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Commentary

Inherited prion diseases

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Almost 70 years ago, the first report of patients dying of a dementing illness in families (1, 2) resembling those described a few years earlier by Jakob was published (3). For decades, this disease, often referred to as Jakob–Creutzfeldt disease, was considered a central nervous system (CNS) degenerative disease of unknown etiology, where most cases were sporadic and a few were of familial origin (4). The transmission of Jakob–Creutzfeldt disease to apes and later monkeys in 1968 transformed thinking about the etiology of this disorder (5) and was accompanied by an inversion of the eponyms to Creutzfeldt–Jakob disease (CJD) (6). The prolonged incubation periods in experimental primates and its presumed relationship to kuru of New Guinea cannibals and scrapie of sheep led to its classification as a slow virus disease (7). With the recognition that ≈10% of the CJD cases are familial (8, 9), the study of prion diseases grew more perplexing. Over the past 15 years, studies on the transmissible pathogen causing CJD of humans and the animal disease scrapie have established that CJD and scrapie are not caused by viruses but are due to prions (10).

The discovery of the prion protein (PrP) in fractions enriched for scrapie infectivity provided the first molecular probe for investigating CJD (11). Like scrapie of animals, patients dying of CJD accumulate a modified form of PrP often designated PrP^{Sc} or PrP^{CJD} in their brains. Molecular cloning of the PrP gene and genetic linkage between scrapie incubation times and codon variations in mice (12, 13) raised the possibility that mutation might feature in the hereditary human prion diseases.

Since the first mutations of the PrP gene were reported 5 years ago in humans dying of familial CJD and Gerstmann–Sträussler–Scheinker disease (GSS) (14, 15), 18 different mutations have been identified. Five of the 18 have been linked genetically to the inherited prion diseases which include familial CJD and GSS (15–19); the cumulative lod (logarithm of odds) score exceeds 30.

The most recent addition to the human prion disease group is fatal familial insomnia (FFI). This disease presents with insomnia, dysautonomia, ataxia, dysarthria, dysphagia, myoclonus, and signs of pyramidal tract dysfunction. In the later stages of the illness, patients exhibit total insomnia, dementia with frontal lobe release signs, rigidity and dystonia, and mutism.

Prior to death patients become stuporous and ultimately comatose (20–23). The progressive insomnia of FFI is characterized by a reduction of the total sleep time (TST) affecting both the non-rapid-eye-movement and rapid-eye-movement (REM) phases of sleep. In general, the diminished TST is unresponsive to medications including benzodiazepines and barbituates. Selective involvement of the thalamus was documented in living patients by positron emission tomography (PET), which showed profound hypometabolism using [^{18}F]2-fluoro-2-deoxyglucose (24). The neuropathological lesions in FFI consist of neuronal dropout and astrocytic gliosis, which preferentially affect the ventral anterior and medial dorsal nuclei of the thalamus (21, 25, 26). Although the cerebral cortex is generally spared, occasionally it exhibits focal spongiform degeneration. Once immunostaining for protease-resistant PrP was found on Western immunoblots, the open reading frame of the PrP gene was sequenced, demonstrating a mutation at codon 178 (17, 21, 27).

The codon 178 mutation was puzzling because it had been previously described in patients with familial CJD who presented with dementia rather than insomnia (28–30). Some investigators thought that another nearby gene segregating with the PrP gene in the Italian families with FFI might be governing the clinical presentation and neuropathology of this inherited prion disease, particularly since these families might have a common ancestor giving rise to a founder effect. To explore the cause of the two distinct clinical presentations in patients carrying the codon 178 mutation, the codon 129 polymorphism was examined because earlier studies showed that homozygosity at this position lowers the age of onset of familial CJD (31) and predisposes to the sporadic form of the illness (32). At position 129, either methionine or valine is commonly found, with methionine being the most common amino acid. In FFI, a methionine residue was always found at 129 on the mutant allele encoding asparagine in place of aspartic acid at 178; in striking contrast, a valine residue at 129 was observed on the mutant allele of patients with familial CJD with the 178 mutation (33). Furthermore, patients homozygous for the 129 polymorphism tended to have an early age of onset. Familial CJD with the Asp-178 → Asn (D178N) mutation and valine at codon 129 is designated CJD(D178N,V129).

Prior to the discovery of the D178N mutation in patients with FFI and the classification of this illness as an inherited prion disease, FFI was classified as a thalamic dementia. That the codon 178 mutation is the cause of FFI is supported by a lod score that exceeds 5 (17). Whether all patients with the D178N mutation and methionine at 129 will manifest their disease as FFI both clinically and neuropathologically remains to be established. Of note is a Tennessee family with the D178N mutation, a methionine residue at 129, and deletion of a single eight-amino acid residue repeat ("octarepeat") (34). While the proband did complain of insomnia initially, it was a minor symptom compared to his dysarthria and ataxia, but no sleep study was performed. By history, dementia was the most prominent feature of the illness in other members of this family, while insomnia or dysautonomia were not mentioned. The histopathological changes seem in this family to be similar to those described in subjects with FFI of a duration of 2 years or more (25). Specifically, among the major thalamic nuclei, the medial dorsal was preferentially affected with neuronal loss and gliosis but not spongiosis. Severe spongiform degeneration was present only in the parahippocampal gyrus, while it was moderate or minimal in the neocortex (C. Vnencak-Jones, M. Johnson, and P. Gambetti, personal communication). It is of interest that the first family with CJD to be described (1, 2, 35) carries the D178N mutation and valine at codon 129 (H. A. Kretzschmar, M. Neumann, and D. Stavrou, personal communication).

To explore why the neuropathology of FFI is restricted to thalamic nuclei, studies of the scrapie isoform of the prion protein designated PrP^{Sc} were performed (36). PrP^{Sc} is distinguished from the cellular isoform (PrP^{C}) by its β-sheet conformation, insolubility, posttranslational formation, and protease resistance (10, 37). Earlier studies with experimental scrapie in

rodents had shown that those regions of the brain exhibiting neuropathological changes contain PrP^{Sc} and that PrP^{Sc} deposition precedes spongiform degeneration (38, 39). Thus, it was reasonable to ask whether PrP^{Sc} in FFI might differ from that in CJD(D178N,V129).

In a recent issue of these *Proceedings*, Monari *et al.* (36) show that PrP^{Sc} after limited proteolysis from FFI cases migrates more rapidly than PrP^{Sc} from CJD(D178N,V129) and CJD(E200K) (CJD with a Glu-200 → Lys mutation) cases. It seems likely that more extensive proteolysis of the N terminus of PrP^{Sc} occurs in FFI based on immunoreactivity with monospecific polyclonal antisera raised against synthetic peptides corresponding to particular segments of PrP^{Sc}. Presumably, this difference in the susceptibility of the N terminus of PrP^{Sc} to limited proteolysis reflects differences in the conformations of the two mutant PrP molecules, one of which contains a methionine at codon 129 and the other a valine. Monari *et al.* suggest that the difference in PrP sequence at codon 129 is responsible for the distinct topographies of the histopathologic lesions that distinguish FFI from CJD(D178N,V129) in accord with studies of mice expressing foreign PrP transgenes (40, 41).

PrP^{C} is likely to be a four-helix bundle protein based on its IR and CD spectra (37) as well as secondary structure predictions; structural models were constructed based on potential sites of helix–helix interactions. From 300,000 possible structures, 4 are thought to be plausible, 1 of which is an X-bundle model that is of particular interest with respect to the point mutations causing the inherited prion diseases and the codon 129 polymorphism (42). Both the codon 178 mutation and the codon 129 polymorphism are among the residues that form a hydrophobic core and, thus, lie in relatively close proximity to each other. How this proximity might feature in altering the conformation of PrP^{C}, which is converted into PrP^{Sc} by a process that involves the unfolding of α-helices and their refolding into β-sheets, is of considerable interest.

No attempt is made here to review all of the inherited human prion diseases, since other recent, comprehensive reviews are available (43–45). Instead, FFI and CJD(D178N,V129) are compared with a few of the more well-studied other inherited human prion diseases. For many years, the unusually high incidence of CJD among Israeli Jews of Libyan origin was thought to be due to the consumption of lightly cooked sheep brain or eyeballs (46, 47). Recent studies have shown that some Libyan and Tunisian Jews in families with CJD have a PrP gene point mutation at codon 200 resulting in a glutamate (E)-200 → lysine (K) change (48, 49). One patient was homozygous for this E200K mutation, but her clinical presentation was similar to that of heterozygotes (49), arguing that familial prion diseases are true autosomal dominant disorders. The E200K mutation has also been found in multiple families in Orava, Slovakia (48), in a cluster of familial cases in Chile (50), in a large German family living in the United States (51), and in British (52) and Japanese families (53).

In contrast to FFI and CJD(D178N,-V129), the age of onset of CJD(E200K) is unaltered by whether or not patients are homozygous for methionine at codon 129 (18). On the other hand, patients with familial CJD due to the insertion of six octarepeats in addition to the five that are found normally appear to develop clinical signs of CNS dysfunction earlier when they are homozygous for methionine at codon 129 (31). The insert at codon 53 containing six octarepeats or 144 bp was initially described in patients with CJD from four families, all residing in southern England (14, 19, 54). The mutation is thought to have arisen through a complex series of events, since the human PrP gene contains only five octarepeats, indicating that a single recombination event could not have created the insert. Genealogic investigations have shown that all four families are related, arguing for a single founder born more than two centuries ago (19). The lod score for this extended pedigree exceeds 11. Studies from several laboratories have demonstrated that two, four, five, six, seven, eight, or nine octarepeats in addition to the normal five are found in individuals with inherited CJD (14, 55–57), whereas deletion of one octarepeat has been identified without the neurologic disease (58–60).

While FFI presents with insomnia and CJD with dementia generally, GSS presents with ataxia. The first point mutation in the PrP gene to be reported in a familial prion disease was a substitution of leucine for proline at codon 102; more importantly, this Pro-102 → Leu (P102L) mutation was shown to be linked genetically to development of GSS with a lod score exceeding 4 (15, 61). The P102L mutation has been found in 10 different families in nine different countries including the original GSS family (62, 63).

Transgenic (Tg) mice expressing the P102L mutation at the corresponding mouse (Mo) codon 101 were constructed. Two Tg lines expressing MoPrP(P101L) ≈8-fold more than wild-type MoPrP were designated Tg(MoPrP-P101L)H mice with the H signifying high levels of the mutant transgene product. A third Tg line expressing MoPrP(P101L) ≈2-fold more than wild-type MoPrP were designated Tg196 mice. Uninoculated Tg(MoPrP-P101L)H mice spontaneously developed CNS degeneration, characterized by clinical signs indistinguishable from experimental murine scrapie and neuropathology consisting of widespread spongiform degeneration, astrocytic gliosis (64), and PrP amyloid plaques. Tg196 remained well for >700 days of age, while Tg(MoPrP) mice overexpressing wild-type MoPrP ≈8-fold have been healthy for >500 days of age (G. Telling and S.B.P., unpublished results).

Brain extracts prepared from Tg(MoPrP-P101L)H and Tg($SHaPrP^{+/+}$)7 mice developing neurologic illness spontaneously transmitted CNS degeneration to many inoculated recipients (65) (K. K. Hsiao, D. Groth, S.-L. Yang, H. Serban, D. Rapp, D. Foster, M. Scott, M. Torchia, S. J. DeArmond and S.B.P., unpublished data). Many Tg196 mice expressing low levels of the mutant MoPrP(P101L) transgene product and some Syrian hamsters developed CNS degeneration between 125 and 500 days after inoculation with extracts from ill Tg(MoPrP-P101L)H mice, while inoculated CD-1 Swiss mice remained well. Ill Tg196 mice exhibit spongiform degeneration and have infectivity as evidenced by serial passage of CNS degeneration in Tg196 mice with incubation periods of >1 year but contain no detectable protease-resistant PrP. Uninoculated Tg($SHaPrP^{+/+}$)7 mice homozygous for the Syrian hamster PrP transgene array develop CNS degeneration beginning at 1 year of age, and extracts from many of these animals transmit neurodegeneration to Syrian hamsters between 125 and 350 days after inoculation (D. Groth and S.B.P., unpublished results). Infectivity from Syrian hamsters with spongiform degeneration and high levels of PrP^{Sc} has been serially passaged in hamsters with incubation periods of ≈75 days.

Spontaneous development of neurologic disease and the generation of infectivity in Tg(MoPrP-P101L)H mice support the contention from genetic linkage studies that PrP gene mutations cause GSS, familial CJD, and FFI. Some investigators have argued that PrP gene mutations render individuals susceptible to a common "virus" (66–68). In this scenario, the putative scrapie virus is thought to persist within a worldwide reservoir of humans, animals, or insects without causing detectable illness. Yet 1 of 10^6 individuals develops sporadic CJD and dies from a lethal "infection," while ≈100% of people with PrP point mutations or inserts appear to develop eventually neurologic dysfunction. Despite numerous attempts to find the putative scrapie virus, none has been found. Whether it will be possible to model FFI and CJD(D178N,V129) in Tg mice remains to be established. Tg($SHaPrP^{+/+}$)7 mice may be a model for sporadic prion disorders (65).

The discovery that FFI is an inherited prion disease clearly widens the clinical

spectrum of these disorders and raises the possibility that many other degenerative diseases of unknown etiology may be caused by prions (27). Studies of the inherited prion diseases have dramatically revised thinking about these illnesses which may have relevance to deciphering the etiologies of some more common CNS degenerative disorders including Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis.

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