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Molecular biology and pathology of prion strains in sporadic human prion diseases

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Abstract

Prion diseases are believed to propagate by the mechanism involving self-perpetuating conformational conversion of the normal form of the prion protein, PrP^C, to the misfolded, pathogenic state, PrP^{Sc}. One of the most intriguing aspects of these disorders is the phenomenon of prion strains. It is believed that strain properties are fully encoded in distinct conformations of PrP^{Sc}. Strains are of practical relevance to human prion diseases, as their diversity may explain the unusual heterogeneity of these disorders. The first insight into the molecular mechanisms underlying heterogeneity of human prion diseases was provided by the observation that two distinct disease phenotypes, and their associated PrP^{Sc} conformers, co-distribute with distinct PrP genotypes as determined by the methionine/valine polymorphism at codon 129 of the PrP gene. Subsequent studies identified six possible combinations of the three genotypes (determined by the polymorphic codon 129) and two common PrP^{Sc} conformers (named types 1 and 2) as the major determinants of the phenotype in sporadic human prion diseases. This scenario implies that each 129 genotype-PrP^{Sc} type combination would be associated with a distinct diseases phenotype and prion strain. However, notable exceptions have been found. For example, two genotype-PrP^{Sc} type combinations are linked to the same phenotype and, conversely, the same combination was found to be associated with two distinct phenotypes. Furthermore, in some cases, PrP^{Sc} conformers naturally associated with distinct phenotypes appear, upon transmission, to lose their phenotype-determining strain characteristics. Currently it seems safe to assume that typical sporadic prion diseases are associated with at least six distinct prion strains. However the intrinsic characteristics that distinguish at least four of these strains remain to be identified.

Keywords

Creutzfeldt-Jakob disease; sporadic fatal insomnia; variably protease-sensitive prionopathy; 129 polymorphism; PrP^{Sc} type; PrP sequencing

Introduction

The existence of prion strains is one of the most controversial and poorly understood facets of prion diseases. The concept of strain, borrowed from virology, was introduced to the field of prion diseases at the time when it was believed that these disorders, known as transmissible spongiform encephalopathies, were caused by viruses or other infectious

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agents yet to be identified. The idea that prion diseases can be associated with different strains of the infectious agent emerged from the observation that animals inoculated with brain homogenates from different scrapie-infected donors consistently developed a disease with distinct incubation times and histopathological lesions, and these differences could be stably propagated in subsequent passages into syngenic hosts. More recently, the apparent analogy between strain phenomena in viruses and prion diseases has been pushed even further with the demonstration that prion strains can undergo apparent mutations and selective amplification, which may lead to drug resistance [43].

While such phenomena are easily explained for viruses carrying nucleic acid that confers upon them the propensity to mutate and undergo selection, the existence of different prion strains has for many years presented a major challenge to the protein-only hypothesis of prion diseases. The latter model asserts that the infectious pathogen is not a virus but a misfolded form of the prion protein, PrP^{Sc}, which self-perpetuates by a mechanism that entails binding to the normal prion protein, PrP^C, and inducing its conversion to the PrP^{Sc} state. A series of recent studies has provided conclusive evidence in support of this protein-only hypothesis [15,32,41,45,73]. Within the context of this model, prion strains are believed to be encrypted in distinct conformations of PrP^{Sc}. This conformational diversity may arise from a number of factors, including the amino acid sequence of substrate PrP^C, the cell and tissue environment where the conversion takes place, and the process leading to the selection of the successful strain from the PrP^{Sc} population initially engendered [13].

Human prion diseases: Heterogeneity

The issue of strains is of particular significance in the case of human prion diseases, for the ultimate cause of the extraordinary diversity of these disorders may lie in strain variability (Table 1 and 2). Human prion diseases are unique in that they may be acquired by infection, be inherited, or arise spontaneously. The critical initial event in the pathogenesis of the form acquired by infection is the interaction between the exogenous PrP^{Sc} and the endogenous PrP^C of the recipient. This interaction results in the formation of endogenous PrP^{Sc} which propagates further by autocatalytic conversion of additional PrP^C molecules, while the original exogenous PrP^{Sc} is cleared. The physicochemical characteristics of the exogenous PrP^{Sc}, the site of the initial conversion, and the tissues and organs involved on the pathway towards brain invasion are among many factors that may contribute to strain variability of the acquired cases. In the inherited prion diseases, the presence of a mutation is thought to alter the properties of PrP^C, promoting its conversion to the PrP^{Sc} state [37]. The combination of specific mutation with the methionine/valine (M/V) polymorphism at codon 129 is the major determinant of strain variation in familial cases [20]. The nature of the etiological events which lead to the idiopathic or sporadic form of prion diseases is less clear. Several scenarios, not mutually exclusive, have been proposed as explanations of the initial PrP^C misfolding and PrP^{Sc} formation. One scenario posits an error in PrP post-translational processing, or the occurrence of a somatic mutation coupled with a weakening in the stringency of the quality control system which is known to occur in aging [21,33,60]. Another scenario presupposes the presence of PrP^{Sc}-like oligomers (denoted PrP* or silent prions) under normal conditions [75]. Following accidental stabilization likely facilitated by aging, PrP* would acquire the capability to convert PrP^C to PrP^{Sc} thereby triggering the disease [16]. Regardless of the etiology, all three forms of prion disease present a remarkable degree of phenotypic heterogeneity, which is likely to be closely related to the diversity of prion strains.

History of human prion strains

The very first evidence of diversity in human prion strains was reported in 1992 in a study describing the linkage of the D178N PrP gene mutation (leading to the substitution of aspartic acid with asparagine at PrP position 178) to a phenotype named fatal familial insomnia (FFI) [44,46,47]. It was observed that the PrP^{Sc} associated with FFI is characterized by an electrophoretic mobility different from that of PrP^{Sc} in sporadic CJD (sCJD) [47]. Subsequent studies described the linkage of the D178N mutation not only to FFI but also to yet another CJD-like phenotype, which was identified as familial (f) CJD^{D178N} [20]. These two phenotypes were found to co-segregate with the genotype of codon 129 present in the mutated allele: FFI was associated with the methionine codon (129M), whereas fCJD^{D178N} segregated with the valine codon (129V) (Figure 1) [20]. Therefore, while the D178N mutation may trigger the disease, codon 129 on the mutated allele appears to determine the basic characteristics of the disease phenotype. These observations provided the first evidence that the 129M/V polymorphism plays an important role in determining the prion disease phenotype. Furthermore, immunoblot examination demonstrated that the protease-resistant PrP^{Sc} in the two conditions had distinct electrophoretic mobilities: in FFI the unglycosylated form of the protease-resistant PrP^{Sc} had an electrophoretic mobility of 19 kDa, whereas in fCJD^{D178N} the corresponding mobility was approximately 21 kDa. These two PrP^{Sc} conformers were subsequently named types 1 (the 21 kDa) and type 2 (the 19 kDa) (Figure 1) [48,55].

FFI, along with sCJDM1 and fCJD^{E200K}, were then transmitted to transgenic (Tg) mice expressing chimera human/mouse PrP with 129M (fCJD^{D178N} could not be transmitted likely because of the mismatch in the 129 residue with the Tg mouse PrP) (Figure 1). The PrP^{Sc} in these infected mice faithfully reproduced electrophoretic mobilities of the inoculum (i.e., 19 kDa in FFI and 21 kDa in fCJD^{E200K} and sCJDM1), and these characteristics were maintained upon second passage [38,71]. Furthermore, the pattern of PrP^{Sc} deposition in the brain was also different in the affected mice inoculated with brain homogenates from FFI, sCJDM1 or fCJD^{E200K}. These findings clearly indicate that the PrP^{Sc} isoforms associated with FFI and fCJD^{E200K} are distinct, representing stable prion strains which can be reproduced even in the absence of the original PrP gene mutations in the host animals.

The realization that the polymorphic codon 129 of the PrP gene could modify the phenotype of familial prion diseases raised the question as to whether the 129 genotype could also affect the phenotype in sporadic prion diseases and thereby explain, at least in part, the phenotypic heterogeneity of these diseases.

Cases with proven sCJD were then subdivided into six groups according to the six possible combination of their PrP^{Sc} type (either 1 or 2) with each of the three possible 129 genotypes (MM, MV or VV). The phenotypes of the six possible combinations were examined for homogeneity within the group and diversity between groups. This exercise led to a new classification of sporadic prion diseases based on molecular rather than clinical or histopathological features, making possible the identification of six distinct phenotypes of sporadic prion disease (Table 2) [18,55,56]. The first and most common phenotype is associated with both the 129MM and 129MV genotypes and with PrP^{Sc} type 1 [sCJDM1(MV)1]; it is often referred to as “classic” CJD. The second and third phenotypes associated with sCJDM2 and sCJDVV1 had not been identified as separate subtypes in previous clinical or pathological classifications. The sCJDM2 phenotype is characterized by the very large and often confluent vacuoles of the spongiform degeneration (SD), whereas the frequently early age at onset defines sCJDVV1. The two phenotypes labeled sCJDMV2 and sCJDVV2 match the phenotype previously labeled as cerebellar or ataxic. Despite their similar clinical features, these two phenotypes can easily be distinguished by

the presence in the cerebellum of kuru-like plaques in sCJDMV2 (but not in sCJDVV2), and their different patterns of cerebellar PrP immunostaining. The sixth phenotype is associated with sFI. This phenotype is virtually indistinguishable from that of FFI and matches the sCJD form previously identified as thalamic. This classification goes a long way towards providing a molecular basis for the phenotypic heterogeneity and in facilitating identification of the PrP^{Sc} strains in sCJD (Table 2).

An alternative classification of PrP^{Sc} types and their pairing with CJD phenotypes has been proposed by Collinge and collaborators [12,26,42]. This classification differs from that of Parchi and colleagues discussed above in two major aspects: 1) It recognizes three (not two) PrP^{Sc} types based on electrophoretic mobility, and 2) it proposes the same nomenclature, i.e. with progressive PrP^{Sc} type numbers, also for PrP^{Sc} isoforms with different ratios of the three PrP glycoforms. In the classification by Parchi and coworkers, PrP^{Sc} types 1 and 2 that show different glycoform ratios are differentiated with letters (Figure 2). Including variant CJD, six human PrP^{Sc} types have been identified according to Collinge and colleagues. As for the associations with the sCJD phenotypes, PrP^{Sc} type 1 would be present in a subtype characterized by a slightly shorter disease duration, which includes sCJDMM and sCJDMV; type 2 and 3 would be found in all three 129 phenotypes in cases generally of long duration. However, the PrP^{Sc} electrophoretic profile in sCJDMM3 and sCJDVV3 apparently differed from that of sCJDMV3.

The studies on classification of sporadic prion diseases and PrP^{Sc} typing also provided insight into the relationship between the 129 genotype and the PrP^{Sc} type. In the original study of Parchi et al over 95% of cases homozygous for methionine at codon 129 (129MM) were found to be associated with PrP^{Sc} type 1, while 86% of sCJD cases associated with PrP^{Sc} type 2 were found to be homozygous for valine or methionine/valine heterozygous (129VV and 129MV) [18,56]. Therefore, the 129MM genotype appears to favor the formation of PrP^{Sc} type 1, whereas type 2 formation is favored by the presence of one or two 129V alleles. The study of the recently discovered cases of sCJD that actually carries both types 1 and 2 further support this notion (Table 3 and see below) [8,59].

Furthermore, amino acid sequencing has revealed that while the major N-termini of PrP^{Sc} types 1 and 2 are at residue 82 and 97, respectively, secondary N-termini are present in both PrP^{Sc} types 1 and 2 (Figure 3) [58]. Interestingly, the secondary N-termini appear to be non-random but rather to correlate with the PrP^{Sc} types and the 129 genotype. They show three main characteristics. First, they are more prevalent in PrP^{Sc} type 2 than in type 1. Second, within each PrP^{Sc} type, secondary cleavages are more prominent in the 129MV heterozygous than homozygous 129 genotypes. Third, they appear to be distributed toward opposite directions in the PrP sequence: in PrP^{Sc} type 1 they appear to involve to a greater extent the PrP sequence that is C-terminal to the main cleavage site, whereas in PrP^{Sc} type 2 they extend more toward the region that is N-terminal to the main cleavage site (Figure 3). Therefore, the 129 genotype may affect not only the main cleavages of the protease resistant fragments of PrP^{Sc} types 1 and 2 but also the number of the secondary cleavages and, therefore, the degree of conformational heterogeneity within each PrP^{Sc} type.

Altogether, available data strongly suggest that PrP^{Sc} type formation and finer variations within each type are influenced by the genotype at codon 129.

Residue 129 polymorphism and strain determination: A biophysical perspective

The fundamentally important role of M/V polymorphism at codon 129 in human PrP^C prompted studies aimed at understanding the effect of this polymorphism on the structure

and biophysical properties of the recombinant prion protein. However, extensive NMR and X-ray crystallographic studies have failed to detect any significant differences in the global and local protein fold between the 129M- and 129V-containing structure [27,40]. Furthermore, residue 129M/V polymorphism was found to have no measurable effect on the global thermodynamic stability, folding or dynamics of the PrP monomer [27]. These findings suggest that the effect of residue 129 on prion diseases is mediated by events taking place at the time of PrP^{Sc} formation, events which probably influence the conformation of the PrP^{Sc} oligomer [27].

Consistent with this view, structural differences which were dependent upon polymorphism were detected by FTIR spectroscopy for amyloid fibrils formed by recombinant PrP 90-231 [2] and recombinant PrP23-144 [30]. An interesting insight into the potential nature of these structural effects was provided by recent crystallographic data for six-residue segments of PrP centered on residue 129. These studies revealed that the crystal structures for M- and V-containing peptides differ in packing within the so-called 'steric zipper' motif [3]. It was suggested that these packing effects could account for differences in disease susceptibility between individuals who are homozygous or heterozygous for either 129M or 129V [3]. However, it is not known whether the structures of these short segments adequately represent structural features within the corresponding region of PrP^{Sc}.

The effect of 129 polymorphism on the structure and biophysical properties of PrP has also been explored in the context of the pathogenic mutation D178N. From early NMR measurements, it was inferred that, in wild-type PrP, Asp178 forms a salt bridge with Arg164 and has the potential to form a hydrogen bond with Tyr128, a residue sequentially adjacent to position 129 [65]. It was also proposed that the Asp→Asn replacement at position 178 would affect this network of hydrogen bonds differentially depending on residue 129 Met/Val polymorphism, providing a rationale for polymorphism-dependent phenotypic variability of inherited prion diseases with D178N mutation. A more detailed insight into structural effects of D178N mutation was provided by recent crystallographic data, which revealed mutation-dependent differences in the loop region (residues R164-S170), as well as polymorphism-dependent differences in the local environment surrounding the altered D178N residue [40]. It was also shown that the polymorphism at residue 129 strongly affects conversion of the D178N variant of recombinant PrP to amyloid fibrils, with the 129M polymorph undergoing substantially faster conversion than the 129V polymorph [2]. Furthermore, fibrils formed by the D178N/129V variant appear to be structurally distinct from those formed by the D178N/129M protein [2]. Altogether, this biophysical insight strongly suggests that by modulating both the conversion process and the conformation of PrP^{Sc}, residue 129V/M polymorphism governs susceptibility to prion disease and prion strain properties.

Prion strains in human prion diseases

Sporadic prion diseases: Creutzfeldt-Jakob disease and fatal insomnia

From the perspective of the disease, prion strains may be viewed as PrP^{Sc} conformers which often (but not necessarily always) are associated with distinct disease phenotypes [4,6,12,61,68,71]. Within the context of this definition, PrP^{Sc} types 1 and 2 definitely fulfill the requirements of distinct prion strains for several reasons. First, they have different physicochemical characteristics such as proteolytic cleavage sites and conformational stability profiles, the features implying different conformations [9,11,36,51,58]. Second, upon inoculation they cause diseases characterized by different incubation times, histopathologies and PrP^{Sc} distributions [5,38,49,69,70]. Finally, they can be faithfully replicated by protein misfolding cyclic amplification (PMCA) [10,31,67]. However, the identification of six phenotypes, five in sCJD and one in sFI (according to the classification

of Parchi and colleagues [56]), raises the question of the number of distinct strains of sCJD [18]. Distinctive clinical, histopathological and PrP immunostaining features of the six phenotypes point to an association with at least six distinct PrP^{Sc} strains. Should this be the case, the strains of sporadic prion diseases would far exceed types 1 and 2. This implies that PrP^{Sc} types 1 and 2, rather than just representing two strains, actually define two categories of strain subtypes, each associated with a distinct phenotype.

Several studies of sCJD subtypes, including transmission to transgenic mice expressing human PrP^C or chimera human/mouse PrP have been reported in the last 15 years [12,25,35,70,71,72]. These studies provide strong evidence that some sCJD subtypes are indeed associated with distinct PrP^{Sc} strains. However, only one recent study has systematically attempted to determine the number of PrP^{Sc} strains associated with the major subtypes of sCJD [5].

Bishop and collaborators tested the strain characteristics of PrP^{Sc} isoforms associated with sCJD (but not sFI) by inoculating sCJD brain homogenates representing the six possible combinations of the 129 genotype and PrP^{Sc} type (i.e., MM, MV and VV each associated with PrP^{Sc} 1 or 2) into knock-in Tg mice expressing human PrP^C with each of the three 129 genotypes [5]. Using incubation times, lesion profiles, and the pattern of PrP^{Sc} intracerebral deposition, this study identified four strains and found them to be associated with the five phenotypes identified in sCJD by Parchi et al [5,56]. Specifically, by bioassay, MM1PrP^{Sc} could not be distinguished from MV1PrP^{Sc} nor could MV2PrP^{Sc} be distinguished from VV2PrP^{Sc}. VV1PrP^{Sc} and MM2PrP^{Sc} could be identified as distinct strains, although both strains appeared to be unusual and difficult to replicate even in their homonymous 129 backgrounds.

The study by Bishop et al [5] has several implications. First, the inability to separate MM1PrP^{Sc} and MV1PrP^{Sc} is consistent with the strong similarity of the sCJD phenotypes associated with these two PrP^{Sc} isoforms. It also supports the finding that these two sCJD PrP^{Sc} isoforms (but not other PrP^{Sc} isoforms including VV1PrP^{Sc}) share the same PK-resistance profile and sensitivity to pH as well as the presence of the same truncated anchorless fragment of 18.5 kDa [50,52]. The lack of distinguishing features suggests two possibilities which are not mutually exclusive. Thus, it is possible that in sCJDMV1 the PrP^{Sc} strain originates exclusively or overwhelmingly from the conversion of 129MPrP^C. Alternatively, the difference in strain between MM1PrP^{Sc} and MV1PrP^{Sc} could be too subtle to generate distinct phenotypes. Indistinguishable physicochemical features have been recently reported for some prion strains, leading to the concept of substrain [13,43]. Indeed, as mentioned above, N-terminal sequencing of PrP^{Sc} isoforms has shown that while MM1PrP^{Sc} and MV1PrP^{Sc} share PK cleavage sites at residues 82 and 78, only MV1PrP^{Sc} is cleaved at the more C-terminal residues 90 and 97, the latter being the major cleavage site of PrP^{Sc} type 2 [58]. Although additional sCJDMV1 cases need to be examined, these extra cleavage sites argue for the presence of a small amount of PrP^{Sc} type 2 in MV1PrP^{Sc}.

A second consideration suggested by the data of Bishop and colleagues is that although by bioassay MV2PrP^{Sc} and VV2PrP^{Sc} were found to have identical strain features, the clinical and pathological differences between sCJDMV2 and sCJDVV2 — the two phenotypes to which they are associated — suggests otherwise. Major neuropathological differences occur in the cerebellum, where in the case of sCJDMV2 PrP^{Sc} kuru plaque-like deposits with the tinctorial characteristics of the amyloid are found along with good preservation of neurons [17,18,55,56]. In contrast, in sCJDVV2 the PrP deposits are loose and amyloid-free, and there is a significant loss of neurons. These contrasting features argue that the mode by which the PrP^{Sc} aggregates form, and possibly the toxicity associated with these aggregates, differ in these two sCJD subtypes. Consistent with the presumed higher toxicity of the PrP^{Sc}

aggregates in sCJDVV2, the clinical duration of the disease is also significantly shorter in this sCJD subtype (6.5 months with 3-18 month range in compared with 17 months with a 5-72 month range in sCJDMV2) [13,56]. According to a recently proposed model of strain pathogenicity, the diverse features that distinguish sCJDMV2 from sCJDVV2 could result from different kinetics of PrP^{Sc} propagation [13]. Furthermore, sequencing has shown that the N-terminus of PK-resistant core of MV2PrP^{Sc} is somewhat more ragged than that of VV2PrP^{Sc}. The presence in MV2PrP^{Sc} of a fragment starting at residue 82, the major cleavage site of PrP^{Sc} type 1, is consistent with the presence of small amounts of PrP^{Sc} type 1 [58]. In agreement with this finding, the electrophoretic profiles of MV2PrP^{Sc} and VV2PrP^{Sc} from most brain regions are easily distinguished by the presence of two highly PK-resistant bands (corresponding to the unglycosylated isoform) in the MV2PrP^{Sc} blot profile and only one such band in the profile of VV2PrP^{Sc} [50,51,59]. Furthermore, the electrophoretic profile of VV2PrP^{Sc} shows an additional fragment of approximately 18 kDa [59]. These findings suggest that, although not distinguishable after transmission to receptive animals, the MV2PrP^{Sc} and VV2PrP^{Sc} isoforms behave differently in the human brain. Distinct physicochemical features may be revealed upon more extensive examination.

The poor transmissibility of both MM2PrP^{Sc} and VV1PrP^{Sc} to Tg mice of all three 129 genetic backgrounds sets these two strains apart, suggesting that they form differently from the other sCJD strains. According to a prevailing hypothesis, the primary structure of the host's PrP^C determines the spectrum of PrP^{Sc} conformations that can be generated in a given species [13,29,43]. However, it is likely that several distinct isoforms of PrP^{Sc} are initially generated when PrP^C converts to PrP^{Sc}. The successful strain is viewed as the result of a selection dictated by the extent to which the rate of conversion and propagation exceeds the rate of clearance for that particular PrP isoform [13]. According to this hypothesis, VV1PrP^{Sc} and MM2PrP^{Sc} might represent isoforms which under some exceptional conditions 'escape' the selection process. This would explain the extreme rarity of these strains (sCJDMM2 and sCJDVV1 combined account for only ~3% of all cases of sCJD [25,56]) as well as the difficulty encountered in replicating them in humanized Tg mice regardless of the 129 background [5].

Finally, it is noteworthy that neither the study of Bishop et al nor any other study on sCJD transmission to humanized Tg mice has found the PrP^{Sc} type 1 described by Collinge et al (that displays a slightly slower gel mobility than the PrP^{Sc} type 1 described by Parchi et al) [5,12,56].

Sporadic CJD with co-occurrence of PrP^{res} type 1 and type 2—It has now been clearly established that, although sCJD subtypes exclusively associated with PrP^{Sc} type 1 or type 2 account for the majority of the cases, the concurrent presence of both PrP^{res} types is significant, varying between 15% and 43% according to the sCJD 129 genotype (Table 3) [8,22,42,59,64,69,72]. The co-occurrence of PrP^{res} types 1 and 2 (hereafter identified as type 1-2) seems to follow certain general patterns. The two PrP^{res} types can be found together in the same brain region or separately in different regions. Regardless whether combined or separate, the topographic distribution of PrP^{res} 1-2 is non-random: it favors specific brain regions such as the cerebral neocortex and the thalamus, but is less common in the cerebellum. Therefore, every brain region can contain both PrP^{Sc} types, though some regions, such as the thalamus, appear to be more apt to propagate both PrP^{Sc} types [8,59]. Again, the representation of types 1 and 2 appears to correlate with the 129 genotype; in sCJDMM1-2 type 1 dominates, whereas in sCJDMV and sCJDVV the opposite is observed, indicating that the strong association of PrP^{Sc} type 1 with the 129MM homozygosity and of PrP^{Sc} type 2 with the presence of at least one 129V allele is also maintained in sCJD1-2 [56,59]. In general, the concomitant presence of PrP^{Sc} types 1 and 2 has the tendency to modify the clinical and histopathological phenotype which exhibits features that are

intermediate between the phenotypes associated with the corresponding sCJD1 and sCJD2 variants [8,59]. This is particularly noticeable in the sCJDMM group, in which case the shift of the phenotype from sCJDMM1 to sCJDMM2 is directly related to the amount of PrP^{Sc} type 2 present in the brain [8]. The PrP^{Sc} type-related shift of the phenotype is much less noticeable for sCJDMV1-2 and sCJDVV1-2, perhaps because the presence of one or two 129V alleles makes the phenotypic features of sCJDMV2 and sCJDVV2 dominant over those of sCJDMV1 and sCJDVV1 [7]. Additional studies are needed to more clearly define the phenotype and PrP^{Sc} characteristics of sCJDMV1-2 and sCJDVV1-2 [7,59]. With regard to PrP^{Sc} strain characteristics in sCJDMM1-2, it is intriguing that the co-existence of PrP^{Sc} types 1 and 2 in the same brain locale seems to alter the characteristics of each of the two PrP^{Sc} types compared to those displayed when they occur separately. For example, the resistance to denaturation of PrP^{Sc} type 1, as determined by the conformation stability immunoassay (CSI), becomes similar to that of PrP^{Sc} type 2, although the cleavage sites of the shifted PrP^{Sc} do not change (Figure 4). A similar effect is also seen after PrP^{Sc} types 1 and 2 are mixed in vitro [8]. Therefore, type 1 and 2 co-occurrence in vivo, as well as the interaction of the two types in vitro, may change some conformational characteristics of PrP^{Sc} type 1 [8]. These findings suggest yet another potential mechanism of strain mutation [1,13,43]. On the whole, the co-occurrence of PrP^{Sc} types 1 and 2 is likely to shed light on the effect that co-occurring strains may exert upon one another as well as on the selection process leading to the presence of two major strains [1,8].

Sporadic fatal insomnia (also occasionally referred to as sCJDMM2 thalamic)

—Presents a special challenge in the assessment of prion strains associated with sporadic prion diseases. Although the clinical and histopathological phenotypes of sFI and sCJDMM2 are quite distinct, both conditions are associated with the 129MM genotype and PrP^{Sc} type 2 [89,56-58]. A most striking variation between these two diseases lies in their contrastive lesion topography. In sFI the thalamus is very severely affected and the cerebral cortex is often almost spared, while the opposite is true for sCJDMM2. This suggests that the histopathology starts, and the pathogenic PrP^{Sc} initially acts, in different brain regions. The lack of correlation of the phenotype with the 129 genotype and the PrP^{Sc} type in these two diseases raises questions regarding the role and nature of additional factors (beyond the 129 genotype and PrP^{Sc} type) as potential determinants of prion strains.

A possible feature distinguishing PrP^{Sc} conformers associated with sFI and sCJDMM2 is the pattern of glycosylation as revealed by two dimensional WB analysis [54]. Whether differences in glycosylation can explain variations in the phenotype remains, however, to be determined [9,63]. Furthermore, variations in the PK-resistant PrP fragments associated with sFI and sCJDMM2 have been reported [52].

Atypical prion diseases: Variably protease-sensitive prionopathy (VPSPr)

A disease affecting the prion protein but exhibiting characteristics different from those of the classic prion diseases was first reported in 2008 [19]. The study of eleven original cases revealed several major features which distinguished this disorder from common sporadic prion diseases. These include: the relatively long duration of nearly two years; clinical presentation more consistent with that of an atypical dementia than sporadic prion disease; the distinctive ladder-like electrophoretic profile and relatively low protease-resistance of the insoluble, disease-associated PrP (PrP^{Dis}) (Figure 2). Due to these characteristics as well as the uncertainty regarding transmissibility, this condition was originally named “protease-sensitive prionopathy”. Another peculiar feature of this condition is that six out of ten cases that could be investigated had a positive family history of cognitive impairment. However, none of these cases was associated with a mutation in the open reading frame of the PrP gene.

All eleven cases of the original report were 129VV. However, a subsequent study revealed that the same disease also affects subjects with the 129MV and 129MM genotypes and the disease was renamed “variably protease-sensitive prionopathy” (VPSPr) (see below) [77]. Currently, 29 cases of VPSPr have been reported: 20 are 129VV, 6 are 129MV, and 3 are 129MM [19,23,28,39,77]. Thus, after the original report of CJD in 1921, VPSPr appears to be the second presumably sporadic disease involving the prion protein that affects all three 129 genotypes [34].

The finding that VPSPr affects the three 129 genotypes raises a number of intriguing questions. One of them is whether each 129 genotype is paired with a distinct phenotype and distinct PrP^{Dis} characteristics as is the case with subtypes of sCJD [18,56]. Clinically the 129VV and 129MV VPSPr cases (the availability of only two 129MM symptomatic cases precludes the comparative clinical study of this subset) differ significantly in duration and the prevalence of myoclonus. Though histopathologically these cases differ only slightly with respect to PrP immunostaining patterns, the major difference lies in the PK resistance of PrP^{Dis} [77]. As originally reported, PrP^{Dis} from 129VV cases is very sensitive to PK digestion, whereas the PK-resistance of PrP^{Dis} from 129MM cases is comparable to that of PrP^{Sc} in classic prion diseases, and the proteolytic resistance of 129MV cases lies somewhere between those of 129 MM and 129 VV cases. This variability in PK resistance that co-distributes with the 129 genotype prompted the addition of “variably” to the original label of “protease-sensitive prionopathy” [77]. Furthermore, although the ladder-like electrophoretic profile is shared by all three VPSPr 129 genotypes, the ratios of the individual bands are different, suggesting that though the same PrP^{Dis} sites are cleaved by PK, their accessibility is dissimilar. Finally, the PrP^{Dis} conformers associated with the three VPSPr genotypes also differ in the degree of immunoreactivity with monoclonal antibodies 3F4 (epitope within residues 106-110) and 1E4 (epitope within residues 97-105) [76,78]. Although these variations may not be as distinctive as they are between the subtypes of sCJD, the three 129 genotypes of VPSPr are associated with distinguishable phenotypes and PrP^{Dis} characteristics.

Of note, there appears to be a drastic difference between VPSPr and classic prion diseases with respect to the prevalence of the three 129 genotypes. In VPSPr the 129MM, 129MV and 129VV genotypes account for 10%, 23% and 67% of all cases, respectively. In contrast 129MM, 129MV and 129VV patients account for 72%, 11% and 17%, respectively, of all sCJD and sFI cases combined [18,56]. Furthermore, in VPSPr, as well as in sCJD, the representations of the three 129 genotypes markedly differ from the prevalence of these genotypes in the general Caucasian population [53]. In sCJD, the overrepresentations of the 129MM and (to a lesser extent) the 129VV genotypes, combined with the underrepresentation of the 129MV genotype, have been attributed to more efficient conformational conversion of PrP^C to PrP^{Sc} in individuals homozygous at codon 129 compared to those heterozygous at this codon [3,53]. This mechanism does not seem to play a role in VPSPr, in which the 129VV genotype appears to pose by far the highest risk, whereas the 129MM genotype appears to be protective.

Altogether, available data argue that PrP^{Dis} associated with VPSPr is distinct from the PrP^{Sc} strains associated with the various subtypes of sCJD, and that the 129 genotype may play a different role in PrP^{Dis} formation [19]. Furthermore, the distinctive phenotypic and PrP^{Dis} features observed among the three 129 genotypes suggest that PrP^{Dis} types associated with these genotypes represent distinct strains (or, at least, sub-strains according to the recently proposed terminology [14,74]). More detailed characterization of these strains awaits the results of ongoing studies on amplification and transmissibility.

Concluding remarks

The assessment of the number of strains associated with human sporadic prion diseases must take into account the observation that both the 129 genotype and the PrP^{Sc} type are the major determinants of the disease phenotype. However, the role of these two determinants is complex and interrelated. First, the genotype at codon 129 appears to be a major determinant of PrP^{Sc} types 1 and 2 which, in turn, are also associated with different phenotypes. However, the three 129 genotypes also may act as determinants of the phenotype in the sCJD associated with PrP^{Sc} types 1 as well as type 2. Since there are three 129 genotypes and two PrP^{Sc} types, one would expect that, at least theoretically, up to six distinct strains could be associated with sCJD and sFI combined, each of them corresponding to one of the six possible pairings of three codon129 genotypes with the two PrP^{Sc} types. However, as discussed in this article, there might be a number of exceptions to this general scenario.

First exception is exemplified by sCJDMM1 and sCJDMV1. Despite the different 129 genotype, these two subtypes of sCJD share the same phenotype and appear to be associated with same strain, suggesting that the 129V allele plays no role in determining the phenotype or the PrP^{Sc} characteristics in sCJDMV1. The opposite exception is sCJDMM2 and sFI, which, despite being associated with two very different phenotypes, share the same combination of 129MM genotype and PrP^{Sc} type 2. Although the mechanism of phenotypic determination in these two diseases is currently unknown, it is reasonable to expect that specific features which discriminate between the two PrP^{Sc} strains associated with sCJDMM2 and sFI do exist and will eventually be found.

Another layer of complexity arises from the inability of propagating to receptive mice the distinct strain properties apparently exhibited by the PrP^{Sc} conformers associated with sCJDMV2 and sCJDVV2 [5]. Again, although the phenotype-determining characteristics of some strains are apparently evident in the human disease, they might be difficult to propagate experimentally, perhaps because of their instability.

Yet another interesting situation is presented by sCJDVV1, in which PrP^{Sc} appears to have all the features of a distinct strain. However, sCJDVV1, like sCJDMM2 and sFI, has a very low prevalence and, like sCJDMM2 is difficult to transmit to humanized Tg mice [5]. Therefore, the strains associated with these three phenotypes are unusual. They might represent variant strains which have escaped the selection process believed to occur during strain formation [13].

Altogether, the picture emerging from these considerations suggests that despite the exceptions previously noted, there are at least six different strains associated with sCJD and sFI, three of which are common while the other three are rare or variant strains (Figure 5).

Despite recent progress in strain-dependent characterization of sporadic human prion diseases, there are still a number of unresolved questions: Can the presumed strains associated with sCJDMM2 and sFI be distinguished based on their physicochemical properties? What mechanism governs the reciprocal influence of PrP^{Sc} types 1 and 2 when they co-occur in sCJDMM1-2? Are some strains inherently unstable and, thus, unable to propagate some of their phenotype-determining characteristics upon transmission? Does PrP^{Dis} associated with VPSPr qualify as a true strain which encompasses three distinguishable sub-strains? Clearly, additional studies are needed to dissect the intricate details of human prion strains. Such studies have potentially broader implications, for recent data suggest that the strain phenomenon may also be relevant to other neurodegenerative diseases associated with protein misfolding and aggregation [62,66].

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Figure 1. Representation of the original identification of two PrP^{Sc} conformers as distinct strains subsequently named types 1 and 2

The two PrP^{Sc} conformers, referred to as types 2 and 1, were respectively associated with fatal familial insomnia (FFI) and Creutzfeldt-Jakob disease (fCJD^{D178N}) (A), two familial prion diseases that share the same D178N mutation in the PrP gene but have distinct phenotypes as demonstrated by the opposite topography of the lesions in thalamus and cerebral cortex (B). PrP^{Sc} types 2 and 1 also show distinct electrophoretic profiles (C), which can be appreciated by the distinct mobilities to kDa 19 and kDa 21 of the lowest electrophoretic bands corresponding to the unglycosylated fragments of PrP resistant to protease digestion. The different electrophoretic mobilities of the two PrP^{Sc} conformers support the notion that they have different conformations represented as different geometric forms (C). PrP^{Sc} types 2 and 1 associated with FFI and fCJD^{E200K} another form of familial CJD was replicated upon inoculation to receptive mice (D), even in the absence of the original PrP gene mutation. Familial CJD^{D178N} could not be transmitted to the same transgenic mice probably because 129M/129V mismatch. ▲: The light blue triangle represents the conformation of the PrP^{Sc} conformer from FFI^{D178N}; ●: The green hexagon represents the conformation of PrP^{Sc} from fCJD^{D178N}; ■: The red square represents the conformation of PrP^{Sc} from fCJD^{E200K} which although being type one as fCJD^{D178N}-associated PrP^{Sc} is presumably different based on the fCJD^{E200K} distinct phenotype.

Figure 2. Western blots of PrP^{Sc} types 1 and 2, and of PrP^{Dis} associated with VPSPR-129MM
 The different electrophoretic mobilities of the PrP^{Sc} types 1 (T1A) and 2 (T2A-C) are evident. The capital letters identify the glycoform ratios that may occur in PrP^{Sc} types 1 and 2. The overrepresentation of the middle band containing the monoglycosylated isoform is indicated with A; B indicates the overrepresentation of the upper band (diglycosylated isoform) and C that of the lowest band (unglycosylated isoform). The ladder-like profile formed by the PrP^{Dis} indicated as VPSPR is from VPSPR-129MM but this profile is shared by the three VPSPR 129 genotypes. All preparations have been treated with proteinase K. The sporadic Creutzfeldt-Jakob disease (sCJD) and variant (v) CJD preparations have been probed with 3F4, while the sporadic fatal insomnia (sFI) and the variably protease-sensitive prionopathy (VPSPR) preparations have been probed with IE4, both monoclonal antibodies to the prion protein.

Figure 3. Diagram of the cleavage pattern of PrP^{Sc} types 1 and 2 associated with the three 129 genotypes

The large arrowheads indicate the major sites of cleavage by proteinase K, which are further identified with the residue numbers of the prion protein (PrP) (lowest bar). The smaller arrowheads indicate the minor cleavage sites. Minor cleavage sites extend more towards the C-terminus in PrP^{Sc} type 1, while they extend more toward the N-terminus in at least 2 of the PrP^{Sc} type 2 preparations (MV2 and VV2). This phenomenon is more prominent when PrP^{Sc} types 1 and 2 are associated with the 129MV genotype indicating that in this association, PrP^{Sc} 1 and 2 N-termini are more ragged.

Figure 4. Conformation stability immunoassay (CSI) of the unglycosylated isoform of PrP^{Sc} from sCJDMM1, sCJDMM2 and sCJDMM1-2

To measure the stability of PrP^{Sc}, increasing concentrations of GdnHCl are used to unfold PrP^{Sc}, which in turn becomes progressively more sensitive to digestion with PK. The concentration of guanidine applied to achieve the digestion of 50% of the PrP^{Sc} with proteinase K (PK) is indicated by [GdnHCl]_{1/2} (shown by the vertical color coded broken lines). The CSI curve shows that the stability of the PrP^{Sc} type 1 when it is present alone as in sCJDMM1 (red hollow diamond) is significantly different from the stability that it shows when it co-occurs with PrP^{Sc} type 2 as in sCJDMM1-2 (green solid triangle) ([GdnHCl]_{1/2} values 2.96M vs. 1.96M, respectively; $p < 10^{-5}$). Furthermore, the CSI curve of the PrP^{Sc} type 1 from sCJDMM1-2 becomes indistinguishable from that of PrP^{Sc} type 2 from sCJDMM2 (black square with hollow circle) and sCJDMM1-2 (blue solid circle), which are similar indicating no change in CSI characteristics between PrP^{Sc} 2 when alone or co-occurring with type 1. CSI profiles are smooth curves best matching data (mean $\pm$ SD) obtained at different concentrations of guanidine.

Figure 5. Proposed relationships between the 129 genotypes, PrP^{Sc} types and subtypes, phenotypes and strains in sporadic Creutzfeldt-Jakob (sCJD) and sporadic fatal insomnia (sFI)

The three 129 genotypes (and possibly other polymorphisms) are believed to influence the formation of PrP^{Sc} type 1 or 2 as the 129MM genotype is preferentially associated with type 1 while 129MV and 129VV are associated with type 2. The six possible combinations of the three 129 genotypes with PrP^{Sc} types 1 and 2 are associated with six distinct disease phenotypes, five belonging to sCJD and one to sFI. The pairing of 129 genotype-PrP^{Sc} type combinations with the phenotypes is not stringent. Notable exceptions are the association of the distinct MM1 and MV1 combinations with one phenotype (MM1/MV1) and the association of the single MM2 combination with two phenotypes (MM2 sCJD and MM2 sFI). It is proposed that the six phenotypes are paired with six distinct prion strains, three of which are relatively prevalent, while three are rare and might result from variations in the strain selection process. The distinct strain proposed to be associated with sCJDMV2 is questionable because of the inability to maintain its distinctive characteristics after transmission [5]. Coexistence of types 1 and 2 associated with the three 129 genotypes and the strains possibly associated with the novel disease variably protease-sensitive prionopathy (VPSP), are not shown for simplicity, and because the presence of true strains in VPSP is currently unclear.

Table 1

Human Prion Diseases: General classification

Form or Etiology	Phenotype
Acquired by infection	Kuru
	vCJD
	Iatrogenic CJD
	Creutzfeldt-Jakob disease
	Fatal familial insomnia
Familial	Gerstmann-Sträussler-Scheinker d.
	Non specific or mixed phenotype
Sporadic	Creutzfeldt-Jakob disease (with five phenotypes)
	Fatal insomnia (FI)
	Variably protease-sensitive prionopathy (VPSPr)? (with three phenotypes)?

Table 2
Classification of sCJD and sFI according to the 129 PrP genotype and PrP type with the corresponding previous nomenclature

Subtype	Phenotypic features	Onset (yrs)/Duration (mo)	Distribution ¹ (%)	Previous Nomenclature
sCJD				
M/M1 M/V1	Typical CJD clinically and pathologically - Typical EEG (83%) - "Synaptic" immunostaining pattern	63.2/3.8	58	Myoclonic Heidenhain
V/V1	Early onset - No typical EEG - Cerebellum spared - Weak "synaptic" immunostaining	46.0/15.3	4	Not described
M/M2 cortical	No typical EEG - Large and confluent vacuoles - Coarse PrP immunostaining - Cerebellum spared	60.3/15.7	9	Not described
M/V2	Axial at onset - Rarely typical EEG - No cerebellar atrophy but kuru plaques - Plaque-like pattern of immunostaining	60.3/17.0	14	Cerebellar or ataxic
V/V2	As M/V2 but no kuru plaques and cerebellar atrophy	60.3/6.6	15	Cerebellar or ataxic
sFI				
M/M2	Clinically and pathologically indistinguishable from FFI	60.3/14	1	Thalamic

¹ Based on 609 cases examined by the National Prion Disease Pathology Surveillance Center, Cleveland, OH, USA

Table 3

Percent distribution of PrP^{Sc} types in sCJD

sCJD subtype	Type 1 or 2 (%)	Type 1-2 (%)
MM1	52	43
MM2	5	
MV1	6	23
MV2	71	
VV1	5	15
VV2	80	

* From an unselected series of 200 sCJD cases, modified from [63].