Prion Proteins with Different Conformations Accumulate in Gerstmann-Sträussler-Scheinker Disease Caused by A117V and F198S Mutations

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Gerstmann-Sträussler-Scheinker disease (GSS) is characterized by the accumulation of proteinase K (PK)-resistant prion protein fragments (PrP^{Sc}) of ~7 to 15 kd in the brain. Purified GSS amyloid is composed primarily of ~7-kd PrP peptides, whose N terminus corresponds to residues W⁸¹ and G⁸⁸ to G⁹⁰ in patients with the A117V mutation and to residue W⁸¹ in patients with the F198S mutation. The aim of this study was to characterize PrP in brain extracts, microsome preparations, and purified fractions from A117V patients and to determine the N terminus of PrP^{Sc} species in both GSS A117V and F198S. In all GSS A117V patients, the ~7-kd PrP^{Sc} fragment isolated from nondigested and PK-digested samples had the major N terminus at residue G⁸⁸ and G⁹⁰, respectively. Conversely, in all patients with GSS F198S, an ~8-kd PrP^{Sc} fragment was isolated having the major N terminus start at residue G⁷⁴. It is possible that a further degradation of this fragment generates the amyloid subunit starting at W⁸¹. The finding that patients with GSS A117V and F198S accumulate PrP^{Sc} fragments of different size and N-terminal sequence, suggests that these mutations generate two distinct PrP conformers. (Am J Pathol 2001, 158:2201–2207)

Prion diseases are neurodegenerative disorders present in both humans and animals.^{1,2} In humans, idiopathic, genetically determined, and transmissible prion disorders have been described.^{1–3} A hypothesized central event in the pathogenesis of these disorders is a conformational change of the normal membrane-associated prion protein (PrP^C) into a pathogenic isoform (PrP^{Sc}).¹ PrP^C is detergent soluble, protease sensitive, and has a predominantly α -helical structure. In contrast, PrP^{Sc} is insoluble in nondenaturing detergents, is relatively resistant to cleavage by proteinase K (PK), and has an increased β -sheet structure.^{4–8}

The methionine (M)/valine (V) polymorphism at the prion protein gene (*PRNP*) codon 129 can influence the clinical phenotype produced by a mutation, this is seen most markedly with the D178N mutation, which can cause Creutzfeldt-Jakob disease (CJD) or fatal familial insomnia depending on the presence of M or V at residue 129.² In CJD, a sporadic or inherited disease characterized by rapid progressive dementia and spongiform degeneration in the cerebrum and cerebellum, two main PrP patterns, PrP^{Sc} type 1 and type 2, have been described.^{9,10} PrP^{Sc} type 1 after deglycosylation is characterized by a PK-resistant C-terminal fragment of 21 kd, whereas PrP^{Sc} type 2 is characterized by a C-terminal protease-resistant peptide of 19 kd.⁹ Gerstmann-Sträussler-Scheinker disease (GSS) is a genetically determined autosomal dominant prion disease with a protracted clinical course, clinically characterized by ataxia and cognitive impairment.³ GSS is caused by mutations P102L, P105L, A117V, G131V, F198S, D202N, Q212P, and Q217R in *PRNP*.^{3,11,12} The pathological hallmark of GSS is the accumulation of PrP, with and without amyloid tinctorial properties, in the brain.³ Our studies have shown that the pattern of PrP^{Sc} isoforms in the multiple GSS variants analyzed is different from that seen in CJD.^{11–13} Patients with CJD present full-length and N-truncated PrP fragments whereas patients with GSS

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Table 1. The Clinical, Genetic, and Pathological Information for Five GSS A117V Patients and One Asymptomatic A117V Mutation Carrier; and for Two GSS F198S Patients is Summarized

Patient	K	AD	Duration, years	Residue 129	G	PrP			Spong	NFT
						Neocortex	Striatum	Cerebellum		
1	SG	61	3	MV	M	+/++	++	—	—	—
2	SG	39	3	MV	F	+/++	++	+/-	—	—
3	SG	32	7	VV	M	+/++	+/++	+/-	—	—
4	SG	33	5	VV	M	+/++	++	+++	—	—
5	AS	45	4	VV	F	+/++++	++	+/-	-/+	—
6	AS	50	N/Aa	MV	M	—	—	+/-	—	—
7	IK	64	6	MV	F	+++	+++	+++	—	+++
8	IK	61	12	VV	F	+++	+++	+++	—	+++

a, Asymptomatic carrier; K, kindred; AD, age at death; G, gender; Duration, years; duration of clinical signs in years; Spong, spongiform degeneration; NFT, neurofibrillary tangles.

Semi-quantitative analysis of lesions: —, absence; +, mild; ++, moderate; +++, severe. Subjects 1 to 6 correspond to two families with GSS A117V and patients 7 and 8 to the Indiana kindred with GSS F198S.

present full-length as well as N- and C-terminal truncated PrP peptides.^{10–13,14,15} Previous studies showed that the amyloid subunit in GSS F198S is a 7-kd peptide with an N terminus at residue G⁸¹.¹⁶ Similar studies in patients from one American and one Alsatian family with GSS A117V revealed that the 7-kd amyloid protein had a major N-terminal cleavage site at residue G⁸¹ and G⁸⁸ to G⁹⁰.^{17–18} In addition, we have reported on the presence of PrP^{sc} isoforms of ~27 to 29, 18 to 19, and 8 kd in brain extracts and microsomal fractions of patients from the Indiana kindred with GSS F198S.¹³

In GSS A117V, previous studies have been contradictory in terms of whether or not PK-resistant PrP is present.^{11,19,20} The aims of the present study are to determine the biochemical characteristics of PrP in several patients and an asymptomatic carrier from two unrelated American families with *PRNP* A117V.^{21,22} We analyzed brain homogenates, microsomal preparations, and purified PrP fractions. In addition, based on the observation that the pattern of digestion of PrP^{sc} depends on the tertiary structure of the protein, we investigated whether PrP conformational isomers are present in phenotypically different GSS variants. To explore this possibility, we determined the N-terminal cleavage sites of the PrP fragments that accumulate in GSS A117V and GSS F198S.

Materials and Methods

The experiments were performed using brain tissue from individuals carrying *PRNP* mutations A117V and F198S. These individuals (seven patients and one asymptomatic carrier) derive from three families and have been studied genetically and neuropathologically.^{21–26} The data on these cases is summarized in Table 1.

Biochemical Assays

Tissue was obtained from six subjects with *PRNP* A117V. Seven samples from the neocortex and four samples from the cerebellum of each of six individuals were analyzed. Tissue obtained from the frontal cortex of all cases was used for subcellular fractionation, including PrP^{sc} purification and sequencing using Edman chemistry.

Tissue was obtained from the frontal cortex and cerebellum of a patient of the Indiana kindred (GSS F198S) heterozygous for the M/V polymorphism at codon 129. In addition, tissues from the frontal cortex, caudate nucleus, and cerebellum of a homozygous V 129 patient from the same kindred were also used for PrP^{sc} purification and amino acid sequencing.

Brain tissues from a patient with sporadic CJD and from one individual dying without neurological disease were used as control.

Brain Extracts

Tissue was homogenized in 9 volumes (10% w/v) of lysis buffer (100 mmol/L NaCl, 10 mmol/L ethylenediaminetetraacetic acid, 0.5% Nonidet P-40, 0.5% Na deoxycholate in 10 mmol/L Tris-HCl, pH 7.4). Samples were resolved in 4 to 12% acrylamide gels and transferred to nitrocellulose membranes as described.^{11,13} Membranes were probed with monoclonal antibody (mAb) 3F4.²⁷ Immunoblot analysis for PrP^{sc} was done after digestion of the homogenates with PK (10 μ g/ml) for 1 hour at 37°C. Selected samples from patient 3 were also digested with 5, 10, 50, and 100 μ g/ml of PK for 1 hour at 37°C.

Subcellular Fractionation

Membrane fractions were obtained as described.^{13,15} In brief, 10% (w/v) homogenates were prepared in 0.32 mol/L sucrose, 5 mmol/L ethylenediaminetetraacetic acid, 3 mmol/L phenylmethylsulfonyl fluoride, 20 mmol/L Tris, pH 7.5. After centrifugation at 6000 $\times g$ for 10 minutes, the pellet (P₁) was discarded; the supernatant (S₁) was centrifuged at 100,000 $\times g$ for 1 hour at 4°C, to obtain a cytosolic fraction (S₂) and a membrane fraction (P₂). To determine the presence of rough endoplasmic reticulum membranes in these preparations, blots from P₂ fractions were probed with mAb 9G10 (Stressgen Biotechnology, Canada) to detect GRP94, a protein normally present in the lumen of the endoplasmic reticulum membrane fraction.²⁸

To examine insoluble and PK-resistant PrP in the membrane fraction, the pellet (P₂) was resuspended in dis-

tilled H₂O, incubated on ice for 20 minutes, and centrifuged at 100,000 × *g* for 1 hour at 4°C. The pellet (P₃) was resuspended in 150 mmol/L NaCl, 2% *N*-lauroylsarcosine (Sarkosyl), 25 mmol/L Tris, pH 7.4, and an aliquot removed for immunoblot analysis. The remaining sample was digested with PK at 100 μg/ml for 1 hour at 37°C. Phenylmethylsulfonyl fluoride was then added to stop PK digestion and the sample was centrifuged at 200,000 × *g* for 1 hour at 4°C, to obtain insoluble and PK-resistant PrP (P₄). The supernatant (S₄) was removed from the pellet (P₄) and protein present in S₄ was precipitated with methanol. The P₄ and S₄ fractions were resuspended in sample buffer and used for Western blot analysis.

Purification of PrP^{Sc}

PrP^{Sc} was purified as described.²⁹ Tissue (1.5 g) was homogenized (1:4) in 0.01 mol/L Na phosphate, pH 7.4, 10% Sarkosyl, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L *N*-ethylmaleimide, incubated 30 minutes at room temperature and spun at 22,000 × *g* for 30 minutes at 10°C. The supernatant (S₁) was collected and centrifuged at 215,000 × *g* for 2.5 hours at 10°C. The pellet (P₂) was resuspended in 200 μl of 50 mmol/L Tris-HCl, pH 8.5, and 1 μl of ribonuclease A (80 μg/μl) was added. After stirring for 1 hour at 37°C, 400 μl of solution B (0.6 mol/L K iodide, 6 mmol/L Na thiosulfate, 1% Sarkosyl, 10 mmol/L Na Phosphate, pH 8.5) were added. The sample was centrifuged at 215,000 × *g* for 1.5 hours at 10°C through a cushion of 20% (w/v) sucrose in solution B (sucrose/sample ratio; 1:4 w/v). The pellet (P₃) was resuspended in 110 μl of phosphate-buffered saline. Selected samples were used for immunoblot analysis before PK digestion. Samples from all cases were digested with PK (100 μg/ml for 1 hour at 37°C).

For deglycosylation, PrP^{Sc} was digested with 5 mU PNGase F (Glyko, Novato, CA) for 2 hours as specified by the manufacturer and analyzed by immunoblot probed with mAb 3F4. In selected samples, polyclonal antibodies AS-6800 raised against synthetic peptides corresponding to residues 89 to 104 (provided by Dr. H. Diringer) and anti PrP-95 to 108 were also used.³⁰ As negative control, filters were probed with the secondary antibody in the absence of primary antibody. An identical protocol was used for the analysis of PrP^{Sc} isolated from a case of CJD.

Protein Sequence Analysis

Purified PrP (ie, non-PK-treated and PK-digested samples) was resolved in 4 to 12% acrylamide gels, transferred to polyvinylidene difluoride Problott membrane (Applied Biosystems, Foster City, CA) and stained with Coomassie blue. The band of interest, as determined by comparison with a Western blot of the same material, was excised and analyzed on an Applied Biosystems model 473A protein sequencer using cycle programs provided by the manufacturer as previously described.³¹

Figure 1. Immunoblot of PrP from five patients with GSS A117V (lanes A–J), from an asymptomatic carrier (lanes K and L), and a CJD control (lanes M and N). Results from frontal cortex are shown for all cases. Samples from lanes A, C, E, G, I, K, and M were not PK treated, whereas samples from lanes B, D, F, H, J, L, and N, were PK treated. PrP was detected with mAb 3F4 and visualized by ECL. The time of exposure to film to detect the ECL product was varied to enable qualitative comparison of samples. Upper panel shows similar total PrP signals for all patients with *PRNP* A117V. In the lower panel lanes M, N were not included because of overexposure of the film.

Results

PrP in Brain Extracts of Subjects Carrying The *PRNP* A117V

Immunoblots of brain extracts from subjects carrying the *PRNP* A117V mutation consistently demonstrated PrP in non-PK-treated homogenates, however considerable heterogeneity of PrP^{Sc} was observed. PrP^{Sc} varied in overall amount and in the relative quantity of the different isoforms in different brain areas of individual patients and among patients. In many samples obtained from the frontal cortex and cerebellum no PrP^{Sc} was detected (data not shown). Nevertheless, PrP^{Sc} was seen in selected samples of the frontal cortex of all patients after digestion with PK (10 μg/ml) (Figure 1; lanes B, D, F, H, and J), but not in samples from the asymptomatic carrier (Figure 1, lane L). In cases 1 to 5 (Figure 1; lanes B, D, F, H, and J), a PrP^{Sc} band of ~14 kd was observed, with patients 1, 2, 3, and 5 usually showing an additional band of ~7 kd (Figure 1; B, D, F, and J). In some blots, a 7-kd band was also seen in patient 4. Extracts obtained from patient 3 showed 7- and 14-kd bands in samples digested with PK at concentrations ranging from 5 to 100 μg/ml (Figure 2). For comparison, we studied in-parallel samples from CJD.

PrP in Subcellular Fractions of Patients with *PRNP* A117V

PrP partitioned with membranes in samples obtained from the frontal cortex of subjects carrying the *PRNP* A117V mutation. Examples from two clinically affected and one asymptomatic carrier are shown in Figure 3; lanes A, D, and G. We observed that the membrane fraction obtained from the affected subjects (1 to 5) contained detergent-insoluble and PK-resistant PrP (ie, PrP^{Sc}) fragments of 7 and 14 kd. PrP^{Sc} present in patients 3 and 4 is shown in Figure 3, lanes B and E. Figure 3 also

Figure 2. Immunoblot of PrP from patient 3 after digestion of frontal cortex brain homogenates with 0, 5, 10, 50, and 100 µg/ml of PK (lanes A-E, respectively). Similarly treated samples from a patient with CJD are shown in lanes F-J. Different times of exposure (1 second, 10 seconds, and 30 seconds) were obtained. PrP was detected with mAb 3F4 and visualized by ECL.

shows that PrP^{Sc} was not detected in the soluble fraction of these patients. Soluble fractions obtained from patients 3 and 4 are shown in Figure 3, lanes C and F. PrP^{Sc} was not detected in membranes or in the soluble fractions of the sample obtained from the frontal cortex of the asymptomatic A117V mutation carrier (Figure 3, lanes H and I). To determine the presence of microsomes, immunoblots were probed with mAb 9G10, directed against the heat-shock protein GRP 94 normally present in the lumen of the endoplasmic reticulum membranes. A positive result was obtained (not shown).

Figure 3. Immunoblot of PrP in membrane fractions obtained from the frontal cortex of patient 3 (lane A), patient 4 (lane D), and from an asymptomatic carrier (lane G). Detergent-insoluble and PK-resistant PrP of ~7 and 14 kd are observed in patients 3 and 4 (lanes B and E), but not in the asymptomatic carrier (lane H). No PrP^{Sc} is observed in the PK-digested soluble fraction in patients or carrier (lanes C, F, and I). PrP was detected with mAb 3F4 and visualized by ECL.

Figure 4. Immunoblot of purified PrP obtained from the frontal cortex of patient 5 (lanes A-C) and from a CJD control (lane D). Samples were either nontreated, ie, no PK or PNGase F treatment (lane A); treated with PK (lanes B and D); or PK- and PNGase F-treated (lane C). PrP was detected with mAb 3F4 and visualized by ECL.

Partially Purified PrP in Subjects Carrying the PRNP A117V Mutation

Because of the variability of detecting abnormal PrP in total brain homogenates and to the fact that previously published protocols are effective in concentrating relatively pure PrP^{Sc}, we analyzed partially purified fractions obtained from the frontal cortex of patients 1 to 5 and from the asymptomatic carrier (case 6). After PK digestion, two major bands of 14 and 7 kd were detected in the patients (ie, patients 1 to 5). A similar pattern was seen when immunoblots were probed with a panel of antibodies raised against the mid-region of PrP (ie, PrP residues 89 to 112), however no signal was seen when the primary antibody was omitted. An example of PrP^{Sc} banding pattern in patient 5 is shown in Figure 4, lane B. No PrP^{Sc} was observed in the partially purified sample obtained from the asymptomatic carrier (not shown).

Analysis of Glycosylation State of PrP^{Sc} Fragments

In CJD and some other prion diseases, PrP^{Sc} is composed of a C-terminal-glycosylated PK-resistant core. The 7-kd PrP^{Sc} fragment seen in GSS A117V is detected with mAb 3F4. Considering the apparent size and immunological profile of this peptide (ie, containing residues 109 to 112, as shown by mAb 3F4 immunoreactivity), we speculated that this peptide would not contain the C-terminal N-linked glycosylation sites at residues 181 and 197. To explore this possibility further, we performed enzymatic deglycosylation on samples obtained from all patients (ie, patients 1 to 5), and showed that an identical mobility is seen in PrP^{Sc} peptides before and after PNGase F treatment. Figure 4, lane C, shows a representative sample obtained from patient 5. This finding supports the concept that glycosylation sites are not present.

PrP Fragments in Non-PK-Treated Samples

We noticed that in selected samples the low-molecular weight PrP fragments (ie, 7 and 14 kd) could be seen before PK digestion. To analyze if the truncated fragments were insoluble in nondenaturing detergent we partially purified PrP in non-PK-digested samples (through ultracentrifugation in the presence of Sarkosyl). In all patients (ie, patients 1 to 5), we observed bands of ~27 to 35, 14, and 7 kd. A sample from patient 5 is shown in Figure 4, lane A. The stoichiometry of the low-molecular weight fragments is different between PK- and non-PK-digested samples. PK digestion increases the amount of the 7- and 14-kd bands. The similar size of the 7-kd fragments in non-PK-treated and PK-treated samples suggest that a similar or identical cleavage site may be present *in vivo* and *in vitro*.

Determination of the N-Terminal Cleavage Site of PrP Fragments in Patients with GSS A117V and GSS F198S

To characterize the primary structure of the 7-kd PrP^{sc} fragment, partially purified and PK-treated PrP obtained from the frontal cortex of patients with GSS A117V (ie, patients 1 to 5) was analyzed using Edman chemistry, after resolution of fragments by gel electrophoresis (see Materials and Methods). Sequence analysis of the 7-kd peptide from patient 3 (for 20 cycles) yielded GQGGGTHSQWNKPS KPKTNM as the major N-terminal sequence corresponding to residues 90 to 109 of PrP. The data were consistent with minor amounts of PrP fragments starting with residues 86, 88, and 92. Analysis of the 7-kd band from the other four patients showed that the major sequence also started with residue 90 of PrP. As stated above, a 7-kd band was also seen in immunoblots obtained from non-PK-digested samples. Sequence analysis of this 7-kd band from patient 4 yielded GXGQGGGTHSQXNKP as the N-terminal sequence corresponding to residues 88 to 102 of PrP.

Patients with GSS F198S and GSS A117V accumulate PrP^{sc} fragments that can be cleaved by PK to generate peptides of different mobility (ie, the small fragment is 7 kd in GSS A117V and 8 kd in GSS F198S) in gel electrophoresis. This observation could be because of the presence of conformational isomers that expose different PrP residues to hydrolysis by PK in the presence of detergents. To explore this possibility, we analyzed the 8-kd PrP^{sc} fragment obtained from the frontal cortex of two patients with GSS F198S (Figure 5). Sequence analysis for 29 cycles yielded GQPHGGGWGQPHGGGWGQGGGTHSQWNKP corresponding to residues 74 to 102 of PrP in both cases. This indicates PK cleavage in the second PrP octarepeat region for GSS F198S, different from the PK cleavage site for GSS A117V.

To analyze the possibility that different PrP conformers might be present in different brain areas, we determined the N-terminal sequence of PrP^{sc} purified from the caudate nucleus (an area with absent or small amounts of amyloid), and the cerebellum (a region with abundant

Figure 5. Immunoblot of purified PrP obtained from the frontal cortex and cerebellum of patient 8 (lanes B and C), from the cerebellum of patient 7 (lane D), and from a CJD control (A). Identical banding pattern was observed in samples obtained from patients 7 and 8. Samples were treated with PK (lanes A–D). PrP was detected with mAb 3F4 and visualized by ECL.

amyloid) in a homozygous 129 V patient with GSS F198S. Similar results through 10 to 15 cycles were observed in both samples indicating G⁵⁸, G⁶⁶, or G⁷⁴ as the N terminus. Although we were not able to determine sufficient residues on the fragments to identify the exact N terminus, based on the size and immunological profile, the simplest interpretation of the data are that the major N terminus is at residue 74, as found in the frontal cortex.

Discussion

This study demonstrates that PrP^{sc} is present in all five patients with GSS A117V but was not detected in the samples analyzed from the asymptomatic carrier. The 14- and 7-kd PrP species were observed in brain extracts and microsome preparations. Whether PrP^{sc} partitioning with membranes is due to protein-protein interaction, protein-lipid interaction, or the formation of sedimentable aggregates in the presence of detergents remains undetermined. PK-resistant fragments of 14 and 7 kd were also seen in purified PrP^{sc} fractions. In all patients (1 to 5), the sequencing of the 7-kd peptide in non-PK- and PK-digested samples, showed a major N-terminal cleavage site between G⁸⁷ to G⁸⁸ and W⁸⁹ to G⁹⁰, respectively.

It is noteworthy that PK digestion changed the stoichiometry of the various PrP peptides observed in the undigested preparations with an increase in the 14- and 7-kd fragments and disappearance of the high-molecular weight isoforms of ~27 to 35 kd. These findings indicate that these patients (1 to 5) accumulate larger PrP isoforms that can be cleaved to smaller, insoluble, PK-resistant fragments. The detection of low-molecular weight peptides in samples that have not been treated with PK, suggest that truncated PrP is generated *in vivo* by hydro-

lysis of pre-existing intermediates. These peptides may be the result of the metabolism of PrP in a pathway associated with amyloid formation, because similarly sized fragments have been found in isolated amyloid cores of patients with GSS.^{16–18}

These data differ from those showing the absence of PrP^{Sc} in patients with GSS A117V.²⁰ It has been proposed that a transmembrane PrP isoform, detected after limited PK digestion at low temperature in the absence of ionic detergents, plays a central role in the pathogenesis of GSS A117V.²⁰ We did not investigate the presence of different topological forms of PrP. Thus, we cannot determine whether the PrP^{Sc} present in the patients analyzed by us (1 to 5) corresponds to the transmembrane, extracellular, secreted, or membrane bound via a phosphatidylinositol anchor PrP species. It is important to note that the amount of PrP^{Sc} in the patients (1 to 5) reported here is significantly smaller than that found in other GSS variants.^{11,13} In agreement with the results presented here, recent studies in an Alsatian patient with GSS A117V¹⁸ showed that the major component of amyloid fibrils was a ~7-kd peptide with ragged N terminus starting mainly at G⁸⁸ and G⁹⁰.¹⁸

The 14- and 7-kd bands, seen in GSS A117V (1 to 5), are detected by antibodies directed to the mid-region of PrP indicating that these fragments include the intact epitope recognized by mAb 3F4 consisting of residues 109 to 112. It has been shown that in a normal human brain, PrP^C is endogeneously cleaved at residues H¹¹¹ or M¹¹².¹⁵ Thus, our results suggest that patients with GSS A117V have an alternative metabolic pathway, leading to the accumulation of N- and C-truncated PrP fragments with abnormal physicochemical properties. The data also suggests that the proteolytic pathway present in these patients with GSS is different from that previously described in CJD.^{10,15} In the latter, N-truncated, C-terminal-ly-intact PrP^{Sc} fragments accumulate in the brain.^{10,15}

To determine whether the ~14-kd fragment could correspond to a glycoform of the ~7-kd peptide, we performed enzymatic deglycosylation and observed no shift in electrophoretic mobility, suggesting that the larger fragment is either nonglycosylated or that sugars at residues N¹⁸¹ and N¹⁹⁷ are not accessible to enzymatic cleavage. Whether the ~14-kd band is an oligomer of the 7-kd peptide or a larger fragment with different N- and C-termini remains to be investigated.

Studies on amyloid fractions have previously shown that patients with GSS F198S accumulate amyloid peptides of ~11 kd spanning residues 58 to 150.³² Further analysis showed that the smallest amyloid subunit in GSS F198S and GSS Q217R corresponds to a 7-kd fragment comprising residues W⁸¹ to Y¹⁵⁰ and W⁸¹ to E¹⁴⁶, respectively.¹⁶ In addition, preliminary data on a patient of an American family with GSS A117V showed a similar fibrillogenic fragment with a ragged N terminus corresponding to W⁸¹, G⁸², and Q⁸³ and the C terminus at E¹⁴⁶.¹⁷ Thus, patients with these GSS variants may accumulate amyloid subunits of similar size and primary structure, despite the different genotypes and phenotypic presentations.

We hypothesized that the purification of PrP^{Sc} may allow the isolation of 1) PrP peptides that have not acquired fibrillogenic properties, 2) peptides that are not metabolized in an amyloidogenic pathway, or 3) peptides that are precluded from the extraction procedure used to isolate amyloid cores. Therefore, to expand our studies we purified PrP^{Sc} from patients of the Indiana kindred with GSS F198S and determined the N-terminal cleavage site of the ~8-kd fragment isolated from areas with and without amyloid accumulation. In all of the samples analyzed the major N-terminal cleavage site corresponded to residue G⁷⁴ of the octapeptide repeat region, suggesting that as yet unidentified local factors may contribute to PrP amyloidogenesis. In view of the fact that the smallest amyloidogenic fragment has a mobility of ~7 kd and N terminus at W⁸¹ and PrP^{Sc} peptides of ~8 kd have an N terminus at G⁷⁴, we speculate that sequential proteolytic cleavage of a precursor PrP^{Sc} fragment generates fibrillogenic peptides in GSS F198S.

In conclusion, the data obtained in the GSS variants analyzed in this study demonstrate that N- and C-truncated PrP isoforms of different size and N-termini, accumulate in GSS A117V and GSS F198S. Based on the principle that the pattern of digestion (ie, protease digestion in the presence of detergents) depends on the tertiary structure of proteins,³³ the data suggest that PrP conformational isomers are present in these patients.

The results show that octarepeats 3 and 4 are an integral part of the 8-kd peptide present in GSS F198S, but not in the 7-kd fragments detected in patients with GSS A117V. The importance of the accumulation of peptides with different N-termini in these GSS variants is unclear at this time. However, other investigators have suggested that Cu²⁺ binds to a structure defined by two of the octarepeats in PrP containing the sequence PHGGGWGQ.³⁴ They proposed that this binding could induce conformational changes in PrP.³⁴ In addition, short peptides corresponding to the octapeptide repeat motif of PrP have been reported to bind Cu²⁺.^{35,36} Moreover, it has been shown that PrP fragments can be transformed from a predominantly α -helical monomeric form to an oligomeric β -sheet-rich secondary structure.^{37,38} Therefore, PrP^{Sc} fragments with a distinct structure could have different neurotoxic properties or a tendency to form aggregates, providing a possible mechanism underlying the differences in phenotypic presentation among GSS variants.

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