

## Brief Report

## PRION PROTEIN CONFORMATION IN A PATIENT WITH SPORADIC FATAL INSOMNIA

JAMES A. MASTRIANNI, M.D., PH.D.,  
RANDAL NIXON, M.D., PH.D., ROBERT LAYZER, M.D.,  
GLENN C. TELLING, PH.D., DONG HAN, M.S.,  
STEPHEN J. DEARMOND, M.D., PH.D.,  
AND STANLEY B. PRUSINER, M.D.

**T**HE human prion diseases include Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker disease, fatal familial insomnia, and the recently described new variant of Creutzfeldt–Jakob disease. Much evidence argues that a post-translational, noncovalent modification of prion protein is the fundamental event in the mechanism underlying these diseases.<sup>1</sup> The normal cellular isoform of the prion protein (PrP<sup>C</sup>) is predominantly  $\alpha$ -helical, is detergent soluble, and is readily digested by proteases. In contrast, the pathogenic isoform (PrP<sup>Sc</sup>) has a substantially  $\beta$ -sheet structure, is insoluble in non-denaturing detergents, and shows relative resistance to proteolytic digestion.<sup>2–4</sup> The protease-resistant core of PrP<sup>Sc</sup>, designated PrP27–30, is usually detectable in humans and animals with prion disease. The relative molecular mass of the protease-resistant segment varies among prion strains and appears to be coupled with or associated with disease-specific phenotypes.<sup>5,6</sup>

Understanding of the prion strains has been advanced by investigations of the genotype–phenotype correlations in inherited forms of prion disease, such as fatal familial insomnia. Eleven kindreds with fatal familial insomnia have been identified.<sup>7–14</sup> Patients from these kindreds usually present with untreatable insomnia, followed by dysautonomia and ataxia, although some variation has been reported.<sup>15</sup> Cognitive function is relatively spared until late in the course of the disease, when diffuse slowing of activity on the electroencephalogram becomes apparent.<sup>10</sup> Neuronal loss and astrocytic gliosis within

the thalamus and olives, and to a lesser degree the cerebellum, are seen. The average age at the onset of the disease is 48 years (range, 25 to 61), and the duration of disease is about 18 months (range, 7 to 33).<sup>8,16,17</sup> PrP27–30 is detectable, though often at low levels, and is usually confined to the thalamus and temporal lobe.<sup>18</sup>

Fatal familial insomnia is caused by a mutation in the *PRNP* gene that results in the substitution of asparagine for aspartic acid at codon 178, in conjunction with the methionine at polymorphic codon 129. When the dominant D178N mutation is coupled with substitution of a valine at position 129, a dementing phenotype, characterized by diffuse spongiosis and widely distributed PrP<sup>Sc</sup>, results. This illness is known as familial Creutzfeldt–Jakob disease.<sup>19</sup> The relative molecular mass of deglycosylated PrP27–30 in familial Creutzfeldt–Jakob disease is approximately 21 kd, whereas that of PrP27–30 in fatal familial insomnia is approximately 19 kd.<sup>20</sup> This difference in size reflects the different conformations adopted by PrP<sup>Sc</sup> in familial Creutzfeldt–Jakob disease and fatal familial insomnia.

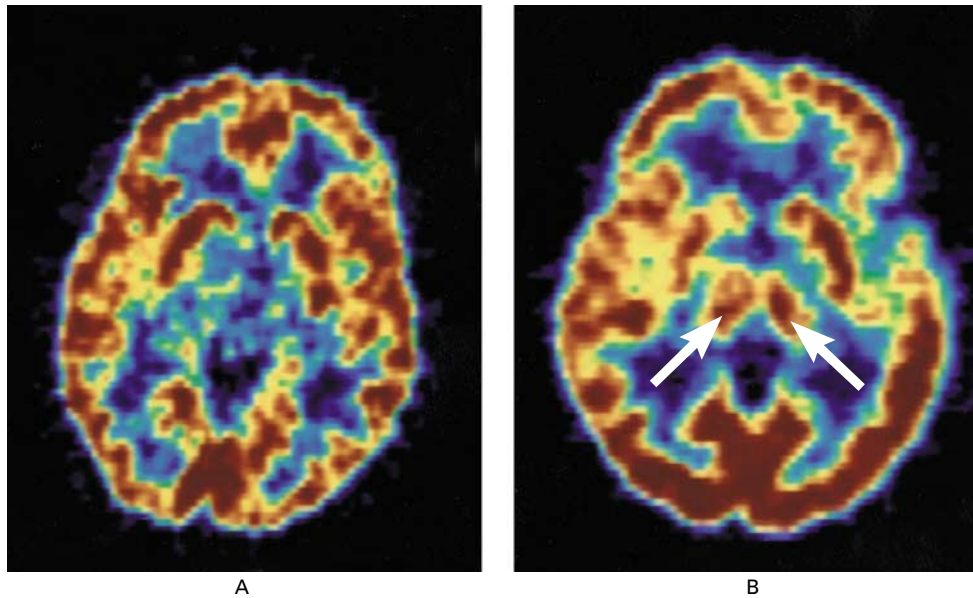
We studied a patient who presented with the typical features of fatal familial insomnia. Pathological examination, molecular genetic and PrP<sup>Sc</sup> conformation studies, and transmission studies to susceptible transgenic mice revealed that this patient carried a strain of prions similar to that in fatal familial insomnia but that he did not carry the *PRNP* mutation that causes it. These findings argue that his disorder was the sporadic equivalent of fatal familial insomnia, which we term sporadic fatal insomnia, and thus support the concept that the prion strain that specifies the clinicopathological phenotypes of the naturally occurring prion disease in humans and of the experimental disorder in mice is determined not by the sequence of the prion protein but rather by the conformation of PrP<sup>Sc</sup>.

### CASE REPORT

A previously healthy 44-year-old man had progressive insomnia. He sought medical advice after one week but was told it was “all in his head.” Heat intolerance soon developed. During the next four months, as his insomnia worsened, he slept an average of one hour per night. Six months after the onset of symptoms, excessive lacrimation developed and he began to have difficulty walking. A neurologic evaluation revealed severe appendicular ataxia. His tandem gait was especially poor, but he could still walk independently. Four months later, he was treated with large doses of hypnotic drugs, which were only briefly beneficial. Loss of the cough reflex, resulting in repeated aspiration, and progressive dysarthria developed. Cognitive function was only slightly impaired, according to results on the Mini–Mental State Examination (score, 28 of 30),<sup>21</sup> although psychomotor slowing was evident, suggesting that a subcortical disease was present. Over the next few months his short-term memory began to deteriorate, and his conversation became repetitious. One year after the onset of symptoms, he was wheelchair-bound because of severe ataxia, his speech was fluent but severely dysarthric, and he began to have difficulty separating dreams from reality. He was admitted to a long-term care facility. No similar neurologic disease was reported in the family.

From the Institute for Neurodegenerative Diseases, Department of Neurology (J.A.M., R.N., R.L., G.C.T., D.H., S.J.D., S.B.P.), and the Departments of Pathology (S.J.D.) and Biochemistry and Biophysics (S.B.P.), University of California, San Francisco; and the Department of Neurology, University of Chicago, Chicago (J.A.M.). Address reprint requests to Dr. Prusiner at the Department of Neurology, Box 0518, University of California, San Francisco, CA 94143-0518.

©1999, Massachusetts Medical Society.



**Figure 1.** [ $^{18}\text{F}$ ]Fluorodeoxyglucose Positron-Emission Tomogram of the Patient with Sporadic Fatal Insomnia (Panel A) and an Age-Matched Control Subject (Panel B).

Metabolic activity within the thalamus of the patient with sporadic fatal insomnia is almost completely absent (yellow and blue signals), as compared with the normal intense activity (red signal) in the control subject (arrows).

The following tests were performed: lumbar puncture; electrolyte analysis; hematologic analysis; urinalysis; liver-function and thyroid-function tests; tests for treponemal antibodies; coagulation studies; tests for anti-Hu, anti-Ri, and anti-Yo antibodies and human immunodeficiency virus antibody; analysis of sedimentation rate; heavy-metal and copper screening; ceruloplasmin tests; serum protein electrophoresis; and a complete serologic study to rule out inflammation. All the results were negative. A magnetic resonance image of the head was normal. Examination of a cerebellar-biopsy sample was unrevealing. On a positron-emission tomogram of the brain, the uptake of [ $^{18}\text{F}$ ]fluorodeoxyglucose within the thalamus was greatly reduced (Fig. 1), a characteristic feature of fatal familial insomnia.<sup>22</sup> Sixteen months after the onset of symptoms, myoclonic jerks developed, the patient became severely hallucinatory and delusional, and he died as a result of aspiration pneumonia. Postmortem studies were performed.

## METHODS

### Genetic Analysis

Genomic DNA was extracted from blood leukocytes by routine methods.<sup>23</sup> A denaturing gradient gel-electrophoresis system that has been previously described was used to screen for mutations in *PRNP*.<sup>24</sup> This system uses five sets of primers to circumvent the problem of single-allele amplification, which could result if a polymorphism in a noncoding region, although rare, was present.<sup>25</sup> In addition, the entire coding region of *PRNP* was sequenced by using the 7-deaza-2'-deoxyguanosine triphosphate method (Sequenase version 2.0, U.S. Biochemical, Cleveland), as described previously.<sup>24</sup>

### Neuropathological Analysis

The left hemisphere of the brain of the patient with sporadic fatal insomnia was removed and coronally sectioned. The right hemisphere was frozen on dry ice for subsequent studies. Formalin-fixed sections were prepared from several brain regions and

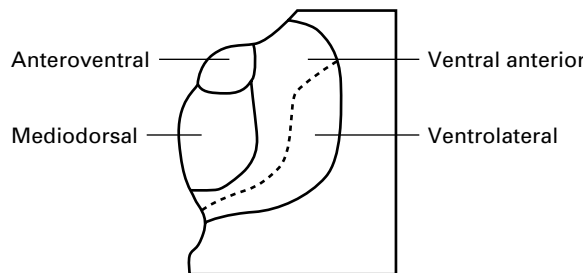
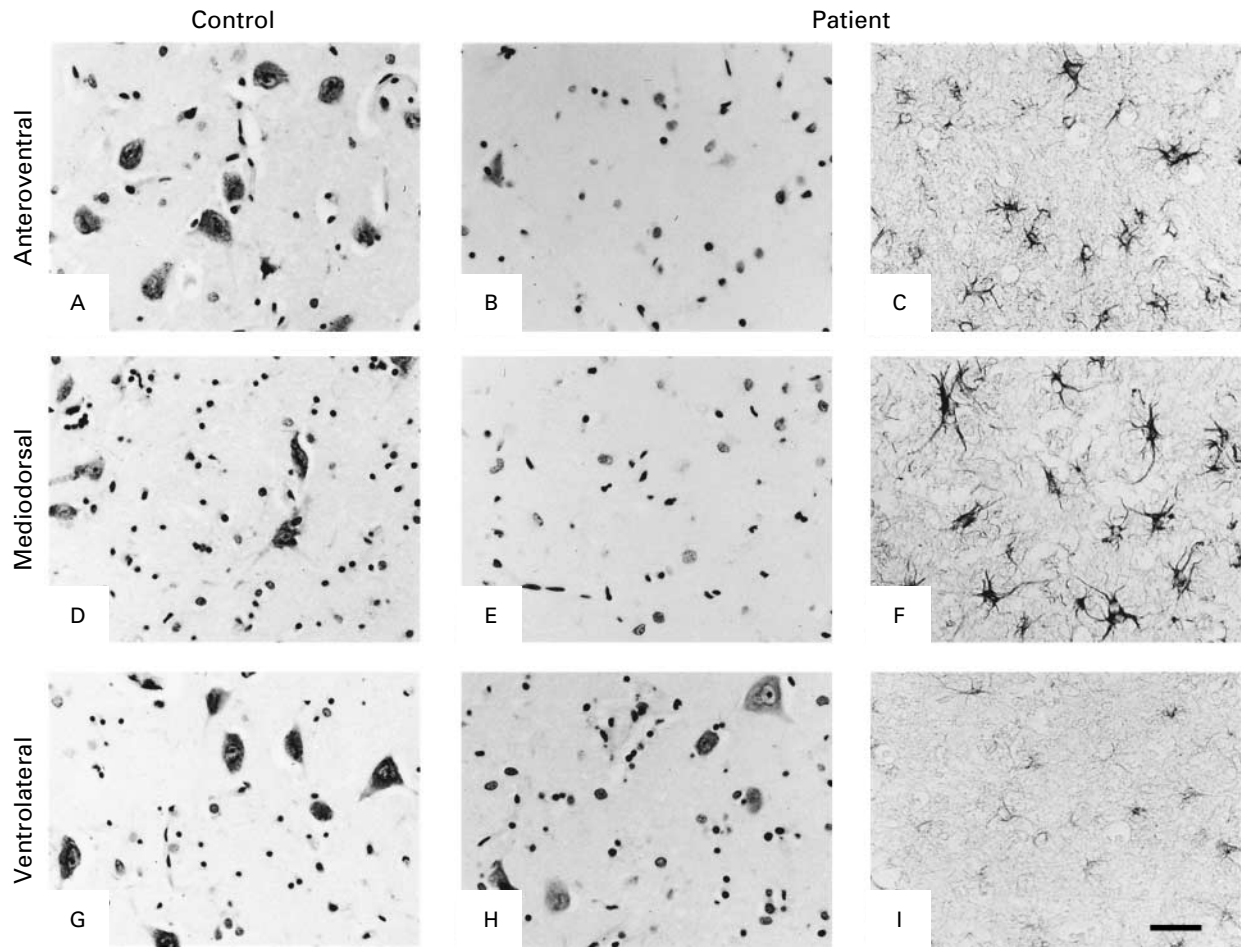
analyzed by staining with hematoxylin and eosin, Bielschowsky's method, Nissl's method, and Luxol fast blue periodic acid-Schiff histochemical stain. Immunoperoxidase staining for amyloid beta-protein and glial fibrillary acidic protein was performed with standard techniques.

### Protein Analysis

Western immunoblotting was performed as described previously,<sup>24</sup> with minor modifications. A 10 percent (wt/vol) homogenate of either human or mouse brain was prepared in 0.5 percent Nonidet P-40 and 0.5 percent sodium deoxycholate; the homogenate was digested with 20  $\mu\text{g}$  of proteinase K per milliliter for one hour at 37°C, and then the reaction was terminated by the addition of 2 mM phenylmethylsulfonyl fluoride. For deglycosylation of PrP<sup>Sc</sup>, sodium dodecyl sulfate (SDS) was added to the digested samples to a final concentration of 1 percent, and the protein was denatured by heating to 100°C for 10 minutes. An aliquot of this preparation was diluted to a final concentration of 0.5 percent Nonidet P-40 and 0.5 percent SDS; *N*-glycosidase F (Boehringer Mannheim, Mannheim, Germany) was added according to the manufacturer's instructions, and the preparation was then incubated overnight at 37°C. PrP<sup>Sc</sup> was recovered by centrifugation at 100,000 $\times g$  for one hour at 4°C. Samples were subjected to SDS-polyacrylamide-gel electrophoresis (SDS-PAGE) in a 16 percent polyacrylamide slab gel. Prion protein was detected with the use of the anti-prion-protein 3F4 monoclonal antibody diluted 1:5000<sup>26</sup> and an enhanced chemiluminescence system (Amersham, Buckinghamshire, United Kingdom).

### Immunoblotting and Histologic Examination of Transgenic Animals

Transgenic mice expressing chimeric mouse-human PrP were deficient for mouse PrP.<sup>27,28</sup> When inoculated intracerebrally with human prions, these mice generate chimeric mouse-human prions in their brains. Nontransgenic mice are generally poor hosts for human prions. Coronal sections of the brain from transgenic ani-



**Figure 2.** Sections of the Thalamus from the Patient with Sporadic Fatal Insomnia and an Age-Matched Control Subject.

There is extensive neuronal loss within the anteroventral and mediodorsal nuclei of the patient (Panels B and E, respectively), as compared with those of the control subject (Panels A and D, respectively). Involvement of the ventrolateral nucleus (Panel H) is reduced but definite. Staining of the same regions for glial fibrillary acidic protein revealed a complementary increase in the size and number of astrocytes in the anteroventral and mediodorsal nuclei, where the pathological changes were greater (Panels C and F, respectively) than in the ventrolateral nucleus (Panel I). Sections were stained by Nissl's method (the bar represents 25  $\mu$ m). The diagram indicates the relative boundaries of the sampled nuclei.

mals inoculated with brain extracts from the patient with sporadic fatal insomnia or from patients with fatal familial insomnia (final wt/vol, 1 percent) were prepared from several regions of the brain for immunoblotting, as previously described.<sup>29</sup> Blots were immunostained with anti-prion-protein monoclonal antibody 3F4<sup>26</sup> after limited digestion with proteinase K for detection of PrP<sup>Sc</sup>.

A semiquantitative method was used to estimate the degree and pattern of spongiform degeneration in the brains of the transgenic mice.<sup>30</sup> Two animals that had been inoculated with brain extract from the patient with sporadic fatal insomnia were examined; one had been inoculated with extract from the thalamus, and one with extract from the frontal cortex.

## RESULTS

## Neuropathological Findings

The pathological findings on examination of the patient's brain were characteristic of those described for fatal familial insomnia in every detail.<sup>10,17,18</sup> Severe loss of neuronal fibers was observed in the anteroventral and mediodorsal nuclei of the thalamus (Fig. 2C and 2F) and, to a much lesser extent, in the ventrolateral nuclei (Fig. 2H and 2I). Only slight vacuolation of the mediodorsal nuclear gray matter was observed on staining with hematoxylin and eosin, and no spongiosis typical of Creutzfeldt–Jakob disease was observed. Profound loss of neurons, proliferation of microglia, and astrocytic gliosis were also evident within the olives (data not shown). Slight vacuolation was present in the white matter of the temporal and occipital lobes, cingulate gyrus, basal ganglia, hippocampus, and midbrain, but vacuolation was notably absent from the white matter of the cerebellum. These changes were suggestive of neuronal-fiber loss rather than typical Creutzfeldt–Jakob disease. Astrocytic gliosis was associated with white-matter vacuolation in the cingulate gyrus, basal ganglia, and hippocampus. There was thinning of the molecular layer and moderate, focal loss of nerve cells from the granular-cell layer of the cerebellum. Axonal degeneration of Purkinje cells was moderate, as evidenced by torpedo formation visualized with silver staining by Bielschowsky's method.

## Genotyping

Denaturing gradient gel electrophoresis was initially used to screen the entire open reading frame of the *PRNP* gene, which revealed a wild-type sequence and coding for methionine at polymorphic codon 129. DNA sequencing of the complete open reading frame of *PRNP* confirmed the presence of a normal GAC trinucleotide, which encodes aspartic acid, at codon 178, and an ATG trinucleotide, which encodes methionine, at codon 129. DNA was also extracted from the thalamus in an attempt to identify a site-specific mutation of *PRNP* that would account for the focal pathological changes; the sequence of the thalamic DNA was also wild type.

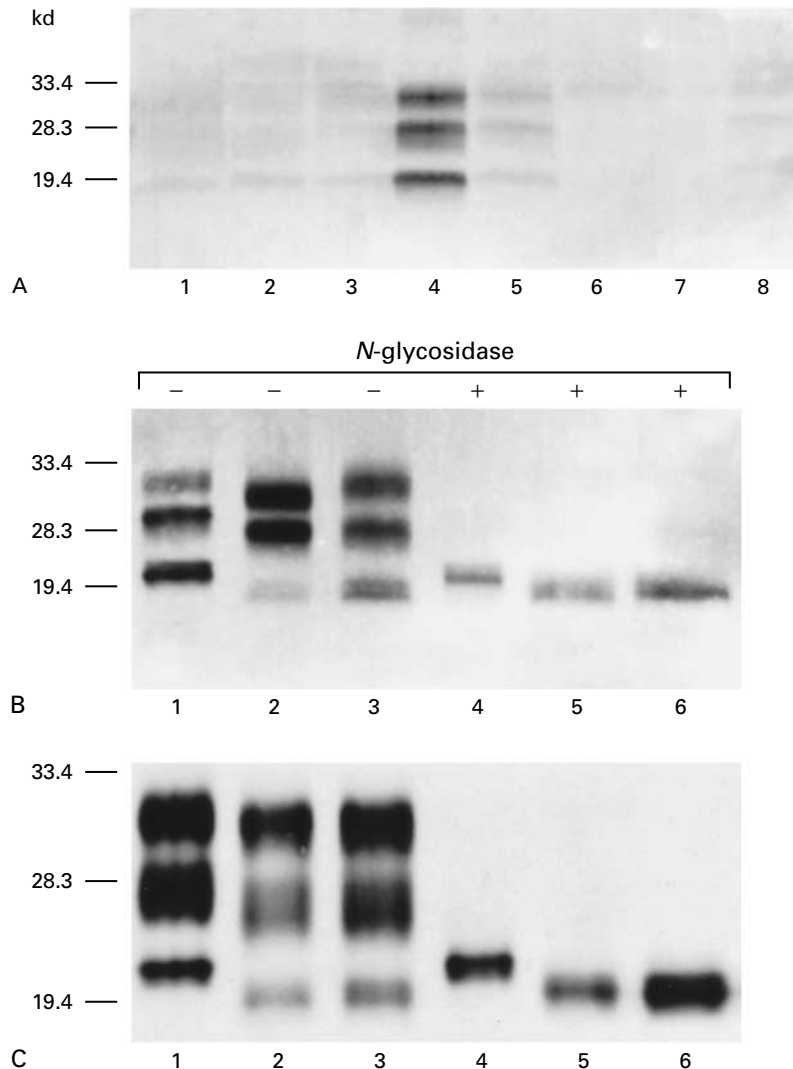
PrP<sup>Sc</sup> Distribution and Conformation

Western immunoblot analysis was performed on tissues obtained from several regions of the brain (Fig. 3). Although PrP<sup>Sc</sup> was not detected in an initial examination of the frontal cortex, it was subsequently detected focally within the thalamus. A five-fold concentration of samples allowed the detection of low levels of PrP<sup>Sc</sup> in extrathalamic regions (Fig. 3A). The highest extrathalamic levels of PrP<sup>Sc</sup> were detected within the subthalamic region, suggesting that the abnormal protein had accumulated focally; however, lower levels were detected within forebrain

structures and the lower medulla, in the region of the olives. The extremely low levels of PrP<sup>Sc</sup> outside the thalamic region suggest that axonal spread in the anterograde direction may account for its distribution. In general, the level of PrP<sup>Sc</sup> in this patient, even in the thalamus, was lower than that in an equivalent sample from a patient with sporadic Creutzfeldt–Jakob disease. Similarly, the levels of PrP<sup>Sc</sup> in patients with fatal familial insomnia have been reported to be lower than those typically found in patients with sporadic Creutzfeldt–Jakob disease.<sup>18</sup>

The glycosylation pattern of PrP<sup>Sc</sup> from the patient with sporadic fatal insomnia differed from that of PrP<sup>Sc</sup> from patients with fatal familial insomnia, a finding that provides further evidence that PrP<sup>Sc</sup> from this patient had the wild-type sequence. The D178N mutation, because of its proximity to the asparagine-linked glycosylation site on residue 181, is thought to account for the predominance of the two glycosylated forms of PrP27–30 in fatal familial insomnia<sup>18</sup> (Fig. 3B) and not in sporadic fatal insomnia. Instead, as is typical with sporadic Creutzfeldt–Jakob disease, equal proportions of glycosylated and unglycosylated forms were observed.

On SDS-PAGE, the molecular mass of the deglycosylated form of PrP27–30 in patients with sporadic Creutzfeldt–Jakob disease with homozygosity for methionine at residue 129 is typically 21 kd, whereas in those with fatal familial insomnia its molecular mass is about 19 kd.<sup>18,20</sup> A 19-kd fragment is also common in patients with sporadic Creutzfeldt–Jakob disease who are heterozygous (methionine and valine) or homozygous for valine at residue 129, but it is rare in patients homozygous for methionine at that residue.<sup>31</sup> We therefore compared the molecular mass of fractions of deglycosylated PrP27–30 isolated from patients with sporadic Creutzfeldt–Jakob disease who were homozygous for methionine at position 129, a patient with fatal familial insomnia with D178N and methionine at position 129, and the patient with sporadic fatal insomnia who was homozygous for methionine at position 129; we found that the latter two fractions comigrated at approximately 19 kd, whereas that of the former migrated at approximately 21 kd (Fig. 3B). These results suggest that PrP<sup>Sc</sup> from sporadic fatal insomnia and PrP<sup>Sc</sup> from familial fatal insomnia possess similar protease-susceptible cleavage sites, which result in the generation of the 19-kd fragment in each. Since the pattern of digestion depends on the tertiary structure of PrP<sup>Sc</sup>, these observations argue that the conformations of wild-type PrP<sup>Sc</sup> in sporadic fatal insomnia and of mutant PrP<sup>Sc</sup> in fatal familial insomnia are similar. Because the wild-type and mutant PrP<sup>Sc</sup> molecules differ in sequence, these findings indicate that the tertiary structure of PrP<sup>Sc</sup> dictates both the clinical and the pathological phenotypes of prion disease.



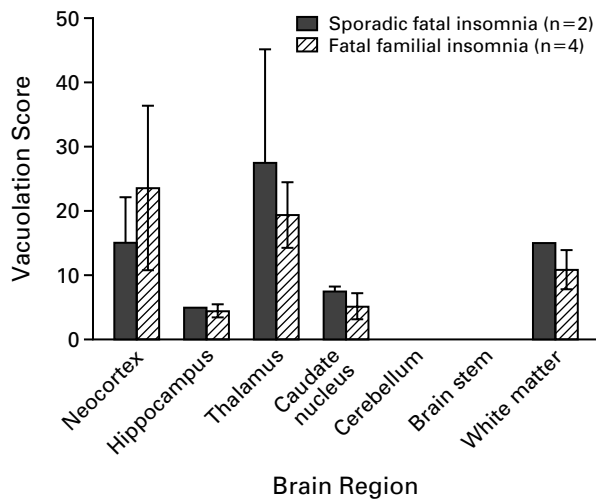
**Figure 3.** Western Immunoblot Analyses of PrP<sup>Sc</sup> from the Brains of Patients and Transgenic Mice.

Panel A shows homogenates prepared from several regions of the brain of the patient with sporadic fatal insomnia: lane 1 shows the frontal cortex, lane 2 the temporal lobe, lane 3 the parietal lobe, lane 4 the thalamus, lane 5 the subthalamus, lane 6 the occipital lobe, lane 7 the cerebellum, and lane 8 the lower medulla. Panel B shows samples of PrP<sup>Sc</sup> from patients with sporadic Creutzfeldt-Jakob disease and homozygosity for methionine at position 129 (lanes 1 and 4), fatal familial insomnia (lanes 2 and 5), and sporadic fatal insomnia (lanes 3 and 6). Lanes 1, 2, and 3 show protease-digested samples with sugar residues intact; lanes 4, 5, and 6 show the same samples after deglycosylation with *N*-glycosidase. Panel C shows PrP<sup>Sc</sup> prepared from the brains of transgenic mice inoculated with brain extracts from the patients in Panel B. Lanes 1, 2, and 3 show protease-digested samples with sugar residues intact; lanes 4, 5, and 6 show the same samples after deglycosylation. Before and after transmission to transgenic mice, the molecular mass of the protease-resistant deglycosylated PrP<sup>Sc</sup> is approximately 19 kd in fatal familial insomnia and fatal familial insomnia-inoculated mice, and 21 kd in sporadic Creutzfeldt-Jakob disease and sporadic Creutzfeldt-Jakob disease-inoculated mice.

### Transmission Studies

Transgenic mice that expressed chimeric mouse-human PrP and that were deficient for mouse PrP were inoculated with extracts from the cortex or the thalamus of the patient with sporadic fatal insomnia. A few mice died before clear signs of central nervous

system dysfunction were identified. The four mice that were inoculated with extracts from the thalamus exhibited signs of neurologic impairment a mean ( $\pm$ SD) of  $180 \pm 2$  days later; the eight inoculated with extracts from the cortex had central nervous system dysfunction after  $221 \pm 6$  days. Brains from



**Figure 4.** Vacuolation Histograms of the Brains of Transgenic Mice after Inoculation with Extracts from Patients with Fatal Familial Insomnia or Sporadic Fatal Insomnia.

The vacuolation score is a semiquantitative estimate of the area of a brain region occupied by spongiform degeneration. It was assessed by a single rater. Values shown are means  $\pm$  SD. Numbers in parentheses are the numbers of mice inoculated.

two of the mice that had been inoculated with extracts from the patient with sporadic fatal insomnia (one received cortex and the other received thalamus) and four that had been inoculated with extracts from patients with fatal familial insomnia were examined histologically in a blinded fashion.

Vacuolation and reactive astrocytic gliosis were present in virtually identical distributions (Fig. 4). Vacuolation was most intense in the thalamus and in the inner half of the neocortex, where thalamocortical afferent fibers terminate. Vacuolation also was present, to a lesser degree, in the outer half of the neocortex and in the caudate nucleus. There was no vacuolation in the cerebellar cortex or brain-stem nuclei. Intense reactive astrocytic gliosis was present in the same locations as vacuolar degeneration. No amyloid plaques were found. PrP<sup>Sc</sup> deposition in the brains of these animals was predominantly in the thalamus and inner half of the neocortex; the brain stem and cerebellum were spared (Fig. 5). The hypothalamus was also relatively unaffected. The corpus callosum and anterior commissure showed a marked deposition of PrP<sup>Sc</sup>.

This pattern of PrP<sup>Sc</sup> deposition was indistinguishable from that observed in the transgenic mice inoculated with prions from a patient with fatal familial insomnia (Fig. 5). These similar patterns of PrP<sup>Sc</sup> accumulation are clearly different from those observed after the transmission of prions from previously described patients with sporadic Creutzfeldt–Jakob disease who were homozygous for methionine at posi-

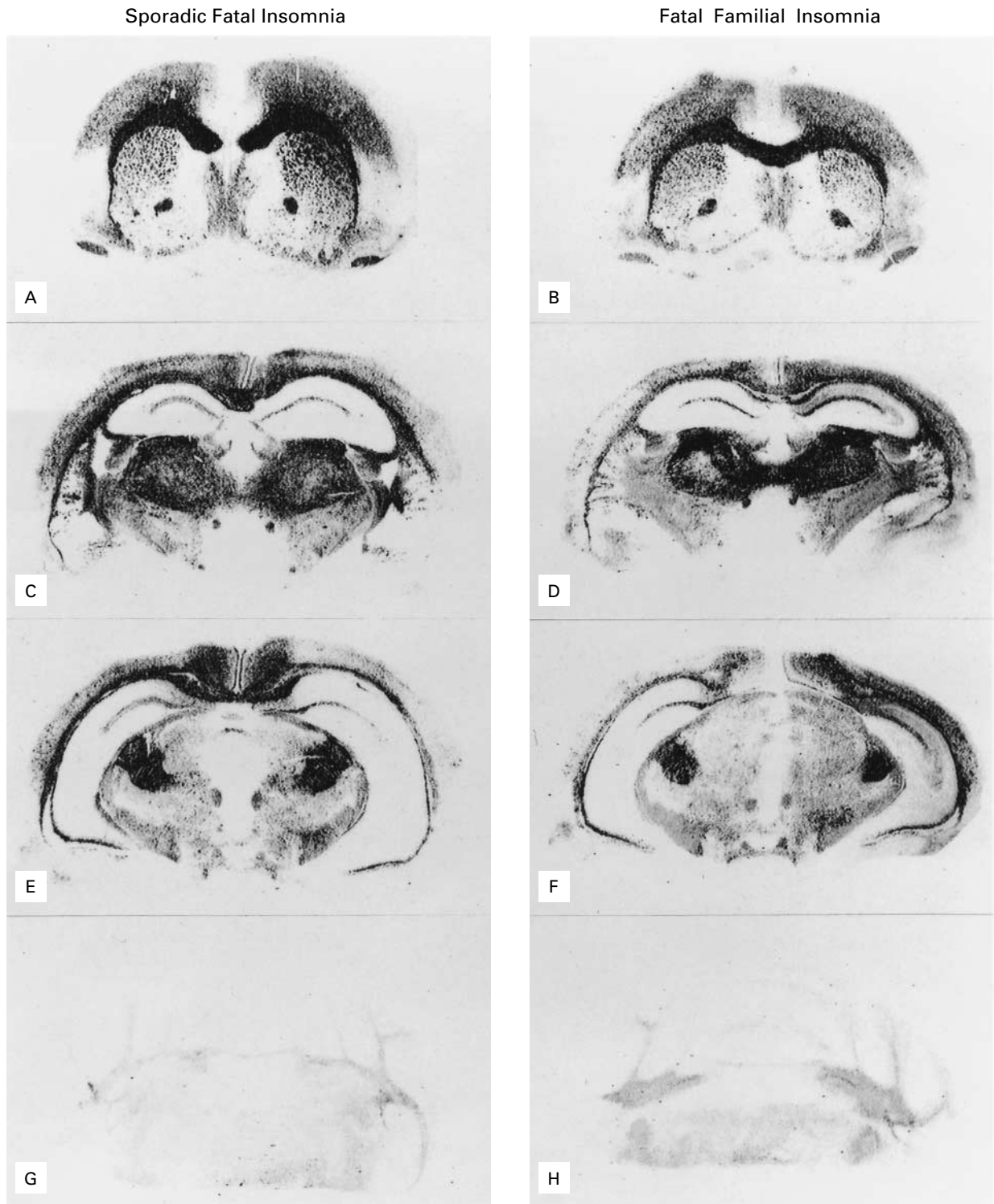
tion 129,<sup>6</sup> patients with familial Creutzfeldt–Jakob disease with an E200K mutation,<sup>6</sup> and patients with familial Creutzfeldt–Jakob disease with valine at position 210I (unpublished data). These results argue that the prion strains associated with sporadic fatal and fatal familial insomnia are indistinguishable even though the sequences of the PrP<sup>Sc</sup> molecules differ at residue 178.

Analysis of the molecular mass of PrP<sup>Sc</sup> from the transgenic mice that had been inoculated with brain extracts from patients with sporadic fatal insomnia or fatal familial insomnia showed that similar conformations were transferred to nascent PrP<sup>Sc</sup>. From the brains of mice inoculated with the human brain extracts, 19-kd fragments of deglycosylated PrP27–30 (Fig. 3C) were found by SDS-PAGE; in contrast, the 21-kd fragments were observed in fractions prepared from mice inoculated with brain extract from a patient with sporadic Creutzfeldt–Jakob disease (Fig. 3B). Thus, the conformation of chimeric mouse–human PrP<sup>Sc</sup> derived from the transgenic mice reflected that of human PrP<sup>Sc</sup> in the brain extracts used as inoculum.

## DISCUSSION

Our results provide evidence that both the clinical and the pathological phenotypes of prion disease are determined by the conformation of PrP<sup>Sc</sup>. The patient with sporadic fatal insomnia had the distinctive clinical and neuropathological features of fatal familial insomnia, yet both of his *PRNP* alleles were wild type. Not only are the clinical manifestations of fatal familial insomnia and sporadic fatal insomnia similar, but PrP<sup>Sc</sup> localizes almost exclusively to the thalamus in both disorders. Furthermore, the molecular mass of PrP<sup>Sc</sup> from the patient with sporadic fatal insomnia was about 19 kd, after limited protease digestion, the same size as that in fatal familial insomnia.<sup>18,20,31,32</sup>

When extracts prepared from the brain of the patient with sporadic fatal insomnia were inoculated into transgenic mice, signs of prion disease developed in the animals. Pathological examination of their brains showed deposition of chimeric mouse–human PrP<sup>Sc</sup> that was confined largely to the thalamus, as was deposition in the transgenic *Prnp*<sup>0/0</sup> mice that were inoculated with extracts of brain affected with fatal familial insomnia.<sup>6</sup> A 19-kd protease-resistant, deglycosylated fragment of chimeric PrP<sup>Sc</sup> was detected in transgenic mice inoculated with brain extracts from patients with either sporadic or familial insomnia, whereas a 21-kd fragment was observed in transgenic mice inoculated with brain extract from a patient with sporadic Creutzfeldt–Jakob disease who was homozygous for methionine at position 129. These studies demonstrate that the strain of human prions associated with sporadic fatal insomnia and containing wild-type PrP<sup>Sc</sup> is indistinguishable from the strain associated with fatal familial insomnia and containing



**Figure 5.** Regional Distribution of PrP<sup>Sc</sup> in the Brains of Transgenic Mice Inoculated with Brain Extracts from Patients with Sporadic Fatal Insomnia (Panels A, C, E, and G) or Fatal Familial Insomnia (Panels B, D, F, and H).

Four regions of the brain are shown: septal regions (Panels A and B), hippocampal and anterior thalamic nuclei (Panels C and D), posterior thalamic nuclei (Panels E and F), and brain stem and cerebellum (Panels G and H). Dark areas represent deposition of PrP<sup>Sc</sup> after PrP<sup>C</sup> had been eliminated by exposure of the membranes to proteinase K.

mutant PrP<sup>Sc</sup>; therefore, sporadic fatal insomnia is a phenocopy and not a genocopy of fatal familial insomnia. Moreover, the same strain of prions can be propagated in a human host who expresses either mutant prion protein (D178N) and methionine at codon 129 or wild-type prion protein and methionine (by homozygosity) at position 129 or in a murine host that expresses chimeric mouse-human prion protein. Since both the disease phenotype and the conformation of PrP<sup>Sc</sup> remained similar even when host sequences of prion protein varied, these findings suggest that the biologic properties of prion strains result from the conformation of PrP<sup>Sc</sup>.

Sporadic fatal insomnia in our patient might have been caused by a somatic D178N mutation in the *PRNP* gene that generated mutant PrP<sup>Sc</sup>, which in turn functioned as a conformational template in the conversion of wild-type PrP<sup>C</sup> into wild-type PrP<sup>Sc</sup>. Alternatively, a sufficient number of wild-type PrP<sup>C</sup> molecules may have been spontaneously transformed into PrP<sup>Sc</sup> molecules with the prion conformation that characterizes sporadic fatal insomnia,<sup>33</sup> resulting in sustained propagation. Whether wild-type PrP<sup>Sc</sup> initially forms in the thalamus or in another region of the central nervous system, or even in another organ, presumably it folds into wild-type PrP<sup>Sc</sup> with the 19-kd conformation that is adopted by the mutant PrP<sup>Sc</sup> that causes fatal familial insomnia. It remains to be determined whether the glycosylation of PrP<sup>C</sup> in the thalamus<sup>34</sup> or a region-specific chaperone, such as protein X,<sup>28,35</sup> is involved in the folding of PrP<sup>Sc</sup> into the 19-kd conformation. The intense deposition of PrP<sup>Sc</sup> observed in the thalamus of experimental mice after transmission of both fatal familial and sporadic fatal insomnia argues for a thalamus-specific targeting mechanism. It seems likely, on the basis of our experience with this patient with sporadic fatal insomnia, that most or all nonfamilial cases of pure thalamic dementia consist of sporadic fatal insomnia, whereas the familial form consists of fatal familial insomnia.<sup>7,9,20,36</sup>

In summary, sporadic fatal insomnia represents the natural occurrence of fatal insomnia in the absence of a mutation in *PRNP* and provides evidence that the conformation of PrP<sup>Sc</sup> is an important determinant of disease phenotype. Whereas conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> involves an increase in  $\beta$ -sheet content, more subtle but clinically important conformational changes appear to underlie the generation of prion strains. Development of sensitive assays to identify these subtle conformational differences<sup>37</sup> will be important in defining the specific phenotypic determinants of PrP<sup>Sc</sup>.

Supported by grants (NS14069, AG08967, AG02132, NS22786, and AG10770) from the National Institutes of Health and by a gift from the Leila Y. and G. Harold Mathers Foundation. Dr. Mastrianni is the recipient of a Clinical Investigator Award (NS01913) from the National Institutes of Health.

*We are indebted to Dr. Pierluigi Gambetti (Case Western Reserve University) for the gracious donation of a brain sample from a patient with fatal familial insomnia; to Dr. William Jagust (University of California, Davis) for assistance in performing and interpreting the positron-emission tomographic study; to Juliana Cayetano for the preparation of histopathological specimens; and to Thomas Lisse for histologic blot preparations.*

## REFERENCES

1. Prusiner SB. Prions. *Proc Natl Acad Sci U S A* 1998;95:13363-83.
2. Oesch B, Westaway D, Wälchli M, et al. A cellular gene encodes scrapie PrP 27-30 protein. *Cell* 1985;40:735-46.
3. Meyer RK, McKinley MP, Bowman KA, Braunfeld MB, Barry RA, Prusiner SB. Separation and properties of cellular and scrapie prion proteins. *Proc Natl Acad Sci U S A* 1986;83:2310-4.
4. Pan K-M, Baldwin M, Nguyen J, et al. Conversion of  $\alpha$ -helices into  $\beta$ -sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci U S A* 1993;90:10962-6.
5. Bessen RA, Marsh RF. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. *J Virol* 1994;68:7859-68.
6. Telling GC, Parchi P, DeArmond SJ, et al. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. *Science* 1996;274:2079-82.
7. Medori R, Tritschler H-J, LeBlanc A, et al. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N Engl J Med* 1992;326:444-9.
8. Medori R, Montagna P, Tritschler HJ, et al. Fatal familial insomnia: a second kindred with mutation of prion protein gene at codon 178. *Neurology* 1992;42:669-70.
9. Peterson RB, Tabaton M, Berg L, et al. Analysis of the prion protein gene in thalamic dementia. *Neurology* 1992;42:1859-63.
10. Gambetti P, Parchi P, Petersen RB, Chen SG, Lugaresi E. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: clinical, pathological and molecular features. *Brain Pathol* 1995;5:43-51.
11. Bosque PJ, Vnencak-Jones CL, Johnson MD, Whitlock JA, McLean MJ. A PrP gene codon 178 base substitution and a 24-bp interstitial deletion in familial Creutzfeldt-Jakob disease. *Neurology* 1992;42:1864-70.
12. Reder AT, Mednick AS, Brown P, et al. Clinical and genetic studies of fatal familial insomnia. *Neurology* 1995;45:1068-75.
13. Silburn P, Cervenakova L, Varghese P, Tannenberga A, Brown P, Boyle R. Fatal familial insomnia: a seventh family. *Neurology* 1996;47:1326-8.
14. Nagayama M, Shinohara Y, Furukawa H, Kitamoto T. Fatal familial insomnia with a mutation at codon 178 of the prion protein gene: first report from Japan. *Neurology* 1996;47:1313-6.
15. McLean CA, Storey E, Gardner RJM, Tannenberga MB, Cervenaková L, Brown P. The D178N (cis-129M) "fatal familial insomnia" mutation associated with diverse clinicopathologic phenotypes in an Australian kindred. *Neurology* 1997;49:552-8.
16. Gambetti P. Fatal familial insomnia: a new human prion disease. Presented at the Prion Diseases in Animals and Humans Symposium, London, September 2-4, 1991.
17. Manetto V, Medori R, Cortelli P, et al. Fatal familial insomnia: clinical and pathologic study of five new cases. *Neurology* 1992;42:312-9.
18. Parchi P, Castellani R, Cortelli P, et al. Regional distribution of protease-resistant prion protein in fatal familial insomnia. *Ann Neurol* 1995;38:21-9.
19. Goldfarb LG, Petersen RB, Tabaton M, et al. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: disease phenotype determined by a DNA polymorphism. *Science* 1992;258:806-8.
20. Monari L, Chen SG, Brown P, et al. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: different prion proteins determined by a DNA polymorphism. *Proc Natl Acad Sci U S A* 1994;91:2839-42.
21. Folstein MF, Folstein SE, McHugh PR. "Mini-Mental State": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975;12:189-98.
22. Perani D, Cortelli P, Lucignani G, et al. [<sup>18</sup>F]FDG PET in fatal familial insomnia: the functional effects of thalamic lesions. *Neurology* 1993;43:2565-9.
23. Sambrook J, Fritsch EF, Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. Plainview, N.Y.: Cold Spring Harbor Laboratory Press, 1989.
24. Mastrianni JA, Iannicola C, Myers R, Prusiner SB. Identification of a new mutation of the prion protein gene at codon 208 in a patient with Creutzfeldt-Jakob disease. *Neurology* 1995;45:Suppl 4:201. abstract.
25. Palmer MS, Collinge J. Mutations and polymorphisms in the prion protein gene. *Hum Mutat* 1993;2:168-73.

26. Kascsak RJ, Rubenstein R, Merz PA, et al. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. *J Virol* 1987;61:3688-93.
27. Telling GC, Scott M, Hsiao KK, et al. Transmission of Creutzfeldt-Jakob disease from humans to transgenic mice expressing chimeric human-mouse prion protein. *Proc Natl Acad Sci U S A* 1994;91:9936-40.
28. Telling GC, Scott M, Mastrianni J, et al. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* 1995;83:79-90.
29. Taraboulos A, Jendroska K, Serban D, Yang S-L, DeArmond SJ, Prusiner SB. Regional mapping of prion proteins in brains. *Proc Natl Acad Sci U S A* 1992;89:7620-4.
30. Carlson GA, Ebeling C, Yang S-L, et al. Prion isolate specified allotypic interactions between the cellular and scrapie prion proteins in congenic and transgenic mice. *Proc Natl Acad Sci U S A* 1994;91:5690-4.
31. Parchi P, Castellani R, Capellari S, et al. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 1996;39:767-78.
32. Parchi P, Capellari S, Chen SG, et al. Typing prion isoforms. *Nature* 1997;386:232-4.
33. Cohen FE, Prusiner SB. Pathologic conformations of prion proteins. *Annu Rev Biochem* 1998;67:793-819.
34. DeArmond SJ, Sánchez H, Yehiely F, et al. Selective neuronal targeting in prion disease. *Neuron* 1997;19:1337-48.
35. Kaneko K, Zulianello L, Scott M, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. *Proc Natl Acad Sci U S A* 1997;94:10069-74.
36. Petersen RB, Goldfarb L, Tabaton M, et al. Fatal familial insomnia and one subtype of familial Creutzfeldt-Jakob disease: effect of a polymorphism on a pathogenic mutation in the prion protein. *FASEB J* 1993;7:A627. abstract.
37. Safar J, Wille H, Itri V, et al. Eight prion strains have PrP<sup>Sc</sup> molecules with different conformations. *Nat Med* 1998;4:1157-65.