NEUROLOGY Discovery

Neurodegeneration in Humans Caused by Prions

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Prion diseases include kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia of humans as well as scrapie and bovine spongiform encephalopathy of animals. For many years, the prion diseases were thought to be caused by viruses despite evidence to the contrary. The unique characteristic common to all of these disorders, whether sporadic, dominantly inherited, or acquired by infection, is that they involve aberrant metabolism of the prion protein. In many cases, the cellular prion protein is converted into the scrapie variant by a process after translation that involves a conformational change. Often the human prion diseases are transmissible experimentally to animals, and all of the inherited prion diseases segregate with prion protein gene mutations.

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Investigations of neurodegeneration in humans and animals caused by prions have produced unexpected and striking findings. For many years, three uncommon human diseases and several animal disorders were labeled transmissible encephalopathies, spongiform encephalopathies, or slow virus diseases.[1-3] These illnesses are transmissible to experimental animals after a prolonged incubation period, and some features of the transmissible pathogen resemble those of viruses.[4-7] Yet early attempts to characterize the infectious pathogen causing scrapie in sheep and goats suggested that these transmissible agents differed from both viruses and viroids.[8,9]

A series of discoveries in the past three decades has led to the molecular and genetic characterization of the transmissible pathogen causing scrapie in animals and a quartet of human illnesses[10-15]: kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia. To distinguish this pathogen from viruses and viroids, the term "prion" was introduced to emphasize its proteinaceous and infectious nature.[16]

An abnormal isoform of the prion protein (PrP), known as the scrapie variant (PrP^{Sc}), is the only known component of the prion.[17,18] Prion protein is encoded by a gene on the short arm of chromosome 20 in humans.[19] The PrP^{Sc} variant differs physically from the normal, cellular isoform (PrP^{C}) by its high β-sheet content, insolubility in detergents, propensity to aggregate, and relative resistance to proteolysis.[20-22] While PrP^{Sc} is formed from PrP^{C} after the polypeptide chain is assembled,[23] attempts to identify a chemical modification that distinguishes the two PrP isoforms have been unsuccessful.[24] In contrast, biophysical studies have shown that PrP^{C} is rich in α-helices and is virtually devoid of β-sheet.[20] The conversion of PrP^{C} into PrP^{Sc} appears to involve the unfolding of α-helices and their refolding into β-sheets. Because infectious prions are composed largely, if not entirely, of PrP^{Sc}, this α-helix to β-sheet structural transition appears to be the fundamental event in the propagation of prions and the pathogenesis of neurodegeneration.

Clinical Manifestations of Prion Diseases

The human prion diseases are manifest as infectious, inherited, and sporadic disorders and are often referred to as kuru, Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia, depending on the clinical and neuropathologic findings (Table 1).

Kuru generally presented as an ataxic disorder, with the first manifestation of the disease being difficulty walking along steep mountain trails in the highlands of New Guinea. Most cases progressed steadily over a year to the point where, because of severe truncal ataxia, patients were no longer able to stand or sit without the aid of a pole implanted in the earth. In the terminal phases of the disease, they often manifested a dementia. With the cessation of ritualistic cannibalism about 40 years ago, kuru has almost disappeared.

Creutzfeldt-Jakob disease usually presents as a de-

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ABBREVIATIONS USED IN TEXT

CpG = phosphodiester bond of deoxycytosine and deoxyguanosine
PrP = prion protein
PrP^{C} = cellular variant of prion protein
PrP^{Sc} = scrapie variant of prion protein

menting illness in the sixth or seventh decade of life. In some cases the disease progresses rapidly, and the patients die within a few months. Most are dead within a year, but a few live as long as 15 years after diagnosis. About 10% of cases of Creutzfeldt-Jakob disease present with an ataxic syndrome that resembles kuru in many respects, but the dementia seems to appear much earlier.

Patients with the Gerstmann-Sträussler-Scheinker syndrome often present with ataxia; often they are initially given a diagnosis of multiple sclerosis and olivopontocerebellar degeneration. The clinical course of the disease can be unpredictable, with the duration varying from a year to two decades. Another form of the syndrome has been labeled "telencephalic Gerstmann-Sträussler-Scheinker," which presents with a dementia much like that of Creutzfeldt-Jakob disease.

The fourth human prion disease is fatal familial insomnia, which begins as an insomnia and autonomic nervous system dysfunction. Late in the course of the illness, which generally lasts about a year, signs of dementia appear.

Infectious forms of prion diseases result from the horizontal transmission of the infectious prions, as occurs in iatrogenic Creutzfeldt-Jakob disease and kuru. Inherited forms, notably Gerstmann-Sträussler-Scheinker disease, familial Creutzfeldt-Jakob disease, and fatal familial insomnia, make up 10% to 15% of all cases of prion disease. A mutation in the open reading frame or protein coding region of the *PRNP* gene has been found in all reported kindred with inherited human prion disease. Sporadic forms of prion disease comprise most cases of Creutzfeldt-Jakob disease and possibly some cases of Gerstmann-Sträussler-Scheinker syndrome.[25] How prions arise in patients with sporadic forms is unknown but has been hypothesized to involve horizontal transmission, somatic mutation of the open reading frame of the *PRNP* gene, and the spontaneous conversion of PrP^{C} into PrP^{Sc}.[2,26] Numerous attempts to establish an infectious link between sporadic Creutzfeldt-Jakob disease and a preexisting prion disease in animals or humans have been unrewarding.[27-29]

Diagnosis of Prion Diseases

A human prion disease should be considered in any patient who has a progressive subacute or chronic decline in cognitive or motor function. Being typically adults between 40 and 70 years of age, patients often have clinical features helpful in providing a premorbid diagnosis of prion disease, particularly sporadic Creutzfeldt-Jakob disease.[30] There is as yet no specific diagnostic test for prion disease in the cerebrospinal fluid. A definitive diagnosis

TABLE 1.—*Human Prion Diseases*

Disease	*Cause*
Kuru	Infection
Creutzfeldt-Jakob disease	
Iatrogenic	Infection
Sporadic	Unknown
Familial	Prion protein mutation
Gerstmann-Sträussler-Scheinker disease	Prion protein mutation
Fatal familial insomnia	Prion protein mutation

of human prion disease, which is invariably fatal, can usually be made from the examination of brain tissue. Over the past four years, knowledge of the molecular genetics of prion diseases has made it possible to diagnose inherited prion disease in living patients using DNA extracted from peripheral tissues.

The classic neuropathologic features of human prion disease include spongiform degeneration, gliosis, and neuronal loss in the absence of an inflammatory reaction. When present, amyloid plaques that stain with α-PrP antibodies are diagnostic. The presence of protease-resistant PrP (PrP^{Sc} or PrP^{CJD}) in the infectious and sporadic forms and most of the inherited forms of these diseases implicates prions in their pathogenesis.

The hallmark of all the prion diseases, whether sporadic, dominantly inherited, or acquired by infection, is that they involve aberrant metabolism of the prion protein.[10] Human prion disease can be rapidly and definitively diagnosed if PrP^{Sc} can be detected immunologically. In the familial forms of the prion diseases, molecular genetic analyses of PrP can be diagnostic and performed on DNA extracted from blood leukocytes after death. Unfortunately, such testing is of little value in the diagnosis of the sporadic or infectious forms of prion disease. In some patients with inherited prion disease, PrP^{Sc} is barely detectable or is undetectable,[31-34] a situation mimicked in transgenic mice, which express a mutant PrP gene and spontaneously develop neurologic illness indistinguishable from experimental murine scrapie.[35] Because molecular genetic analyses of *PRNP* genes in patients with unusual dementing illnesses are readily performed, the diagnosis of inherited prion disease can often be established where there was either little or no neurologic abnormality,[36] atypical neurodegenerative disease,[32] or misdiagnosed neurodegenerative diseases,[37] including Alzheimer's disease. Although the horizontal transmission of neurodegeneration to experimental hosts was for a time the "gold standard" of prion disease, it can no longer be used as such. Some investigators have reported that frequently inherited prion diseases from humans cannot be transmitted experimentally to animals (rodents) despite the presence of a pathogenic mutation in the *PRNP* gene.[7] Other investigators have been able to accomplish this using apes and monkeys as hosts.[38]

In summary, the diagnosis of prion or prion protein disease may be made in patients on the basis of the presence of PrP^{Sc}, the mutant *PRNP* genotype, or appropriate immunohistologic procedures and should not be excluded

in patients with atypical neurodegenerative diseases until one or preferably two of these examinations have been done.[36,39,40]

Inherited Human Prion Diseases

Genetics were first thought to have a role in Creutzfeldt-Jakob disease with the recognition that about 10% of cases are familial.[41,42] The discovery of the PrP gene and its linkage to scrapie incubation times in mice[43] raised the possibility that mutation might feature in the hereditary human prion diseases. A proline (P)→leucine (L) mutation at codon 102 was shown to be linked genetically to the development of Gerstmann-Sträussler-Scheinker disease with a lod score exceeding 3 (Figure 1).[11,12,36,44-70] This mutation may be due to the deamination of a methylated deoxycytosine (C) coupled to deoxyguanosine (G) through a phosphodiester bond (CpG) in the germline DNA encoding PrP, which results in the substitution of deoxythymine for deoxycytosine. The P102L mutation has been found in ten different families in nine different countries including the original family with Gerstmann-Sträussler-Scheinker disease.[43,71]

An insert of 144 base pairs at codon 53 containing six octarepeats was initially described in patients with Creutzfeldt-Jakob disease from four families, all residing in southern England (Figure 1).[15,40,49] The mutation is thought to have arisen through a complex series of events because the human *PRNP* 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.[15] 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 persons with inherited Creutzfeldt-Jakob disease,[49,50,72,73] whereas the deletion of one octarepeat has been identified without the neurologic disease.[45,46,51]

The unusually high incidence of Creutzfeldt-Jakob disease among Israeli Jews of Libyan origin was thought for many years to be due to the consumption of lightly cooked sheep brain or eyeballs.[74,75] Recent studies have shown that some Libyan and Tunisian Jews in families with Creutzfeldt-Jakob disease have a *PRNP* gene point mutation at codon 200 resulting in a glutamate (E)→lysine (K) substitution.[56,63] One patient was homozygous for the E200K mutation, but her clinical presentation was similar to that of heterozygotes,[63] arguing that familial prion diseases are true autosomal dominant disorders. The E200K mutation has also been found in Slovaks originating from Orava in north-central Slovakia,[56] in a cluster of familial cases in Chile,[76] in a large German family living in the United States,[77] and in British[78] and Japanese families.[79] Some investigators have argued that the E200K mutation originated in a Sephardic Jew whose descendants migrated from Spain and Portugal at the time of the Inquisition.[76] It is more likely that the E200K mutation has arisen independently multiple times by the deamidation of a methylated CpG as described earlier for the P102L mutation.[12,63] In support of this hypothesis are historical records of Libyan and Tunisian Jews indicating that they are descended from Jews living on the island of Jerba, where Jews first settled around 500 BC, and not from Sephardim.[80]

Many families with Creutzfeldt-Jakob disease have been found to have a point mutation at codon 178, resulting in an aspartic acid (D)→asparagine (N) substitution.[80,81] In these patients and in those with the E200K mutation, PrP amyloid plaques are rare; the neuropatho-

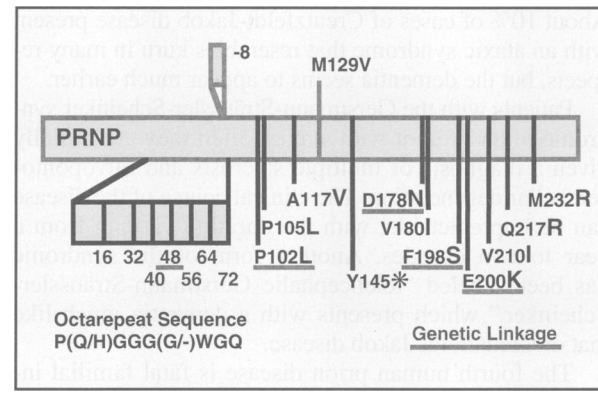

Figure 1.—Human prion protein gene (*PRNP*). The open reading frame is denoted by the large gray rectangle. Human *PRNP* wild-type polymorphisms are shown above the rectangle, and mutations that segregate with the inherited prion diseases are given below. The wild-type human prion protein gene contains five octarepeats P(Q/H)GGG(G/-)WGQ from codons 51 to 91.[44] Deletion of a single octarepeat at codon 81 or 82 is not associated with prion disease[45-47]; whether this deletion alters the phenotypic characteristics of a prion disease is unknown. There are common polymorphisms at codons 117 (alanine [Ala]→Ala) and 129 (methionine [Met]→valine [Val]); homozygosity for Met or Val at codon 129 appears to increase susceptibility to sporadic Creutzfeldt-Jakob disease.[48] Octarepeat inserts of 16, 32, 40, 48, 56, 64, and 72 amino acids at codons 67, 75, or 83 are designated by the small rectangle below the open reading frame. These inserts segregate with familial Creutzfeldt-Jakob disease, and substantial genetic linkage has been demonstrated where sufficient specimens from family members are available.[36,49-55] Point mutations are designated by the wild-type amino acid preceding the codon number and the mutant residue follows—that is, P102L. These point mutations segregate with the inherited prion diseases, and genetic linkage (underlined mutations) has been shown where sufficient specimens from family members are available. Mutations at codons 102 (proline [Pro]→leucine [Leu]), 117 (Ala→Val), 198 (phenylalanine [Phe]→serine [Ser]), and 217 (glutamine [Gln]→arginine [Arg]) are found in patients with Gerstmann-Sträussler-Scheinker disease.[12,43,55-62] Point mutations at codons 178 (aspartate [Asp]→asparagine [Asn]), 200 (glutamate [Glu]→lysine [Lys]), and 210 (Val→isoleucine [Ile]) are found in patients with familial Creutzfeldt-Jakob disease.[56,63-66] Point mutations at codons 198 (Phe→Ser) and 217 (Gln→Arg) are found in patients with Gerstmann-Sträussler-Scheinker syndrome who have prion protein amyloid plaques and neurofibrillary tangles.[11,67] Additional point mutations at codons 145 (tyrosine [Tyr]→Stop[*]), 105 (Pro→Leu), 180 (Val→Ile), and 232 (Met→Arg) have been recently reported.[68,69] The single-letter codes for amino acids are as follows: A = alanine, D = aspartate, E = glutamate, F = phenylalanine, I = isoleucine, K = lysine, L = leucine, M = methionine, N = asparagine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, Y = tyrosine. (Reproduced with permission from Prusiner.[70])

logic changes generally consist of widespread spongiform degeneration.

Insomnia has been described recently in multiple Italian families with the D178N mutation.[32] The neurologic disorder in these patients with fatal familial insomnia is restricted to selected nuclei of the thalamus. It is unclear whether all patients with the D178N mutation or only a subset have sleep disturbances; some of these patients have been diagnosed as having thalamic dementia.[82] Sequencing studies of DNA show that the allele with the D178N mutation encodes a methionine at position 129 in those with fatal familial insomnia, whereas a valine is encoded at position 129 in familial Creutzfeldt-Jakob disease.[83] The discovery that fatal familial insomnia is an inherited prion disease clearly widens the clinical spectrum of these disorders and raises the possibility that many other degenerative diseases of unknown cause may be caused by prions.[32]

Like the E200K and D178N(V129) mutations, a valine (V)→isoleucine (I) mutation at *PRNP* codon 210 produces Creutzfeldt-Jakob disease with classic symptoms and signs.[64,84] This V210I mutation is probably also incompletely penetrant.

Other point mutations at codons 105, 117, 145, 198, 217, and possibly 232 also segregate with inherited prion diseases.[43,57,67-69] Patients with a dementing or telencephalic form of Gerstmann-Sträussler-Scheinker disease have a mutation at codon 117. These patients and some in other families were once thought to have familial Alzheimer's disease, but are now known to have prion diseases on the basis of PrP immunostaining of amyloid plaques and *PRNP* gene mutations.[85-87] Patients with the codon 198 mutation have numerous neurofibrillary tangles that stain with antibodies to τ and have amyloid plaques[85-87] that are composed largely of a PrP fragment extending from residues 58 to 150.[88] A genetic linkage study of this family produced a lod score exceeding 6.[11] The neurologic disorder of two patients of Swedish ancestry with the codon 217 mutation[89] was similar to that of patients with the codon 198 mutation.

Patients with the Gerstmann-Sträussler-Scheinker syndrome have been reported who have a substitution of leucine for proline at PrP codon 105.[68] A patient with PrP amyloid plaques and a prolonged neurologic illness spanning almost two decades was found to have an amber mutation of the *PRNP* gene resulting in a "stop" codon at residue 145.[69] Staining of the plaques with α-PrP peptide antisera suggested that they might be composed exclusively of the truncated PrP molecules. That a PrP peptide ending at residue 145 polymerizes in amyloid filaments is to be expected, as an earlier study showed that the major PrP peptide in plaques from patients with the F198S mutation was an 11-kd PrP peptide beginning at codon 58 and ending at about 150.[88] Furthermore, synthetic PrP peptides adjacent to and including residues 109 to 122 readily polymerize into rod-shaped structures with the tinctorial properties of amyloid.[90-93]

Nomenclature for the Inherited Human Prion Diseases

Although each of the *PRNP* mutations is associated with a typical clinical presentation as noted earlier, there are a sufficient number of exceptions that a particular mutation in a single pedigree can present with symptom complexes typical of Creutzfeldt-Jakob disease in some patients and the Gerstmann-Sträussler-Scheinker syndrome in others. Because we now know the molecular basis of the disorders, it seems preferable to name them according to the mutation and to no longer refer to them as familial Creutzfeldt-Jakob disease, the Gerstmann-Sträussler-Scheinker syndrome, or fatal familial insomnia. Once the *PRNP* gene mutation has been determined, then we suggest that prion disease (P102L) be used in place of ataxic Gerstmann-Sträussler-Scheinker syndrome, such as that found in the original family with that disorder,[71,94] prion disease (E200K) instead of familial Creutzfeldt-Jakob disease in Libyan Jews, and prion disease (D178N, M129) instead of fatal familial insomnia (Table 2). These designations describe the precise causes of the disorders and remove any possible ambiguities.

The need to designate the inherited prion diseases by their mutations (molecular lesions) is emphasized by the vastly different clinical presentations and postmortem neurologic disorders noted in four afflicted members of a family with prion disease (6 octarepeat insert).[40] One of the four family members with the insert had a classic case of Creutzfeldt-Jakob disease and had pronounced spongiform change in the cerebral cortex, whereas another

TABLE 2.—*Inherited Prion Diseases of Humans*

Proposed Designation	*Alternative Name*
Inherited prion disease (P102L)	Gerstmann-Sträussler-Scheinker disease
Inherited prion disease (P105L)	Gerstmann-Sträussler-Scheinker disease
Inherited prion disease (A117V)	Gerstmann-Sträussler-Scheinker disease
Inherited prion disease (Y145Stop)	Gerstmann-Sträussler-Scheinker disease
Inherited prion disease (D178N)	Familial Creutzfeldt-Jakob disease, familial fatal insomnia
Inherited prion disease (V180I)	Gerstmann-Sträussler-Scheinker disease
Inherited prion disease (F198S)	Gerstmann-Sträussler-Scheinker disease
Inherited prion disease (E200K)	Familial Creutzfeldt-Jakob disease
Inherited prion disease (V210I)	Familial Creutzfeldt-Jakob disease
Inherited prion disease (Q217R)	Gerstmann-Sträussler-Scheinker disease
Inherited prion disease (octarepeat insert)	Familial Creutzfeldt-Jakob disease

presented with ataxia and had numerous PrP amyloid plaques at autopsy. The second case might have been called Gerstmann-Sträussler-Scheinker disease with hesitation. The third and fourth members of the family died in hospitals with the diagnosis of dementia, but had no spongiform change at autopsy and were not given the diagnosis of Creutzfeldt-Jakob disease.

Human *PRNP* Gene Polymorphisms

At *PRNP* codon 129, a polymorphism encodes either methionine (M) or valine (V) (Figure 1).[95] This polymorphism appears able to influence prion disease expression not only in inherited forms, but also in iatrogenic and sporadic forms of prion disease.

Susceptibility to infection may be partially determined by the *PRNP* codon 129 genotype,[96] analogous in principle to the incubation-time alleles in mice.[96,97] Population frequencies for the codon 129 polymorphism in whites are 12% V/V, 37% M/M, and 51% M/V.[48] In 16 patients (15 white, 1 black) from the United Kingdom, the United States, and France with iatrogenic Creutzfeldt-Jakob disease from contaminated growth hormone extracts, eight (50%) were V/V, five (31%) were M/M, and three (19%) were M/V.[96] Thus, a disproportionate number of patients with iatrogenic Creutzfeldt-Jakob disease were homozygous for valine at *PRNP* codon 129. Heterozygosity at codon 129 may provide partial protection. Whether these associations are strongly significant awaits statistical analysis of larger samples. Thousands of children who received pituitary growth hormone extracts are still at risk for the development of Creutzfeldt-Jakob disease. Fortunately, the use of genetically engineered growth hormone will eliminate this iatrogenic form of the disease.

No specific mutations have been identified in the *PRNP* gene of patients with sporadic Creutzfeldt-Jakob disease. Patients with the sporadic form, however, are largely homozygous at codon 129.[48] This finding supports a model of prion production that favors PrP interactions between homologous proteins, as appears to occur in transgenic mice expressing Syrian hamster PrP inoculated with either hamster prions or mouse prions,[10,98,99] as well as

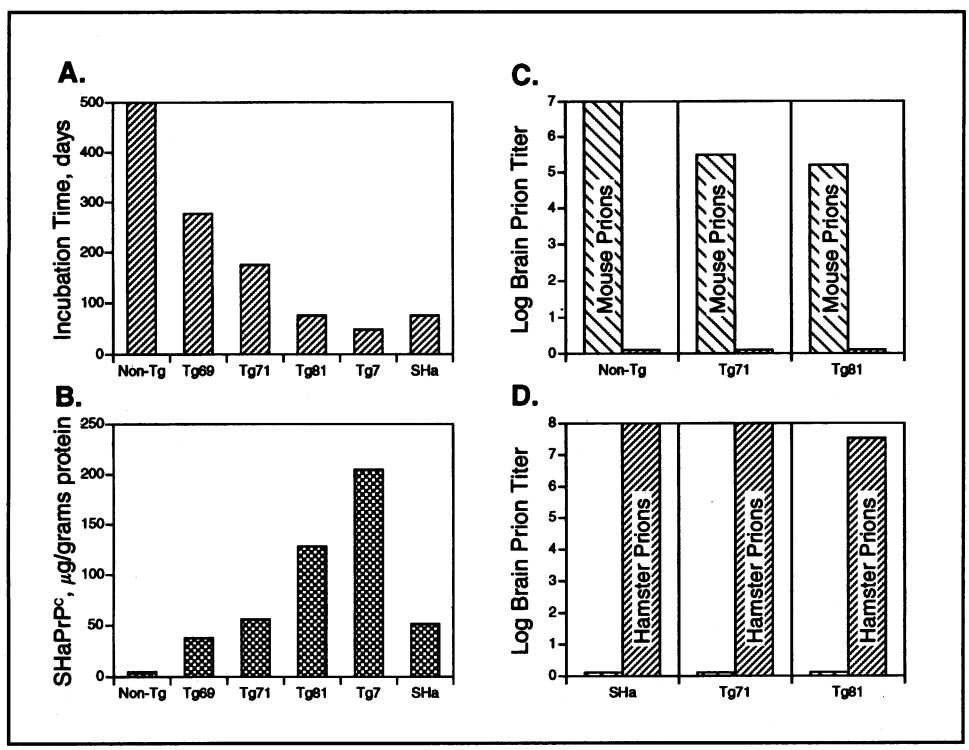

Figure 2.—Transgenic (Tg) mice expressing Syrian hamster (SHa) prion protein (PrP) exhibit species-specific scrapie incubation times, infectious prion synthesis, and neurologic abnormalities.[99] **A,** Scrapie incubation times are shown in nontransgenic mice (Non-Tg) and 4 lines of Tg mice expressing SHaPrP and Syrian hamsters inoculated intracerebrally with about 10^7 of a median infectious dose of Sc237 prions serially passaged in Syrian hamsters. The 4 lines of Tg mice have different numbers of transgene copies: Tg69 and Tg71 mice have 2 to 4 copies of the SHaPrP transgene, whereas Tg81 have 30 to 50 and Tg7 mice have more than 60. Incubation times are the number of days from inoculation to the onset of neurologic dysfunction. **B,** Brain Syrian hamster cellular prion protein ($SHaPrP^C$) levels are shown in Tg mice and Syrian hamsters. The $SHaPrP^C$ levels were quantitated by an enzyme-linked immunoassay. **C,** Prion titers were measured in brains of clinically ill animals after inoculation with mouse prions. Brain extracts from Non-Tg, Tg71, and Tg81 mice were bioassayed for prions in mice (left) and hamsters (right). **D,** Prion titers were measured in brains of clinically ill animals after inoculation with SHa prions. Brain extracts from Syrian hamsters as well as Tg71 and Tg81 mice were bioassayed for prions in mice (left) and hamsters (right). (Reproduced with permission from Prusiner.[110])

transgenic mice expressing a chimeric mouse/hamster PrP transgene inoculated with "artificial" prions.[100]

Ataxia develops as an early sign in about 15% of patients with sporadic Creutzfeldt-Jakob disease, accompanied by dementia.[101] Most but not all patients with ataxia have compact (kuru) plaques in the cerebellum.[102] Patients with ataxia and compact plaques have a protracted clinical course that may last as long as three years. The molecular basis for the differences between Creutzfeldt-Jakob disease of shorter and longer duration has not been fully elucidated, but some preliminary analyses have suggested that patients with protracted, atypical clinical courses are more likely to be heterozygous at codon 129.[103,104]

Homozygosity at codon 129 has been reported to be associated with an earlier age of onset in patients with the inherited prion disease caused by the 6 octarepeat insert, but not that caused by the E200K mutation in Libyan Jews.[14,105] As noted earlier, the phenotype for fatal familial insomnia is found in patients with the D178N mutation who encode a methionine at codon 129 on the mutant allele, whereas those with dementing illness (familial Creutzfeldt-Jakob disease) encode a valine at 129.[83] Homozygosity for either M or V at codon 129 is thought to be associated with an earlier age of onset for the D178N mutation.

Barriers for the Transmission of Prion Diseases

The passage of prions between species is a stochastic process characterized by prolonged incubation times.[106] Prions synthesized de novo reflect the sequence of the host PrP gene and not that of the PrP^{Sc} molecules in the inoculum.[107] On subsequent passage in a homologous host, the incubation time shortens to that recorded for all subsequent passages, and it becomes a nonstochastic process. The species-barrier concept is of practical importance in assessing the risk for Creutzfeldt-Jakob disease developing in humans after the consumption of scrapie-infected lamb or from cattle with bovine spongiform encephalopathy.[108,109]

To test the hypothesis that differences in PrP gene sequences might be responsible for the species barrier, transgenic mice expressing Syrian hamster (SHa) PrP were constructed.[98,99] The PrP genes of Syrian hamsters and mice encode proteins differing at 16 positions. Incubation times in four lines of transgenic SHaPrP mice inoculated with mouse prions were prolonged compared with those observed for nontransgenic control mice. Inoculation of transgenic SHaPrP mice with SHa prions showed abrogation of the species barrier, resulting in abbreviated incubation times due to a nonstochastic process (Figure 2-A).[98,99,110] The length of the incubation time after inoculation with SHa prions was inversely proportional to the level of $SHaPrP^{C}$ in the brains of transgenic SHaPrP mice (Figure 2-B).[99] The levels of $SHaPrP^{Sc}$ in the brains of clinically ill mice were similar in all four transgenic SHaPrP lines inoculated with SHa prions. Bioassays of brain extracts from clinically ill transgenic SHaPrP mice inoculated with mouse prions revealed that only mouse prions but no SHa prions were produced (Figure 2-C). Conversely, inoculation of transgenic SHaPrP mice with SHa prions led to only the synthesis of SHa prions (Figure 2-D). These findings contend that the amino acid sequence of PrP^{Sc} in the inoculum specifies whether mouse PrP or $SHaPrP^{C}$ will be converted into PrP^{Sc} in recipient transgenic SHaPrP mice. Furthermore, the production of PrP^{Sc} appears to involve the formation of an intermediate complex between homotypic PrP^{C} and PrP^{Sc}.

The neurologic abnormalities of transgenic SHaPrP mice were also found to be determined by the genetic origin of prion inoculum. Mouse prions injected into transgenic SHaPrP mice produced a neurologic abnormality characteristic of mice with scrapie. A moderate degree of vacuolation in both the gray and white matter was found, but amyloid plaques were rarely detected. Inoculation of transgenic SHaPrP mice with SHa prions produced intense vacuolation of the gray matter, sparing of the white matter, and numerous SHaPrP amyloid plaques characteristic of Syrian hamsters with scrapie.

Transgenic Mice Overexpressing Wild-type PrP Genes

During transgenetic studies, we discovered that uninoculated older mice harboring high copy numbers of wild-type PrP transgenes derived from Syrian hamsters, sheep, and PrP-B mice spontaneously developed truncal ataxia, hind-limb paralysis, and tremors.[111] These transgenic mice showed a profound necrotizing myopathy involving skeletal muscle, a demyelinating polyneuropathy, and focal vacuolation of the central nervous system. The development of disease was dependent on transgene dosage. For example, transgenic $(SHaPrP^{+/+})7$ mice homozygous for the SHaPrP transgene array regularly developed disease between 400 and 600 days of age whereas hemizygous transgenic $(SHaPrP^{+/0})7$ mice developed disease only after more than 650 days.

Attempts to demonstrate PrP^{Sc} in either muscle or brain were unsuccessful, but the transmission of disease with brain extracts from transgenic $(SHaPrP^{+/+})7$ mice inoculated into Syrian hamsters did occur. These Syrian hamsters had PrP^{Sc} as detected by immunoblotting and spongiform degeneration (D. Groth and S. B. Prusiner, unpublished data, June 1993). Serial passage with brain extracts from these animals to recipients was observed. De novo synthesis of prions in transgenic $(SHaPrP^{+/+})7$ mice overexpressing wild-type $SHaPr^{Pc}$ provide support for the hypothesis that sporadic Creutzfeldt-Jakob disease does not result from infection, but rather is a consequence of the spontaneous, although rare, conversion of PrP^{C} into PrP^{Sc}. Alternatively, a somatic mutation in which mutant $SHaPrP^{C}$ is spontaneously converted into PrP^{Sc}, as in the inherited prion diseases, could also explain sporadic Creutzfeldt-Jakob disease. These findings as well as those described earlier for transgenic mice with mouse PrP(P101L) strongly suggest that prions are devoid of foreign nucleic acid, in accord with many earlier studies that use other experimental approaches.[10]

Conclusions and Prospects for Therapy

Knowledge accrued from the study of prion diseases may provide an effective strategy for defining the causes and dissecting the molecular pathogenesis of the more common neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Advances in the molecular genetics of Alzheimer's disease and amyotrophic lateral sclerosis suggest that, like the prion diseases, an important subset is caused by mutations that result in nonconservative amino acid substitutions in proteins expressed in the central nervous system.

At present, there are no effective therapies for the treatment of prion diseases. These disorders are invariably fatal. The inherited prion diseases can be prevented by genetic counseling coupled with prenatal DNA screening, but such testing may present ethical problems. For example, during the childbearing years, prospective parents are generally symptom free and may not want to know their own gene types. The apparent incomplete penetrance of some of the inherited prion diseases makes predicting the future for an asymptomatic person uncertain.[14,63,76]

Ablation of the PrP gene in transgenic Prn-$p^{0/0}$ mice has not affected the development of these animals, and they remain healthy at 2 years of age.[112] Because the absence of PrP^{C} expression does not provoke disease, we can conclude that scrapie and other prion diseases are a consequence of PrP^{Sc} accumulation rather than an inhibition of PrP^{C} function. To date, the function of PrP^{C} remains unknown.

The resistance of Prn-$p^{0/0}$ mice to prions and their failure to propagate scrapie infectivity have prompted suggestions that gene therapy or antisense oligonucleotides might ultimately provide an effective therapeutic approach.[113,114] Mice heterozygous (Prn-$p^{0/+}$) for ablation of the PrP gene have prolonged incubation times when inoculated with mouse prions. This finding is in accord with studies of transgenic SHaPrP mice where decreased SHaPrP expression was accompanied by prolonged incubation times.[99]

Because the delivery of therapeutic polynucleotides to the central nervous system remains problematic, the most effective therapy may evolve from the development of drugs that block the conversion of PrP^{C} into PrP^{Sc}. Because the fundamental event in both the formation of PrP^{Sc} and the propagation of prions seems to be the unfolding of α-helices and their refolding into β-sheets,[20] drugs targeting this structural transformation would seem likely to be efficacious.

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