Sporadic human prion diseases: molecular insights and diagnosis

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Human prion diseases can be sporadic, inherited, or acquired by infection. Distinct clinical and pathological characteristics separate sporadic diseases into three phenotypes: Creutzfeldt-Jakob disease (CJD), fatal insomnia, and variably protease-sensitive prionopathy. CJD accounts for more than 90% of all cases of sporadic prion disease; it is commonly categorised into five subtypes that can be distinguished according to leading clinical signs, histological lesions, and molecular traits of the pathogenic prion protein. Three subtypes affect prominently cognitive functions whereas the other two impair cerebellar motor activities. An accurate and timely diagnosis depends on careful clinical examination and early performance and interpretation of diagnostic tests, including electroencephalography, quantitative assessment of the surrogate markers 14-3-3, tau, and of the prion protein in the CSF, and neuroimaging. The reliability of CSF tests is improved when these tests are interpreted alongside neuroimaging data.

Introduction

Despite their rarity, prion diseases, formerly named spongiform encephalopathies, are the focus of continued interest from the scientific community. These diseases were first described in the early 1920s, but were not widely studied until 1966, when they were shown to be transmissible in a virus-like manner, except for having a long incubation time.[1,2] Transmissibility conferred to prion diseases the unique characteristic of being both infectious and inherited; it also posed the major challenge of identification of the novel and unconventional infectious agent. Because the infectious agent is resistant to inactivation and can transmit disease not only between people but also from animals to people, prion diseases are a serious threat to public health; hence, prion surveillance centres have been established in many countries.

In 1982, Stanley Prusiner proposed the protein-only hypothesis, which stated that the infectious agent was a protein that he named proteinaceous infectious particle or prion. He suggested that prions are formed from the normal or cellular prion protein (PrP^C) by the acquisition of an abnormal conformation.[3]

In the past 5 years, researchers have shown that a prion-like formation mechanism, whereby a pathogenic protein is generated from its normal isoform through a change in conformation, might also apply to proteins associated with major neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, tauopathies, Huntington's disease, and amyotrophic lateral sclerosis, and to systemic amyloidoses such as type 2 diabetes and amyloidosis A.[4–8] The latest, although still controversial, data suggest that PrP^C, perhaps through its conversion to prion, might act as the receptor for amyloid β protein, mediating synaptic dysfunction in Alzheimer's disease and possibly other disorders.[9,10] Prions and other alternatively folded proteins might also be involved in normal cell function, including the formation of biochemical memory at the synapse.[4,11] Therefore, the term prion currently applies to not only a disease-specific pathogen but also to a mechanism that could have a wide and crucial role in pathological and physiological processes.

Human prion diseases present a formidable diagnostic challenge to clinicians, mainly because of their rarity and substantial heterogeneity. The annual incidence of all human prion diseases is generally reported to be about 1 case per million individuals.[12] The incidence peaks at nearly 6 cases per million between the ages of 65 and 74 years, and prion disease accounted for about 1 in 8500 deaths in the USA between 1999 and 2008 (L Schonberger, Centers for Disease Control and Prevention, personal communication).[12] The heterogeneity of human prion diseases is attributable not only to the presence of three forms with distinct causes—sporadic, inherited, and acquired by infection (table 1)—but also to the many diverse phenotypes that make up the sporadic form.

In this Review, we briefly assess the causes and pathogenesis of human prion diseases and then focus on clinical and diagnostic aspects of sporadic prion diseases, in particular Creutzfeldt-Jakob disease (sCJD), the most common of these diseases. We review clinical aspects, including diagnostic tests, with an emphasis on features that might improve accuracy and timeliness of diagnosis.

Basic mechanisms

Human PrP^C is 209 (residues 23–231 of the polypeptide predicted by the cDNA sequence) aminoacids long and has two sites of non-obligatory N-linked glycosylation that can generate three glycoforms: diglycosylated, monoglycosylated, and unglycosylated.[13] Domains with relevant secondary structures are located in the C-terminal region (around residues 121–231) and include two short sequences with a β-sheet conformation and three longer sequences that form α-helices. In cells, PrP^C is synthesised in the endoplasmic reticulum and then processed along the secretory pathway; it is transported through the Golgi apparatus and predominantly transferred to the plasma membrane where, linked by a

	Previous nomenclature
Familial	
Phenotype	
CJD	Unchanged
Fatal familial insomnia	Not described
Gerstmann-Sträussler-Scheinker disease	Unchanged
Heterogeneous or mixed phenotype	Unchanged
Sporadic	
Phenotype	
CJD 129MM1 and CJD 129MV1*†	Myoclonic and Heidenhain¶
CJD 129VV1	Not described
CJD 129MM2	Not described
CJD 129MV2	Cerebellar or ataxic
CJD 129VV2	Cerebellar or ataxic
Fatal insomnia‡	Thalamic
Variably protease-sensitive prionopathy§	Not described
Acquired by infection	
Phenotype	
Kuru	Unchanged
Variant CJD	Not described
Iatrogenic CJD	Unchanged

CJD=Creutzfeldt-Jakob disease. *The molecular classification is based on the genotype at codon 129 of the prion protein (PrP) gene and the type (based on electrophoretic mobility), 1 or 2, of the scrapie PrP (PrP^{Sc}). Hence, MM, MV, and VV indicate the patient's homozygosity for methionine (M) or valine (V), or MV heterozygosity at codon 129. †129MM and 129MV associated with PrP^{Sc} type 1 have the same phenotype. ‡So far, all patients with sporadic fatal insomnia have been 129MM2. §Patients with variably protease-sensitive prionopathy could have any one of the three 129 genotypes; they are all associated with a PrP^{Sc} that shows an electrophoretic profile different from those of PrP^{Sc} types 1 and 2. ¶Also called classic; the amyotrophic form has not been identified; the panencephalopathic form is thought to have the longest duration of any of the subtypes of sporadic CJD.

Table 1: **Molecular classification and previous nomenclature of human prion diseases**

Panel: **Proposed functions of the normal or cellular prion protein (PrP^{C})[14]**

- Copper binding (copper serving as a cofactor for an undetermined PrP^{C} enzymatic activity)
- Signalling receptor (binding to neural cell adhesion molecule)
- Signal transduction (caveolin1-dependent coupling to tyrosine kinase Fyn)
- Role in neuronal growth and survival (protection against Bax-mediated cell death)
- Synaptic regulation (as a receptor or receptor-related in $GABA_A$-ergic inhibitory synapses)
- Sleep and circadian rhythms regulator
- Inhibitory regulation of NMDA receptors
- Interaction with stress inducible protein 1
- Regulation of cell differentiation and apoptosis
- Maintenance of peripheral nerve myelin[15]
- Receptor for amyloid β in Alzheimer's disease and possibly for other amyloids[9,10]

glycosylphosphatidylinositol anchor, it hangs into the extracellular space.[13] PrP^{C} is soluble in detergents, monomeric, and sensitive to protease digestion; the panel lists its many proposed functions.[14]

The crucial conformational transition that converts PrP^{C} into an infectious and pathogenic isoform, commonly identified as scrapie PrP (PrP^{Sc}, from the first animal prion disease identified), is thought to entail extension of the β-sheet structures at the expense of one or more α-helices.[16] Extension of the β-sheet region confers insolubility to detergents, resistance to protease digestion, and the propensity to aggregate. However, the presence of protease-sensitive but detergent-insoluble PrP^{Sc} has also been reported.[17,18] This observation adds to the complexity of the PrP^{Sc} isoforms and human prion diseases.

Causes and pathogenesis

Whereas the causes of prion diseases that are inherited or acquired by infection are known, the cause of sporadic prion diseases is speculative. In the inherited form, the formation of PrP^{Sc} is favoured by the presence of a mutation that destabilises the mutated native PrP; in the form acquired by infection, exogenous PrP^{Sc} acts as template for generation of endogenous PrP^{Sc}.

Of the many causal mechanisms proposed for sporadic prion diseases, the failure of the system known as the quality control complex or proteostasis network is the most compelling.[19,20] The cellular quality control complex includes sets of chaperone proteins that help newly formed polypeptides to achieve the proper conformation, and their clearance compartments, such as the ubiquitin-proteasome system and lysosomes, which dispose of polypeptides that fail to adopt the native structure. Although misfolded proteins are produced under normal cellular conditions as the result of intrinsic errors in protein biosynthesis, these errors are likely to increase with ageing, overwhelming the capacity of the quality control complex. Furthermore, ageing itself can diminish the stringency and efficiency of the complex.

In sporadic prion diseases, the failure of the quality control complex is thought to result in the abundant presence of misfolded PrP in the cell, with the propensity to aggregate and replicate by templated conversion of PrP^{C}.[21] This initial event is likely to occur in small regions or even in one cell. The newly formed PrP^{Sc} would then propagate to other regions of the brain by self-replication, probably along nerve pathways. When it reaches susceptible brain regions, PrP^{Sc} impairs function and causes tissue damage and symptomatic disease. Although rare, this scenario must be fairly fixed and repetitive because the types and topographies of the lesions associated with the types and subtypes of sporadic prion diseases are constant (appendix). Another scenario predicts the normal existence of an off-pathway isoform that is intermediate between PrP^{C} and PrP^{Sc}, named insoluble PrP (iPrP) or silent prion.[22,23] The failure of the

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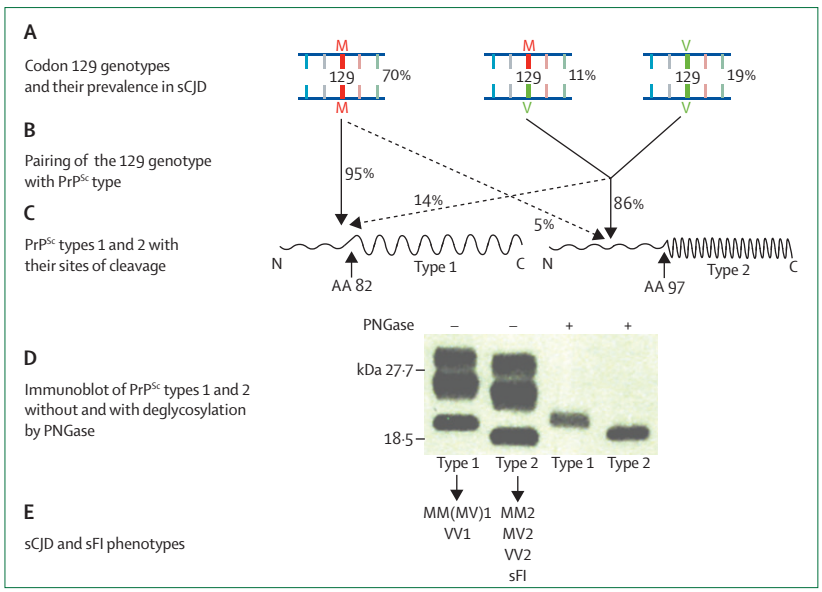

Figure 1: **Correlations between the PrP genotype, as determined by the MV polymorphism at codon 129, and PrP^{Sc} types 1 and 2**

(A) Diagrammatic representation of each of the three 129 genotypes (MM, MV, and VV) with their average relative prevalence in all subtypes of sCJD. (B) PrP^{Sc} type 1 is associated with the 129MM genotype in about 95% of cases, whereas MV and VV genotypes are associated with PrP^{Sc} type 2 in about 86% of cases. (C) Diagrammatic representation of PrP^{Sc} types 1 and 2; each consists of an amino-terminal region (N) of different size that is protease-sensitive and is digested down to amino acid (AA) 82 in type 1 and to amino acid 97 in type 2 (arrows). The different cleavage site is thought to be the result of the different conformation in PrP^{Sc} types 1 and 2. (D) Types 1 and 2 PrP^{Sc} have distinct electrophoretic mobilities because of the different size of their respective protease-resistant fragments (type 2 being smaller than type 1) and are easily distinguished by their different migration on electrophoresis, especially after cleavage of the sugars by the enzyme peptide N glycosidase F (PNGase). (E) Both 129 genotype and PrP^{Sc} types are thought to act as determinants of the phenotypes of sporadic prion diseases that are commonly identified with letters and numbers to indicate the associated genotype and PrP^{Sc} type. PrP^{Sc}=scrapie prion protein. M=methionine. V=valine. sCJD=sporadic Creutzfeld-Jakob disease. sFI=sporadic fatal insomnia.

control mechanisms that under normal conditions keep iPrP secured would convert iPrP into PrP^{Sc} and trigger the prion diseases.[24]

Molecular classification

Distinct characteristics separate sporadic prion diseases into three phenotypes: CJD, fatal insomnia, and variably protease-sensitive prionopathy (VPSPr). CJD accounts for more than 90% of all cases of sporadic prion disease. Not long after it was first described, it became apparent that CJD included various clinical and histopathological forms. Common classifications listed the following forms: myoclonic, Heidenhain or amaurotic, classical or dyskinetic, thalamic, cerebellar or ataxic, amyotrophic and panencephalopathic (table 1).[25]

This heterogeneity was at least partly explained when we and others reported that the methionine (M) to valine (V) polymorphism at codon 129 of *PRNP* could affect the disease phenotype and some of the characteristics of the disease-associated PrP^{Sc} (figure 1).[26–30] Hence, the clinical and histopathological features of sCJD differ depending on whether the patient is homozygous for methionine (MM) or valine (VV), or heterozygous at this codon.[25,30] Furthermore, more than 90% of patients with sCJD who are 129MM have type 1 PrP^{Sc} whereas more than 80% of patients who are 129VV and 129MV have PrP^{Sc} type 2 (figure 1).[25,30] The two types of PrP^{Sc}, either of which is associated with most prion diseases, are distinguished by their electrophoretic mobilities after protease digestion (figure 1). The different mobilities result from the different sites of cleavage by the protease in the two types of PrP^{Sc}, which are likely to result from distinct conformations.[28,31] These findings have led to the theory that the 129 genotype favours the formation or selection of particular PrP^{Sc} conformers, which then affect the disease phenotype.[31]

Grouping of all cases of sporadic prion diseases according to the pairing of the 129 genotype with the PrP^{Sc} type led to the recognition of six disease phenotypes, five of which pertain to sCJD and one to sporadic fatal insomnia (variably protease-sensitive prionopathy is not associated with PrP^{Sc} 1 or 2), which match most of the subtypes previously described according to clinical features, but also include novel ones (table 1, appendix).[25,29–34] The codistribution of distinct 129 genotype-PrP^{Sc} type combinations with distinct disease phenotypes, however, is not stringent. For example, the sCJD subtype identified as sCJD MM1 has clinical, histopathological, and PrP^{Sc} characteristics that are almost indistinguishable from those of the sCJD MV1 subtype, despite having a distinct 129 genotype.[25,30,33,34] By contrast, the sCJD MM2 subtype and sporadic fatal insomnia, both of which affect 129MM patients and are associated with PrP^{Sc} type 2, have very different disease phenotypes.[30–33] The mechanisms underlying these apparent discrepancies have not been identified.[31]

This classification has been challenged because many cases of prion disease have PrP^{Sc} belonging to both types 1 and 2.[35–39] Although this issue has not been fully resolved, the co-occurrence of the two PrP^{Sc} types—eg, in the subtype identified as sCJDMM1–2—seems to be associated with a hybrid phenotype mirroring the mixture of the two phenotypes present when types 1 and 2 occur separately, as in sCJD MM1 and sCJD MM2.[34,38] Furthermore, at least in sCJDMM1–2, the presentation of the two phenotypes associated with PrP^{Sc} types 1 and 2 is directly related to the amounts of the co-occurring PrP^{Sc} types 1 and 2.[38] Therefore, the co-occurrence of PrP^{Sc} types seems to strengthen the notion that the PrP^{Sc} type at least partly determines the phenotype.

An alternative classification of sCJD subtypes, which is based on the same principle of 129 genotype-PrP^{Sc} type pairing but which recognises several (rather than two) PrP^{Sc} types, has also been proposed.[40,41]

Clinical diagnosis

For the practical purpose of enabling a prompt diagnosis, sporadic prion diseases can be separated into three groups: the cognitive subtypes, including sCJD MM1 and

	sCJD cognitive type			sCJD ataxic type		sCJD (all subtypes)
	MM1/MV1[30,39]	MM2[30,38,44]	VV1*[30,45]	VV2†[30,39] (n=103)	MV2[30,46] (n=85)	(n=515)
Age at onset (years)	66 (42–91)	66 (49–82)	43 (19–71)	64 (41–83)	62 (40–81)	64 (19–91)
Duration (months)‡	4 (1–24)	14 (3–24)	19 (4–72)	6 (3–18)	17 (4–43)	8 (1–72)
Presentation						
Cognitive decline	192/273 (70%)	23/23 (100%)	26/27 (96%)	28/96 (29%)	20/27 (74%)	289/446 (65%)
Ataxia	106/273 (39%)	3/23 (13%)	0/27	94/96 (98%)	22/27 (81%)	225/446 (50%)
Psychiatric	63/273 (23%)	2/23 (9%)	9/27 (33%)	10/96 (10%)	9/27 (33%)	93/446 (20%)
Visual signs	74/273 (27%)	0/23	0/27	3/96 (3%)	0/27	77/446 (17%)
Aphasia	63/273 (23%)	7/22 (32%)	1/27 (4%)	2/96 (2%)	3/27 (11%)	76/445 (17%)
Advanced stage						
Cognitive decline	257/277 (93%)	18/18 (100%)	27/27 (100%)	47/47 (100%)	53/53 (100%)	402/422 (95%)
Ataxia	147/277 (53%)	8/18 (44%)	6/12 (50%)	47/47 (100%)	53/53 (100%)	261/407 (64%)
Psychiatric	91/277 (33%)	12/18 (67%)	20/27 (74%)	10/47 (21%)	31/53 (58%)	164/422 (39%)
Visual signs	113/277 (41%)	4/18 (22%)	0/12	0/47	13/53 (25%)	130/407 (32%)
Aphasia	97/277 (35%)	13/18 (72%)	4/12 (33%)	0/47	19/53 (36%)	133/407 (33%)
Parkinsonism§	69/277 (25%)	13/18 (72%)	6/12 (50%)	3/47 (6%)	40/53 (75%)	131/407 (32%)
Pyramidal	166/277 (60%)	15/18 (83%)	7/12 (58%)	23/47 (50%)	18/53 (34%)	229/407 (56%)
Myoclonus¶[30,38,47]	205/211 (97%)	15/18 (83%)	11/27 (41%)	39/59 (66%)	39/53 (74%)	309/368 (84%)
EEG sensitivity‖[48]	73% (189)	24–44% (21)**	0–42% (27)**	13% (59)	8% (52)	44% (348)
CSF sensitivity‖[49]						
14.3.3	100% (108)	40% (5)	100% (4)	100% (23)	100% (2)	95% (142)
Tau	97% (115)	53% (15)	100% (4)	100% (23)	100% (6)	88% (163)
MRI sensitivity‖[50]	80% (49)††	93% (15)	100% (2)	60% (15)	100% (8)	81% (89)

Data are mean (range), n/N (%), or % (N). sCJD=sporadic Creutzfeldt-Jakob disease. M=methionine. V=valine. PrP^{Sc}=scrapie prion protein. EEG=electroencephalogram. *Cali I, and Gambetti P, unpublished (15 cases). †Cali I, and Gambetti P, unpublished data (17 cases). ‡Duration is time from first symptom to death. §Including other types of dyskinesia. ¶Rare at presentation. ‖95% CIs not available; data should be interpreted with reference to sample size. **Ranges are the variations reported. ††sCJDMV1 alone has a sensitivity of 100%, on the basis of six cases.

Table 2: **Clinical features of sCJD and its subtypes, by 129 genotype-PrP^{Sc} type**

sCJD MV1, sCJD MM2, and sCJD VV1; the ataxic subtypes, including sCJD VV2 and sCJD MV2 (table 2); and sporadic prion diseases with non-CJD phenotypes, which include sporadic fatal insomnia and VPSPr.[32,33,42,43] The last two diseases will be considered separately because their non-CJD phenotypes pose special diagnostic challenges (table 3; appendix).

Cognitive subtypes

In the cognitive group, sCJD MM1 or sCJD MV1 (the classic forms) are by far the most common, accounting for 55–70% of sporadic prion diseases (table 2).[30,47] At onset, sCJDMM1 and sCJDMV1 typically present with symptoms that are well defined and seemingly easy to recognise. The patient, most commonly aged between 50 and 70 years, shows rapidly progressive multidomain cognitive impairment and confusion, occasionally accompanied by cortical visual disturbances, ataxia, and spontaneous or induced myoclonus. Patients often have a gaze that expresses apprehension or fear and shows heightened reactivity to external stimuli. This syndrome is frequently preceded by mild psychiatric symptoms such as malaise, anxiety, mood changes, and diminished ability to concentrate. Extrapyramidal and cerebellar signs occasionally occur at onset but the neurological examination can also be unremarkable. Less common presentations include a prominent ataxia eclipsing the cognitive impairment, concomitant epilepsy, and visual deficits such as field defects, distortion, and cortical blindness. Neurological signs can be unilateral.

The disease progresses rapidly, often going from the poorly defined prodromal syndrome to unmistakable cognitive, behavioural, and motor abnormalities in a matter of days. This presentation and the rapid progression should prompt the inclusion of prion disease in the differential diagnosis. MRI, CSF examination, and EEG recording should then be done promptly; in most cases, these three tests together will establish the diagnosis of sCJD and rule out several other rapidly progressing brain disorders. Within a few weeks the patient develops severe pyramidal and extrapyramidal signs, invariably followed by vegetative state and death on average 4 months after onset.[30,39]

The sCJD MM2 and sCJD VV1 subtypes are rare, accounting for 2–10% and 1–4%, respectively, of cases of sCJD (P Gambetti, unpublished, table 2).[30,47] They can be difficult to distinguish from each other on the basis of the initial clinical signs.[30,38,44,45] Both subtypes present with

	Sporadic familial insomnia	Variably protease-sensitive prionopathy			
	MM2 (n=31)	VV (n=21)	MV (n=9)	MM (n=3)*	All genotypes (n=33)
Age at onset (years)	46 (13, 24–74)	67 (9, 48–77)	74 (5, 65–81)	78 (12, 64–87)	70 (9, 48–87)
Duration (months)†	24 (13, 10–73)	18 (15, 10–60)	34 (25, 7–73)	41 (9, 10–73)	24 (10, 7–73)
Presentation					
Cognitive decline	13/31 (42%)	12/21 (57%)	6/9 (67%)	0/3	18/33 (55%)
Ataxia	13/31 (42%)	0/21	0/9	1/3 (33%)	1/33 (3%)
Insomnia	9/31 (29%)	..	..	..	..
Psychiatric	8/31 (26%)	14/21 (67%)	6/9 (67%)	1/3 (33%)	21/33 (64%)
Visual signs	7/31 (23%)	..	..	..	..
Dysautonomia	1/31 (3%)	..	..	..	..
Aphasia	..	11/21 (52%)	1/9 (11%)	1/3 (33%)	13/33 (39%)
Parkinsonism	..	2/21 (10%)	0/9	1/3 (33%)	3/33 (9%)
Advanced stage					
Cognitive decline	31/31 (100%)	21/21 (100%)	9/9 (100%)	3/3 (100%)	33/33 (100%)
Ataxia	22/31 (71%)	10/21 (48%)	2/9 (22%)	1/3 (33%)	13/33 (39%)
Insomnia	14/31 (45%)	..	..	..	..
Psychiatric	14/31 (45%)	18/21 (86%)	6/9 (67%)	1/3 (33%)	25/33 (76%)
Visual signs	13/31 (42%)	..	..	..	..
Pyramidal signs	9/31 (29%)	..	..	..	..
Dysautonomia	6/31 (19%)	..	..	..	..
Aphasia	..	12/21 (57%)	1/9 (11%)	2/3 (67%)	15/33 (45%)
Parkinsonism	..	8/21 (38%)	3/9 (33%)	3/3 (100%)	14/33 (42%)
Myoclonus	32% (30)	12% (16)	22% (9)‡	100% (2)‡	22% (27)‡
EEG sensitivity§	7% (27)	0% (16)	25% (4)	50% (2)	9% (22)
CSF sensitivity§¶	13% (15)	37% (8)	0% (4)	50% (2)	21% (14)
MRI sensitivity§‖	8% (26)**	5% (20)	0% (9)	0% (2)	3% (31)

Data are median (SD, range), n/N (%), or % (N). M=methionine. V=valine. PrP^{Sc}= prototypic scrapie prion protein. EEG=electroencephalogram. *An additional proven case was asymptomatic at death. †Duration is time from first symptom to death. ‡All in the late phase of disease. §95% CIs not available; data should be interpreted with reference to sample size. ¶14-3-3 and tau tests combined. ‖As determined by the presence of alterations typical of Creutzfeldt-Jakob disease. **Single-photon emission CT or PET scans were positive in all eight patients who underwent this examination.

Table 3: **Clinical features of sporadic familial insomnia and subtypes of variably protease sensitive prionopathy, by 129 genotype-PrP^{Sc} type**

cognitive impairment and patients can remain monosymptomatic for several months.[44,45]

In sCJD MM2 the presentation occasionally includes amnestic aphasia and apraxia. Although the general course is long compared with classic sCJD (14 months on average), the cognitive impairment progresses rapidly and evolves into severe dementia about 5 months after onset. In this initial phase, MRI is abnormal in most patients, whereas concentrations of CSF 14-3-3 and tau proteins are increased in about half of cases. Therefore, when these tests are positive, they can help to exclude other rapidly degenerative dementias. The EEG periodic sharp wave complexes are present in less than half of cases in the intermediate and advanced stages.[30,44] A plethora of signs including myoclonus, pyramidal and extrapyramidal deficits, and vegetative alterations, which arise 5 or more months after onset, might more clearly lead to the diagnostic suspicion of CJD.

In sCJD VV1 the cognitive impairment progresses more slowly than in sCJD MM2 and is occasionally associated with personality changes. Hypertonia, ataxia, and myoclonus develop 7 months or more after onset. The distinctive feature of sCJD VV1 is the relatively young age at onset, on average 41 years, more than 20 years earlier than sCJD MM1 (table 2).[30,45] This feature, along with behavioural changes and slow progression, can lead to the misdiagnosis of variant CJD (vCJD). However, several tests can be used to distinguish between these diagnoses. In sCJD VV1, MRI shows increased hyperintensity in the cerebral cortex or basal ganglia, or both, whereas vCJD is characterised by the distinctive bilateral pulvinar sign even at early stages.[45,51] CSF tests for tau and 14-3-3 are positive in all cases of sCJD VV1, but in less than 50% of cases of vCJD.[52] The genotype at *PRNP* codon 129 is MM in almost all cases of vCJD, and tonsil biopsy consistently shows PrP^{Sc} in vCJD but not in sCJD.[53]

Ataxic subtypes

The sCJD VV2 and sCJD MV2 subtypes of the ataxic group are less prevalent than is sCJD MM1, but combined still account for about a third of cases of sCJD; they

differ from sCJD MM1 in that they often present with ataxia, although cognitive impairment is also common, especially in sCJD MV2 (table 2).[30,46] Ataxia and cognitive deterioration can be the only symptoms for several months before extrapyramidal involvement and myoclonus are detected at later stages. Typically, in the earliest phase of the disease the patient reports mood changes (or other minor mental symptoms), dizziness, mind going blank, and unsteady gait. The paucity of presenting signs, the late appearance of myoclonus, and the rarity of EEG findings make the diagnosis of CJD in these cases difficult.[30,46] However, both 14-3-3 and tau CSF tests are highly sensitive in both subtypes and, along with MRI, they should be requested promptly in cases presenting with cognitive impairment or ataxia, or both.[46,49] At least two of these three tests have been reported to be positive in almost all cases.[46] Although the presentation is similar in these two subtypes, dementia and ataxia worsen more rapidly in sCJD VV2 than in sCJD MV2, consistent with the course of sCJD VV2 being 2–3 times shorter than that of sCJD MV2. In agreement with the similar lesion distribution, MRI examination cannot separate these two subtypes because both show prominent hyperintensity in basal ganglia and thalamus, but MRI is helpful to distinguish them from sCJD MM1, in which the thalamus is minimally or not involved.[44,54]

Sporadic fatal insomnia

Sporadic fatal insomnia (also referred to as the thalamic form of CJD) is associated with clinical features that mimic those of fatal familial insomnia, its inherited form (table 3). Sporadic fatal insomnia is the rarest type of sporadic prion disease. On the basis of 31 proven cases (18 published, 11 examined at the National Prion Disease Pathology Surveillance Center [NPDPSC] in Cleveland, OH, USA, and two at the Carlo Besta Neurological Institute in Milan, Italy), the average age at onset and duration are 46 years and 24 months, respectively.[32,33,55,56] The clinical presentation is heterogeneous, but most often includes cognitive impairment and ataxia, whereas psychiatric and visual signs are less common and dysautonomia is rare. At early stages, sleep abnormalities are not commonly reported but they are also rarely investigated anamnestically or at clinical examination. As the disease progresses, all presenting signs become more severe and pyramidal signs might arise (P Gambetti and colleagues, unpublished).[55]

The presence of rapidly progressing cognitive impairment and behavioural or mood changes associated with ataxic signs, and especially with sleep abnormalities, should lead to the inclusion of sporadic fatal insomnia in the differential diagnosis. Suspicion of sporadic fatal insomnia should prompt a polysomnographic study and possibly cerebral blood flow-single photon emission CT (CBF-SPECT) or PET. Whereas standard EEG, CSF tests, and MRI are not helpful for diagnosis of this disorder, polysomnography has been diagnostic in all five cases in which this test was done, showing reduction of sleep-related EEG activities, such as K-complexes and spindles, even in the early phase of the disease. PET examinations have consistently shown thalamic hypometabolism, associated with variable participation of cerebral cortical regions (table 3; P Gambetti and colleagues, unpublished).[55]

Variably protease-sensitive prionopathy

VPSPr is estimated to account for about 2–3% of cases of sporadic prion diseases.[42,43] However, it might be more common than present data suggest; because the diagnosis of prion disease often is not considered at presentation, many cases of VPSPr might be classified as atypical dementias and not further investigated. We are aware of 34 cases (30 published and four definitely diagnosed at the NPDPSC).[42] The distribution of these cases among the three genotypes at codon 129 is lopsided: 21 (62%) are 129VV, nine (26%) are 129MV, and four (12%) are 129MM (table 3). This distribution is quite different from that of the general white population (8% VV, 49% MV, and 43% MM) and that of patients with sCJD (19% VV, 11% MV, and 70% MM) suggesting that the role of the 129 genotype as a risk factor is different in VPSPr and sCJD.[43] The median age at onset for the three genotypes combined is 70 years. Patients with genotype 129VV have the youngest median age at onset (67 years), followed by 129MV (74 years), and 129MM (78 years). Overall median disease duration is 24 months (distribution by genotype: 129VV, 18 months; 129MV, 34 months; 129MM, 41 months).[42]

The clinical presentation is characterised by one or more components of a triad comprising psychiatric signs (psychosis or behaviour and mood changes), speech deficit, and cognitive decline, often with prominent involvement of frontal lobe functions. This onset is followed by progressive motor impairment, especially parkinsonism and ataxia. As per the distribution of clinical signs according to the 129 genotype, the three VPSPr-129MM cases lacked psychiatric signs; aphasia, parkinsonism, and ataxia were rare or absent in VPSPr-129MV and, except for aphasia, were rare or absent in VPSPr-129VV. Myoclonus occurs with all three genotypes, but is rare in patients who are 129VV, and progressively more common in those who are 129MV and 129MM. CSF was negative for the 14-3-3 protein in all cases tested except two, one 129VV and one 129MM. Periodic complexes on EEG were absent in all 129VV cases, but have been reported in one of four 129MV cases, and in one of two 129MM cases. MRI showed diffuse brain atrophy in all VPSPr cases except for one 129VV case, which showed hyperintensity of the putamen 8 months after disease onset. About 60% of VV cases, one 129MM case, and no 129MV cases have been associated with probable familial occurrence of dementia.

The differential diagnosis of VPSPr and sCJD is generally not problematic in the absence of a rapid course and myoclonus. The consistent finding of brain atrophy at

neuroimaging, the lack of typical EEG recording, and the negative 14-3-3 and tau tests in VPSPr further separate these two diseases. This combination of findings could explain why normal pressure hydrocephalus, diffuse Lewy body disease, and frontotemporal dementia are the initial diagnoses commonly made in cases of VPSPr. A prion disease is generally suspected at a later stage because of the relatively rapid progression compared with that of other dementias and dementia-like illnesses.

The early distinction of VPSPr from non-Alzheimer's dementias is difficult. Behavioural changes followed by cognitive impairment of frontal type or language deficit make VPSPr similar to the frontotemporal dementias or Pick's complex.[57] Alternatively, the frequent appearance of motor impairment, most often parkinsonism and ataxia, is consistent with subcortical dementias such as normal pressure hydrocephalus and diffuse Lewy body dementia.

The clinical disease duration of frontotemporal dementia (3–17 years) is substantially longer than the 0·6–6 year duration of VPSPr.[57] Furthermore, in this disorder, MRI and PET show selective atrophy of either the frontal or temporal lobe.[58–60]

The classic triad of urinary incontinence, gait disturbance, and frontal-type dementia that distinguishes normal pressure hydrocephalus even in the early stages is not present in VPSPr; no reported cases of VPSPr include urinary incontinence among the early signs, although gait disturbance and frontal-type cognitive impairment are common.[61] Therefore, the presence of urinary incontinence should be carefully investigated. VPSPr can be further distinguished at MRI examination by the absence of severe enlargement of the lateral ventricles associated with some degree of transependymal migration of CSF to periventricular regions, and the absence of sustained clinical improvement after CSF withdrawal that typifies normal pressure hydrocephalus.

Finally, VPSPr does not show the characteristic signs of diffuse Lewy body dementia, including fluctuation of clinical signs, vivid hallucinations, and marked hypometabolism in the occipital association cortex and primary visual cortex detected by PET and SPECT.[62] VPSPr should be viewed as a distinct component in the differential diagnosis of atypical dementias, especially frontotemporal dementias, and the diagnosis of VPSPr should be considered in patients with non-Alzheimer's dementia that are difficult to classify.

Diagnostic procedures

Four clinical tests are widely used in the diagnosis of prion diseases: EEG recording, CSF analysis, brain biopsy, and brain MRI (tables 2, 3). Sequencing of the *PRNP* gene is done to search for a mutation when the inherited form is suspected, or to exclude subtypes of prion disease invariably associated with a specific genotype at codon 129, such as vCJD and sporadic fatal insomnia (both almost invariably associated with 129MM).

EEG

The typical EEG in sCJD is characterised by periodic or pseudoperiodic sharp wave complexes (PSWC). These complexes tend to arise in the middle and late stages of disease; diffuse slowing and frontal rhythmic delta activity are often recorded in early stages.[63] The diagnostic reliability of PSWC in sCJD varies according to the sCJD subtype (tables 2, 3).[48] Triphasic waves resembling PSWC can occur in other neurological disorders such as anoxic and hepatic encephalopathies.[64–66]

CSF testing

The 14-3-3 and tau proteins seem the most reliable among several CSF diagnostic markers for prion diseases.[49,67–69] The CSF 14-3-3 test is usually done by immunoblot and is not quantitative. Therefore, a substantial percentage of tests are excluded as ambiguous. The overall reported sensitivity and specificity of the western blot test for the 14-3-3 protein in sCJD are 85–97% and 84–97%, respectively (tables 2, 3), but these findings were obtained in analyses of very small cohorts of patients.[68,69] In a population of 420 patients suspected of having a prion disease who were subsequently examined at autopsy, the 14-3-3 test had 95% sensitivity, but only 28% specificity.[49]

The tau test is slightly better than the 14-3-3 test.[49] The corresponding values for tau on the same population are: 86% sensitivity, 67% specificity, and 79% for both positive and negative predictive values, showing substantially better specificity than the 14-3-3 test. The predictive values of the tau test are inversely related to the differential between the amount of tau detected and the cutoff value, which is generally about 1150 pg/mL. Thus, the negative predictive values are 83% and 54% when tau is in the 0–799 pg/mL and 800–1149 pg/mL ranges, respectively.[49] This variability in predictive values should be considered when weighing the diagnosis in difficult cases of suspected sCJD.

Diverse disorders, such as Alzheimer's disease, multiple infarcts, brain neoplasms, and encephalitides, can lead to false positive results in these CSF tests.[49] The reliability of 14-3-3 and tau tests can improve with compliance to four principles: CSF contamination by blood should be avoided, because presence of blood increases false positive tests; CSF should be obtained as soon as sCJD is suspected; if one or both tests are negative, they should be repeated 2–3 weeks later; and MRI should precede the request of the 14-3-3 and tau tests and should be used for interpretation of the results of these tests, because pathology can be discriminated with MRI in many cases and hence it can help differential diagnosis.[49]

A CSF test called real-time quaking-induced conversion was introduced in 2010.[70] It is based on rapid

detection of minute amounts of PrP^{Sc} by in-vitro conversion of recombinant PrP^{c} acting as substrate. This test has been reported to have sensitivity greater than 83–87% and 100% specificity, but these data are based on small numbers of patients.

Brain biopsy

Immunochemical examination of fixed or frozen brain tissue obtained by biopsy remains the only way to confirm the diagnosis of sporadic prion disease in living patients. Less invasive procedures allowing the detection of PrP^{Sc} in the olfactory mucosa and skeletal muscle have not been extensively validated and adopted.[71–73] However, brain biopsy should be done with caution because the diagnosis of prion disease on the basis of careful clinical assessment and interpretation of CSF and MRI data is fairly accurate, and establishment of a definitive diagnosis of prion disease migth not necessarily benefit the patient. The notable exception being when a treatable disorder cannot be excluded by any other means.

MRI

The importance of MRI in the diagnosis of sCJD has increased in the past decade. MRI has benefited from the improved quality of diffusion-weighted imaging (DWI) sequence acquisition and the spread of electronic image display. Two types of MRI are currently used: DWI, including mean diffusivity parametric maps, and the fluid attenuated inversion recovery (FLAIR) sequence. DWI is the most sensitive and specific MRI sequence for early diagnosis of sCJD and it has rapidly replaced the use of FLAIR, which is still helpful for the differential diagnosis of sCJD and other rapidly progressive dementias (figure 2).[74]

The finding of asymmetric hyperintensity on DWI in at least three cortical non-contiguous gyri or in the striatum (caudate and rostral part of the putamen), or both, is highly suggestive of sCJD. In most cases signal abnormalities are associated with moderately reduced water diffusivity on mean diffusivity maps. On the basis of these criteria, MRI has proven to be an accurate diagnostic test (figure 2, tables 2–4).

DWI signal hyperintensities are detected in the neocortex and striatum in more than 65% of cases, in the neocortex alone in less than 20%, and in the striatum alone in 10% of cases; less than 5% show no signal abnormalities.[76] Other regions, such as the thalamus, can also be involved. MRI signal abnormalities are present at clinical onset in most cases, and can precede the onset of symptoms. MRI abnormalities diagnostic of CJD have been reported in at least one case of definite sCJD 2 months before clinical onset, when CSF tests were still negative.[77] As the disease progresses, signal alterations generally become more extensive and often spread to new regions and to contralateral symmetrical areas.[78,79] In the advanced stage of CJD, especially in cases of long duration, dilatation of the ventricles and cortical thinning are common features, whereas the DWI and FLAIR signal hyperintensities can disappear.[78,79]

To distinguish sCJD subtypes with MRI is challenging despite differences in the topology and type of lesions between subtypes. Distinct MRI lesion patterns have been reported in sCJD MM1 (and sCJD MV1) when compared with sCJD MV2 and sCJD VV2.[54] The thalamus, which is almost unremarkable in sCJD MM1, can be greatly affected in sCJD MV2 and sCJD VV2.

The most likely cause of false positive and negative results is the presence of imaging artifacts, absence of DWI in the imaging protocol, and reader's inexperience. Most frequent artifacts are attributable to susceptibility

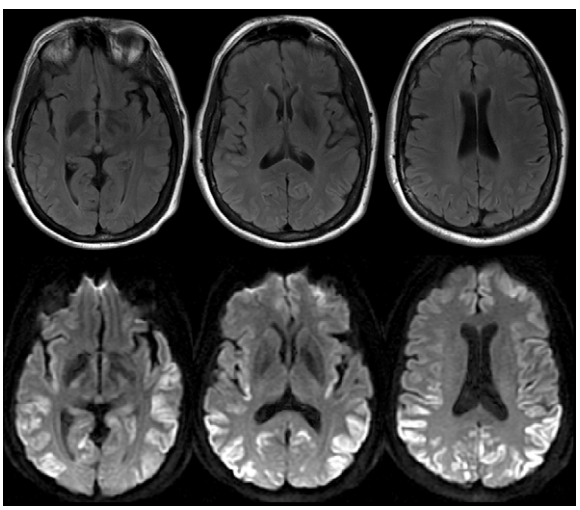

Figure 2: **FLAIR and DWI MRI in a patient with sCJD**
The top row shows FLAIR MRI, and the bottom row shows DWI MRI. The patient had the sCJD subtype with methionine homozygosity at codon 129 of *PRNP* and PrP^{Sc} type 1-2 (sCJDMM1–2). Note the much more prominent signal hyperintensity in the cortical ribbon on DWI than in FLAIR images and the extensive asymmetrical involvement of the temporal and parietal lobes. The cortex in the left frontal lobe and bilateral insula are mildly involved. The striatum and thalamus show no signal abnormality. FLAIR=fluid attenuated inversion recovery. DWI=diffusion-weighted image. sCJD=sporadic Creutzfeld-Jakob disease.

	Zerr and colleagues, 2009[50]	Vitali and colleagues, 2011[75]	NPDPSC (unpublished) and Bizzi and colleagues, 2010[76]
MRI sequence	DWI and FLAIR	DWI and FLAIR	DWI and FLAIR
sCJD (n)	120	48	154
Negative controls (n)	68	29	76
Sensitivity	83%	96%	91%
Specificity	82%	93%	92%
Accuracy	82%	95%	91%

NPDPSC=National Prion Disease Pathology Surveillance Center. DWI=diffusion-weighted imaging. FLAIR=fluid attenuated inversion recovery. sCJD=sporadic Creutzfeldt-Jakob disease.

Table 4: **Proficiency of MRI**

Search strategy and selection criteria

We searched our own reference collection and PubMed for English language publications from 1968 to March, 2012, with the keywords "CJD", "prion", "quality control", "conformational diseases", "fronto-temporal dementia", "MRI", "14-3-3", and "tau". Reports were selected on the basis of their relevance, timeliness, and clarity.

differences and poor control of eddy currents. Artifacts commonly occur in regions near the skull base; they can be minimised by acquiring sequences in several planes and doing quantitative diffusivity measurements.

Criteria based on the higher hyperintensity of the pulvinar region of the thalamus (pulvinar sign) relative to that of the anterior putamen have been issued to distinguish the MRI signal abnormalities of variant CJD from those of sCJD.[51] A few non-prion rapidly progressive dementias show DWI abnormalities that mimic those of sCJD. These include autoimmune limbic encephalitis, extrapontine myelinolysis, Wilson disease, Wernicke's encephalopathy, encephalitides in acute phase, and focal epileptic status.[75]

New MRI diffusion acquisition schemes with many diffusion times and heavier diffusion weightings are likely to be introduced shortly. Imaging at ultra-high magnetic field (7·0 Tesla) might improve spatial resolution, tissue contrast, and sensitivity. These advances might allow clinicians to discriminate microscopic differences in vacuolar size and lesion topography and ease identification of the main sCJD subtypes. More importantly, these and future developments, such as the efficient use of ligands, might lead to earlier diagnosis than is currently possible.

Conclusions and future perspectives

Substantial factual and conceptual progress has been made on most aspects of prion diseases during the past 5 years. The basic mechanism of formation of PrP^{Sc}, the pathogenic and infectious prion protein, has been established; and the notion that the initial formation of PrP^{Sc} might be localised and followed by the propagation of PrP^{Sc} throughout the brain, which could account for part of the incubation period, is becoming more accepted. Even more importantly, evidence increasingly shows that there are many diseases based on protein misfolding, including most neurodegenerative diseases, which might share a prion-like mechanism of propagation.

This information seems sufficient to mount several approaches to treatment. Furthermore, basic treatment strategies are likely to be common to neurodegenerative diseases based on protein misfolding. However, no progress has been made on clinical treatment (appendix). Perhaps globally coordinated research into all rational therapeutical approaches should be implimented, following the successful blueprint of the Human Genome Project. PrP^{Sc}-targeted immunotherapy, which can be modelled on the strategies currently used for Alzheimer's disease, is a promising treatment strategy, because passive immunisation with antibodies to PrP^{C} and active mucosal vaccination can protect rodents against prion infection from a peripheral source.[80,81] Furthermore, humanised versions of antibodies successfully tested in in-vivo models could be available in the near future for clinical trials in prion diseases (appendix).

Successful treatment will depend on early diagnosis, especially in the rapidly progressing prion diseases. In a genetically determined prion disease, the diagnosis could be made by PET about a year before symptom presentation, offering a window of opportunity for treatment to prevent clinical disease.[82] Presymptomatic diagnosis of sCJD by MRI has also been reported.[77] More reliable diagnostic approaches are expected soon. They will use more powerful MRI technology and CSF tests based on direct detection of PrP^{Sc} rather than surrogate markers. It is tempting to envision future non-invasive diagnostic technologies—involving imaging or abnormal protein detection, or both—that can identify neurodegenerative diseases at a presymptomatic stage with one test. In the meantime, clinicians should take full advantage of clinical examination supported by prompt and combined use of the available MRI and CSF diagnostic tests that we have described.

Contributors

PG designed the structure of the Review, wrote the introduction, basic mechanisms section, and the conclusions, and edited the report. GP wrote the clinical diagnosis and diagnostic procedures sections, provided searches of published work, data collection, and created the tables. AB wrote the MRI section and made suggestions and contributed to the discussion of pertinent data. GF co-wrote the treatment section, collected data, and contributed to the editing of the report. JGS contributed to the general design of the Review and to the introduction. FT provided information about treatment, critically reviewed the sections about clinical diagnosis and diagnostic procedures, and edited and reviewed the report.

Conflicts of interest

We declare that we have no conflicts of interest.

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