A PrP cerebral amyloid angiopathy has not been reported in diseases caused by PRNP mutations or in human transmissible spongiform encephalopathies; we propose to name this phenotype PrP cerebral amyloid angiopathy (PrP-CAA).

Abnormal isoforms of the prion protein (PrP) are found in sporadic and iatrogenic Creutzfeldt–Jakob disease, kuru, and hereditary disorders caused by mutations in the PrP gene (PRNP). Parenchymal deposition of PrP amyloid is the hallmark of Gerstmann–Sträussler–Scheinker disease (GSS), a dominant disorder associated with specific PRNP mutations (1). The composition of PrP amyloid has been determined in GSS with mutations at codons 117, 198, and 217 (2, 3). The major components of amyloid are N- and C-terminally truncated PrP fragments of approximately 7 and 11 kDa, spanning residues 81–144/150 and 88–150, respectively (2, 3). These fragments originate from the mutant alleles (2). In GSS with mutation at codon 198 or 217, deposition of PrP amyloid in the parenchyma is associated with neurofibrillary lesions composed of paired helical filaments (PHFs) (1).

A mutation at codon 145 of PRNP (4) resulting in a stop codon (Y145Stop) provides a condition in which PrP is synthesized as a truncated isoform. The C terminus of this PrP is similar to the C terminus of PrP fragments that result from mutations at PRNP codons 117, 198, and 217. Such a protein is devoid of glycosylation sites and the signal sequence for the glycosyl-phosphatidyl inositol anchor. Here, we report that this molecular defect results in deposition of vascular PrP amyloid that coexists with neurofibrillary le-

Fig. 1. Genetic analysis of patient. A fragment of PRNP was amplified by PCR and subjected to digestion with Mse I to detect the codon 145 mutation (Left) or Mse II to determine the genotype at codon 129 (Right). P, patient lane (heterozygous at codon 145, Met/Met at codon 129); C, control lane (normal at codon 145, Val/Val at codon 129).

Abbreviations: PrP, prion protein; PRNP, PrP gene; GSS, Gerstmann–Sträussler–Scheinker disease; CAA, cerebral amyloid angiopathy; PHFs, paired helical filaments; AD, Alzheimer disease; mAb, monoclonal antibody.

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Neuropathology. Tissue blocks from cerebral cortex, subcortical nuclei, midbrain, pons, medulla, and cerebellum were fixed in 4% formaldehyde and embedded in paraffin. Ten-micrometer-thick sections were stained with hematoxylin and eosin, the Heidenhain–Woelecke method for myelin, the Bodian method for neurofibrils, and Congo red and thioflavin S for amyloid. For immunohistochemistry, 10-μm-thick sections were incubated with (i) antisera raised to synthetic peptides homologous to human PrP residues 23–40 (PrP23–40), 90–102 (PrP90–102), 127–147 (PrP127–147), and 220–231 (PrP220–231) (6), 1:100 dilution; (ii) monoclonal antibody (mAb) 3F4, which recognizes an epitope corresponding to residues 109–112 of human PrP, 1:500 dilution (7, 8); (iii) mAb Alz50, which recognizes an epitope including residues 2–10 of τ protein (9, 10), 1:5 dilution; (iv) phosphorylation-dependent anti-τ mAbs AT270 (1:500) (11), AT8 (1:500) (12), AT180 (1:500) (11), and PHF1 (1:5) (13, 14), which recognize phosphorylated Thr-181, Ser-202/Thr-205, Thr-231, and Ser-396/Ser-404, respectively, and mAb AT100 (1:500) (15), which recognizes phosphorylated τ at a presently unknown site (12); (v) mAbs 10D5 (1:100), 6E10 (1:2000), and 4G8 (1:2000), raised against peptides corresponding to residues 1–28, 1–17, and 17–24 of amyloid β (Aβ) (16, 17); and (vi) an antiserum to glial fibrillary acidic protein (BioGenex Laboratories, San Ramon, CA) (1:50). For immunodetection, the unlabeled peroxidase-antiperoxidase (Sternberger Monoclonals, Baltimore) and avidin–biotin complex (Boehringer Mannheim) methods were used. Specificity of the immunoreactions was determined as described (6).

For electron microscopy, 30-μm-thick paraffin sections of cerebral cortex were deparaffinized and rehydrated. Immunogold labeling with PrP90–102, 1:3 dilution, and with PHF1, undiluted, was carried out using 10-nm colloidal gold particles (Goldmark Biologicals, Phillipsburg, NJ) conjugated to goat anti-rabbit IgG or goat anti-mouse IgG at a dilution of 1:20.

Biochemistry. Amyloid was isolated from gray matter, and proteins were extracted with formic acid and analyzed by Western blot with 3F4 (1:5000 dilution) and PrP23–40, PrP90–102, PrP127–147, and PrP220–231 (1:1000 dilution) as described (2).

Fig. 2. (A) Vessel walls cut in the longitudinal and transversal planes show fluorescent amyloid deposits in cerebellar cortex. (B–H) Parenchymal and leptomeningeal vessel walls as well as perivascular tissue show PrP deposits in cerebral cortex. (I) Schematic representation of normal (Top) and mutant (Middle) PrP. Residue numbers and amino acids are indicated above and below the diagrams, respectively. Map of PrP (Bottom) shows the regions used in constructing synthetic peptides for the production of polyclonal antibodies; ●, immunopositivity; ○, no immunoreactivity. (A, thioflavin S; B, C, E, and G, immunostaining with antiserum PrP90–102; D, immunostaining with mAb 3F4; H, immunostaining with antiserum PrP220–231; A, ×90; B, ×100; C, ×230; D, ×100; E, ×130; F, ×370; G, ×330; H, ×120.)
RESULTS

Genetic Analysis. Mae I digestion of a PRNP fragment amplified from DNA extracted from frozen brain tissue resulted in two cleavage fragments and an undigested fragment (Fig. 1), thus confirming heterozygosity for the codon 145 mutation (4). Since the genotype at codon 129 can influence the phenotype of PrP-associated diseases (1, 5), we determined the codon 129 genotype. The patient was homozygous for methionine at codon 129 (Fig. 1). The codon 145 mutation was confirmed in the paraffin-embedded tissue.

Neuropathology. Diffuse atrophy of the cerebrum and dilation of the lateral ventricles were severe. Microscopically, the two most characteristic lesions were (i) amyloid deposits in parenchymal and leptomeningeal vessels and in the perivascular neuropil and (ii) neurofibrillary lesions in cerebral gray matter. Neuronal loss and gliosis were severe.

Amyloid and PrP Immunoreactivity. Amyloid angiopathy involved primarily the walls of small and medium-sized vessels (Fig. 2A) of the cerebral and cerebellar gray matter and to a lesser extent the leptomeningeal vessels; amyloid was also prominent in the perivascular space and the surrounding parenchyma. Amyloid was immunoreactive with anti-PrP antibodies (Fig. 2 B–G) but not with anti-Aβ antibodies. Vessel-associated PrP deposits were conspicuous in gray matter of cerebrum and cerebellum but were only occasionally observed in the brainstem. While PrP deposits in the capillaries were conspicuously present in the vessel walls, in the arterioles they were abundant in the adventitia, relatively sparing the media.

Vascular PrP-immunoreactivity was detected occasionally in the leptomeninges (Fig. 2E), but it was rare in the white matter.

Amyloid was immunostained by PrP90–102 (Fig. 2 B–G), PrP127–147, and 3F4, but it was not reactive with PrP23–40. Immunolabeling was also observed with antiserum PrP220–231 and was more prominent and consistent in cerebellar than in cerebral cortex (Fig. 2H). The hypothetical diagram in Fig. 2I shows the normal and mutant proteins as well as the regions against which the antibodies were produced.

By electron microscopy, amyloid was composed of straight 8- to 10-nm filaments; bundles of radially oriented amyloid filaments were seen adjacent to the vessel wall, whereas haphazardly oriented filaments were numerous within the wall of vessels internally to the basement membrane (Fig. 3A–C). Amyloid fibrils were decorated by PrP90–102, which also labeled amorphous material intermingled with the fibrils (Fig. 3B).

Characterization of Amyloid Protein. Immunoblot analysis of proteins extracted from amyloid showed that the smallest subunit migrated as a broad band at ~7.5 kDa (Fig. 4). This band was immunoreactive with antibodies to epitopes located between residues 90 and 147 of PrP and was unreactive with PrP23–40 and PrP220–231 (Fig. 4). Amyloid fractions also contained higher molecular weight peptides migrating as poorly resolved bands of 12–16 and 22–30 kDa (Fig. 4A). These bands exhibited the same pattern of immunoreactivity as the 7.5-kDa peptide, suggesting that they consisted primarily of polymers of amyloid protein.

Neurofibrillary Lesions and τ Immunoreactivity. Neurofibrillary tangles, neuropil threads, and dystrophic neurites were numerous in the cerebral gray matter (Fig. 5). Most affected was the hippocampus (Fig. 5 A–D), where the majority of pyramidal and granule cells were involved. In the pyramidal layer, numerous extracellular tangles were seen.

Neurofibrillary lesions were recognized by all anti-τ mAbs (Fig. 5 B–F). AlZ50 recognized only intracellular neurofibrillary lesions (Fig. 5C). All antibodies stained a large number of neurit threads, although with various degrees of intensity (Fig. 5 F–J). AT8 recognized the largest number of neurit threads, whereas AlZ50 recognized the smallest number. In

![Fig. 3.](image)

(A and B) Blood vessel wall and adjacent parenchyma show presence of amyloid fibrils. Amyloid deposits are composed of haphazardly oriented filaments within the vessel wall (arrowhead) and bundles of radially oriented filaments in the parenchyma adjacent to the vessel wall (arrows). (C) Immunoelectron microscopy shows that amyloid fibrils and adjacent amorphous material are labeled by antiserum PrP-90–102. (A, ×2000; B, ×5300; C, ×64,800.)

![Fig. 4.](image)

(A) Western immunoblot analysis of proteins extracted by formic acid from amyloid fibrils, fractionated on a Sephacryl G-100 column, and probed with mAb 3F4. Lanes 1–5, fractions obtained by gel filtration; lane 0, unFractionated material. The amyloid subunit migrates as a broad band centered at 7.5 kDa (lane 5). (B) Immunoblot analysis of the amyloid protein (fraction 5 from gel filtration) with antibodies to various PrP regions. The amyloid protein is immunoreactive with antibodies recognizing PrP residues 90–147 (lanes b–d) but is unreactive with antibodies to N- and C-terminal domains (lanes a and e, respectively).
many instances, abnormal neurites immunolabeled by Alz50, AT8, and PHF1 were closely associated with parenchymal amyloid deposits and amyloid-laden blood vessels, resulting in a picture of neuritic immunolabeling that overlapped with PrP immunopositivity (Fig. 5 D and E). Neurofibrillary lesions were composed of PHFs with a periodicity of 70–80 nm and were decorated by mAb PHF1 (Fig. 6).

**DISCUSSION**

The point mutation TAT to TAG (tyrosine to stop) at codon 145 in conjunction with a Met-129 codon of PRNP is associated with PrP amyloid deposition in cerebral vessels and with τ-positive neurofibrillary lesions. We propose to name this newly recognized neuropathologic phenotype PrP cerebral amyloid angiopathy (PrP-CAA). In PrP-CAA, the vascular amyloid in tissue sections and the 7.5-kDa fragment contained in the purified amyloid fractions were consistently labeled by antibodies to epitopes included in the PrP sequence spanning residues 90–147. The pattern of immunoreactivity indicates that the major PrP amyloid protein of Y145Stop is truncated at the N and C termini. Truncation of the C terminus of the protein occurs at a similar site (i.e., between residues 144 and 150) in all GSS variants in which the amyloid protein has been analyzed (2, 3). Thus, the formation of a PrP peptide with a C terminus around residue 150 appears to be important for amyloid formation. This concept is supported by *in vitro* studies with synthetic peptides homologous to various segments of PrP showing that the sequence spanning residues 106–147 is highly fibrillogenic (18). On the basis of immunochemical data, truncation at the N terminus in PrP-CAA most likely occurs within the octapeptide repeat region, as observed in GSS variants (2, 3).

In PrP-CAA, amyloid-laden vessels were also labeled by antibodies against the C terminus of PrP. The absence of C-terminal fragments of PrP from purified amyloid suggests that the fragments are not incorporated into amyloid filaments, or are removed during the extraction procedure, or contribute to amyloid in an amount not detectable by our procedure. Deposition of PrP and/or PrP fragments other than the amyloid protein has previously been observed in GSS (2, 6, 19). Previous studies showed that the amyloid protein originates from the mutant allele (2); however, it was not possible to establish the allelic origin of the fragments containing the N and C termini. In Y145Stop, the PrP peptides containing the C terminus probably derive from wild-type PrP, suggesting that it can also be involved in the pathologic process. Furthermore, the finding that immunoreactivity with antibodies to the C terminus was more intense in cerebellar than in cerebral cortex suggests that there are regional differences in PrP processing.
PrP cerebrovascular amyloidosis (congophilic angiopathy), a feature not previously observed in human diseases characterized by cerebral PrP amyloid deposition, has been reported in sheep with natural scrapie (20). However, despite some similarities in immunohistochemical characteristics, the lesions found in Y145Stop were widespread and not rare and focal as in scrapie. In addition, we have been studying, neuropathologically and genetically, a GSS patient who presents not only the PrP parenchymal deposits typical of that disease but also frequent PrP deposits in the cerebellar capillaries.

Immunohistochemical and biochemical studies of cerebral amyloidoses have revealed that vascular deposits are produced by at least four known proteins: PrP, amyloid-β, cystatin C, and transthyretin (21, 22). Two additional types of cerebral amyloid angiopathy are caused by as yet unknown proteins (23, 24). In all forms, the vascular involvement may be both cerebral and leptomeningeal. At variance with these conditions, in PrP-CAA arterioles show minimal involvement of the media. The origin of the PrP vascular amyloid precursor in PrP-CAA may include (i) a neuronal source, (ii) the cerebrovascular system itself (i.e., endothelial cells), and (iii) a circulating protein. The presence of vascular amyloid in cerebral and cerebellar gray matter and its absence in white matter may indicate that neurons are an important source of the amyloid precursor. Alternatively, cortical vessels might have some unique property not shared by white matter vessels. On the other hand, PrP mRNA is found not only in neurons but also in other cells of the central nervous system, including endothelial and meningeal cells (25). Thus, it is possible that the amyloid deposited in leptomeningeal and parenchymal vessels is produced by endothelial cells. In other types of cerebrovascular amyloidosis, such as hereditary cerebral hemorrhage of Icelandic and Dutch types as well as AD, it is believed that the amyloid deposited in vessels derives from soluble precursors (21). The PRNP stop codon 145 creates a C-terminally truncated molecule that lacks the asparagine-linked carbohydrates at positions 181 and 197 and the glycosyl-phosphatidylinositol anchor. It is conceivable that this protein, when produced outside the brain parenchyma, might be soluble and cross the blood–brain barrier. A soluble form of the cellular PrP has been isolated from cerebrospinal fluid of normal individuals (26).

The other major neuropathologic feature of PrP-CAA is the presence of abundant neurofibrillary lesions in most gray-matter structures of the cerebral cortex. These are composed of PHFs and could be labeled with antibodies that recognize the N terminus of τ and multiple phosphorylation sites in τ. Thus, the neurofibrillary lesions seen in this condition are very similar if not identical to those observed in AD. However, the pathology in PrP-CAA stands in contrast to that observed in hereditary cerebral hemorrhage, Dutch type, where an Aβ amyloid angiopathy is found in the absence of neurofibrillary lesions (27). Neurofibrillary tangles and neuripil threads have been reported in GSS variants with large amounts of amyloid—namely, GSS with mutations at PRNP codons 198 and 217, where the topographic association of neurofibrillary lesions and PrP amyloid is striking (1, 28). In PrP-CAA, the association of PrP amyloid with neurofibrillary lesions also does not appear to be a casual one. In many instances, abnormal neurites labeled with antibodies to phosphorylated epitopes in τ coexisted with PrP amyloid in the neocortex surrounding blood vessels, suggesting that the dysorphic angiopathy may affect the neuronal cytoskeleton.

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