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Molecular pathology, classification, and diagnosis of sporadic human prion disease variants

Piero Parchi, Daniela Saverioni

IRCCS Istituto delle Scienze Neurologiche and Dipartimento di Scienze Neurologiche, Università di Bologna, Via Foscolo 7, 40123 Bologna, Italy

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Abstract

Human prion diseases are a unique group of transmissible neurodegenerative diseases that occur as sporadic, familial or acquired disorders and show a wide range of phenotypic variation. The latter has been attributed to the existence of distinct strains of the agent or prion, and to the genetic background of the host, namely the primary sequence of the gene encoding the prion protein, which is the site of mutations and polymorphisms. The characterization of distinct isoforms of the abnormal prion protein in the brain of affected patients, which has been shown to correlate with the disease phenotype, has recently led to the concept of molecular strain typing, in which the different prion protein isoforms or "types", possibly enciphering the strain variability in their conformation, may serve as surrogate markers for individual prion strains. In sporadic Creutzfeldt-Jakob disease, the most common human prion disease, there are at least six distinct clinico-pathological disease phenotypes that largely correlate at a molecular level with two prion protein types with distinctive physicochemical properties and the genotype at the methionine/valine polymorphic codon 129 in the prion protein gene. Recent results of transmission studies indicate that five prion strains with distinctive biological properties can be isolated from these six disease variants. It has also been shown that about a third of sporadic cases show a mixed phenotype and the co-occurrence of prion protein types. The origin of prion strains and their co-occurrence as well as the mechanisms underlying the strain-specific neuronal targeting remain largely unexplained and their understanding constitute, together with the development of successful therapies and more sensitive and specific clinical biomarkers, the major challenges that this disease poses for the future.

Key words: *CJD, FFI, neurodegenerative dementia, prion protein, polymorphism, phenotype, strain, transmission.*

Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are rapidly progressive neurodegenerative disorders that affect most species of mammals. In humans, they comprise Creutzfeldt-Jakob disease (CJD) [25,77,78,132], Gerstmann-Sträussler-Scheinker disease (GSS) [59], and the recently described variable protease-sensitive prionopathy (VPSPr) [193]. Fatal insomnia (FI) [116], and kuru [111] are also usually reported as distinct disease entities, although recent studies have shown that their molecular features and transmission properties fall within the wide phenotypic spectrum of CJD [109,130,133].

Human prion diseases are clinically characterized by rapidly progressive neurological signs involving mul-

Communicating author:

Piero Parchi MD, PhD, Department of Neurological Sciences, Universtity of Bologna, Via Foscolo 7, 40123, Bologna, Italy, fax: 39-051-2092958; e-mail: piero.parchi@unibo.it

tiple systems and, neuropathologically, by spongiform change, neuronal loss, glial activation, and abnormal PrP deposition, which sometimes forms mature amyloid plaques. Unlike other neurodegenerative disorders characterized by protein misfolding and aggregation that usually either occur sporadically or are linked to pathogenic single gene mutations, prion diseases can be also exogenously acquired. Furthermore, all forms of prion disease can be transmitted experimentally, irrespectively of their apparent distinct aetiology. Like conventional infectious pathogens, the agent causing prion diseases, usually referred to as prion, naturally occurs in a variety of different strains: these are defined as natural isolates of infectious prions characterized by distinctive clinical and neuropathological features, which are faithfully recapitulated upon serial passage within the same host genotype [2].

Sporadic prion diseases, particularly sporadic CJD (sCJD), are the most common in humans, accounting for more than 80% of all cases and having an incidence of about 1.5 cases per million. Genetic forms (also called inherited or familial prion diseases) are of autosomal dominant inheritance, account for about 10% of prion cases, and are linked to mutations in the prion protein (PrP) coding sequence [33]. Accidental transmissions to humans are responsible for rare acquired forms such as iatrogenic and variant CJD (iCJD and vCJD, respectively) [20,184].

The pathogenesis of prion diseases is strictly related to a host-encoded glycoprotein of unknown function, the cellular prion protein (PrP^C), which is encoded by the *PRNP* gene. Prion diseases do not develop or transmit in absence of PrP^C expression, and are all characterized by tissue deposition of an abnormal, partially protease-resistant isoform (PrP^{Sc}) of PrP^C [26,150]. PrP^{Sc} represents the only molecule thus far associated with infectivity, and according to the prion hypothesis, the essential, if not the exclusive, component of the transmissible agent [38,150]. PrP^C misfolding leading to PrP^{Sc} formation involves consistent changes in the secondary structure of the protein with part of the α-helical structure turning into β-sheet [150]. The generated PrP^{Sc} aggregates and forms deposits throughout the brain that are associated with spongiform change, glial activation and neuronal loss.

The present review focuses on sporadic human prion diseases. After a brief analysis of aspects of the biology and molecular pathology of prions which are relevant to the understanding of the human disease, the distinctive features of the sporadic human disease variants or subtypes and the issues related to their diagnosis and classification are described.

Genetics and cell biology of the prion protein

In humans, the prion protein gene (*PRNP*) belongs to the *Prn* gene family, which also includes its paralogues *Prnd* (encoding Doppel) and *Sprn* (encoding Shadoo). It is located in the short arm of chromosome 20, spans 16 kb and contains two exons separated by an intron. The first exon contains untranslated sequences, while the second includes the open reading frame (ORF) encoding for PrP^C. PrP^C is constitutively expressed in the central nervous system (CNS), predominantly in neurons but also in glial cells, and in various non-neuronal tissues, particularly in leukocytes and other cell lines of the immune system. The fact that the *PRNP* sequence is conserved among species suggests that PrP^C is involved in a key function that has developed early during evolution [43,103].

PrP^C is translated in the rough endoplasmic reticulum (ER) as a 253-aminoacid peptide, which subsequently undergoes post-translational modifications in its transition through the ER itself and the Golgi apparatus. These modifications include the removal of a 22-aminoacid signal peptide at the N-terminal end, the formation of a disulphide bond and the addition of a glycosylphosphatidylinositol (GPI) anchor at the C-terminal end after the removal of the last 23 residues. Finally, complex oligosaccharides are attached at residues Asn 181 and Asn 197, although the addition of these glycans does not occur obligatory. As a consequence, three differently glycosylated forms of the protein, also termed glycoforms, are formed: the diglycosylated, monoglycosylated and unglycosylated PrP^C. After crossing the ER and the Golgi apparatus, PrP^C localizes at the cell surface where it is anchored by the GPI [65]. Both in the cell and at the cell surface, PrP^C is associated with specific membrane lipid microdomains, enriched in cholesterol and sphingolipids, called detergent-resistant membranes or lipid rafts. The membrane-spanning form of PrP^C is in a dynamic equilibrium with an internalized form, which is recycled from the cell surface by endocytosis. During this inverse route, which is thought to be mediated by a specific subgroup of lipid rafts termed caveolae-like domains (CLD) [85,181], PrP^C cleavage at residue 112 generates a truncated C-terminal fragment [41]. The biological significance of PrP^C internalization is not known, although some evidence indicates that it is induced,

at least in part, by copper ions [19,96,139,141]. In summary, the association with lipid rafts seems to play a primary role in driving PrP^C localization in the cell, by providing a sort of "shuttle" which carries it from the ER to the cell surface and vice versa. Other studies, however, have shown that the association with lipid microdomains is not essential for PrP^C transport to the plasma membrane [163], but rather important for its correct folding [162]. In particular, cholesterol would act as a molecular chaperone in the PrP^C folding process mediated by lipid rafts.

The structure of mature human PrP^C comprises a poorly definite domain at the N-terminal end of the protein (which spans ~100 residues), a globular domain in the central portion (residues 125-228) and a short flexible C-terminal domain, ending with the GPI anchor (residues 229-230/231) [33]. The N-terminal domain contains the so-called repeat region (residues 51-91) which consists of a 9-aminoacid sequence followed by a tandem repeat of four copies of a 8-aminoacid sequence (PHGGGWGQ). This region is involved in copper ions binding [18,75]. The globular domain is composed of three α-helices and two antiparallel β-sheets, separated by short loops and kept together in their final tertiary structure by interactions between the exposed amino acidic lateral chains that are in close contact with each other when the protein is correctly folded [33].

PrP^C physiological function remains largely elusive. Weissman *et al*. [27] made the important observation that *PRNP* knock-out in mice do not significantly affect brain development and function, although some abnormalities were described later [158] in other *PRNP*-null mice, who developed ataxia and Purkinje cell loss, due to a rearrangement in the regulatory sequences of the paralogue gene *Prnd* encoding *Doppel* after *PRNP* deletion [1]. Current evidence suggests that PrP^C is involved in several regulatory mechanisms, like neuron survival or apoptosis, neurite outgrowth, synapses formation and function, and myelinic fibres maintenance, and that these effects are possibly mediated by the involvement of PrP^C in signal transduction [1]. In this regard, PrP^C expression, cross-linking or interactions with other proteins have been shown to affect the activity of several proteins involved in cell signalling pathways [1]. Based on the strong affinity of PrP^C for copper and the effect of copper ions on the protein internalization into the cell, a role for PrP^C in copper metabolism and in particular in its removal from synapses has also been proposed [71].

PrP^C-PrP^{Sc} conversion and neurotoxicity

The central event in prion disease pathogenesis is thought to be the formation of PrP^{Sc}, an abnormal PrP^C conformer which preferentially accumulates in the CNS. According to the protein-only or prion hypothesis [149], PrP^{Sc} formation works as a template-assisted process, in which PrP^{Sc} (the template) drives PrP^C refolding into the pathogenic conformation [74,91].

PrP^{Sc} is identical in its primary sequence to the PrP^C molecule from which it has originated, but its secondary structure is significantly different, since during PrP^{Sc} formation, some α-helical structures turn into β-sheet [40,125,154]. The resulting abnormally misfolded isoform is distinguishable from the normal isoform because of (i) its insolubility in non denaturing detergents and (ii) its partial resistance to protease digestion that usually involves the C-terminal moiety beyond residues 80-100. Exceptions to this rule, however, particularly for the latter property are increasingly documented. Indeed, variable relative amounts of a protease-sensitive form of PrP^{Sc} have been recently detected in a number of naturally occurring prion diseases [10,56,80,131,147].

It remains controversial exactly how and where in the cell the interaction and the conversion of PrP^C to PrP^{Sc} occur. Early studies pointed to the cell surface as the putative site of PrP^{Sc} formation [16,39,175]. Subsequent reports, however, have suggested that also the ER plays a role in the conversion process, probably after PrP^{Sc} is internalized [11]. In genetic prion diseases, in particular, the ER could be the primary site where the mutant PrP, already predisposed to a misfolded conformation, converts into PrP^{Sc} [65]. There are also studies suggesting that PrP^C-PrP^{Sc} conversion may occur in CLDs or clathrin-coated pits [85,143,173,181]. Like other pathogens, prions might use lipid rafts to enter the cells and possibly to initiate and/or propagate the PrP^C-PrP^{Sc} conversion process that facilitates prion amplification. The role of lipid rafts in the conversion process is also suggested by the findings that both PrP^C and PrP^{Sc} are associated with them [7,8,17,117,175,176] and that the elimination of PrP^C from them prevents PrP^{Sc} formation [85,176]. More recent studies, however, have highlighted the possibility that lipid rafts favour the conversion by bringing together PrP^{Sc} and PrP^C, rather than by triggering PrP^C refolding [31]. Indeed, given their role in PrP^C folding and stabilization of its conformation, lipid rafts may even prevent PrP^C transconformation. According to this view, the conversion

would, thus, occur only after PrP^C exits these domains. Finally, other studies also suggest that lipid rafts do not provide the environment in which PrP^C-PrP^{Sc} refolding occurs, but rather promote PrP^{Sc} aggregation and fibrillization once the pathogenic misfolded protein has been produced at other sites (reviewed in [145]).

In prion diseases, PrP^{Sc} accumulation can be detected in the CNS in form of deposits of different size and organization, ranging from oligomers to fully blown fibrillary amyloid, a property shared by other protein aggregates associated with neurodegeneration, like amyloid-β in Alzheimer's disease [52]. PrP^{Sc} accumulation, by inducing spongiform change and microglial activation, followed by neuronal loss and astroglial proliferation is believed to be the main pathogenic event responsible for the neurodegeneration. PrP^{Sc} formation and aggregation and the spread of neurotoxicity throughout the brain, however, requires PrP^C expression, since neurons lacking PrP^C are preserved from degeneration even if they neighbour the infected cells [106,107]. In this context, in addition to a PrP^{Sc} gain of function mechanism, also a loss of function secondary to PrP^C depletion [160] could be hypothesized as the molecular basis of neurodegeneration in prion diseases, even if this aspect remains poorly understood, due to the lack of conclusive data concerning the function of PrP^C.

Prion strains and their molecular basis

It has been known for many years that mammalian prions, like conventional infectious agents, naturally occur in a variety of strains. The first demonstration of this phenomenon was obtained in goats experimentally inoculated with distinct scrapie isolates: some animals became drowsy, while others had a scratching syndrome [138]. Based on the length of incubation time following inoculation, the type and distribution of lesions, and the pattern of intracerebral deposition of PrP^{Sc}, a variety of scrapie strains were subsequently identified after passage through inbred mouse lines [22,24,55,70]. The wide variety of scrapie strains have been traditionally seen as the major challenge to the protein only hypothesis [54]. Indeed, to accommodate the prion hypothesis and the strain phenomenon, PrP^{Sc} must act as an informational molecule. Although the strain properties are clearly not encoded by PrP primary sequence (strains can be propagated in animals with the same *PRNP* genotype, and re-isolated after passages in intermediate species with distinct *PRNP* haplotypes), it has been clearly demonstrated that PrP^{Sc} exists in a number of molecular subtypes that show differences in size and glycosylation pattern, degree of protease resistance, aggregation state, or conformational stability [12,13,46,127,129,136,155,169, 170,177, for a review of more recent studies see also 2]. According to many scientists in the field who believe in the prion hypothesis, these data represent a strong argument for the contention that the molecular basis of strain variation lies within the structure of PrP^{Sc}. Bessen and Marsh first embraced this idea when they showed that two strains of transmissible mink encephalopathy (TME), propagated in inbred Syrian hamsters, gave rise to PrP^{Sc} molecules with distinct electrophoretic mobility and degree of resistance to protease digestion [13]. The two TME strain-specific PrP^{Sc} were subsequently propagated *in vitro* through non-genetic mechanisms [12], leading to the conclusion that different conformers of PrP^{Sc} may faithfully self-propagate and cause distinct clinico-pathological diseases [12]. Later, Caughey and colleagues, using infrared spectroscopy, provided also some direct evidence that the two distinct TME strains are associated with PrP^{Sc} molecules that differ in conformation in regions with β-sheet secondary structure [37].

Data in support of the idea that, also in human prion diseases, pathologically distinct phenotypes may result from different conformers of PrP^{Sc}, came from the study of sCJD. Two types of PrP^{Sc} (types 1 and 2) differing in relative molecular mass and glycoform ratio in conjunction with the codon 129 polymorphism, were shown to largely determine the clinico-pathological variability of sCJD [129,133]. By demonstrating the transmission of two human disease phenotypes associated with PrP^{Sc} types 1 and 2, in syngeneic animals, Telling *et al*. [177] provided the first definitive demonstration of prion strain variation in humans. They inoculated brain homogenates from subjects affected by familial FI (linked to PrP^{Sc} type 2) or familial CJD (linked to PrP^{Sc} type 1) in transgenic (Tg) mice, and demonstrated not only a faithful reproduction of PrP^{Sc} biochemical features of the original inoculum in the recipient animal, but also a different intracerebral distribution of lesions.

A rather indirect approach that has been increasingly used in recent years to study the strain-specificity of PrP^{Sc} structure, is based on the early work by Kocisko *et al*. [92], which analysed the progressive denaturation of PrP^{Sc} in the presence of increasing concentrations of chaotropic salt guanidine hydrochloride (GdnHCl). Several authors used this approach to char-

acterize animal prion strains [58,142,155,178] and synthetic prions [97,98], and observed a correlation between PrP^{Sc} conformational stability (i.e. sensitivity to denaturation) on one hand, and the degree of infectivity or the converting activity of PrP^{Sc} on the other [38,58,142]. Along this line, Pirisinu *et al*. [146] have recently set up a novel assay which allowed them to distinguish between PrP^{Sc} isoforms related to distinct TSE variants, based upon the variable propensity to be denatured by GdnHCl measured through the change in solubilization rather than in the degree of resistance to protease digestion. By avoiding the protease-digestion step, the method may allow for the characterization of the so-called protease-sensitive PrP^{Sc} ($sPrP^{Sc}$), an abnormal isoform of PrP^{Sc} that is indistinguishable from PrP^{C} on the protease digestion assay despite retaining the abnormal conformation. $sPrP^{Sc}$ is increasingly found to be associated to animal and human prion diseases: two recently characterized prion diseases, atypical scrapie [10] and protease-sensitive prionopathy [56], along with some phenotypic subtypes of GSS [80,131,147] have been shown to only accumulate $sPrP^{Sc}$ together with protease-resistant N- and C-terminally truncated unglycosylated PrP^{Sc} fragments in the 7-14 kDa range. According to some authors, $sPrP^{Sc}$ may contribute up to 90% of thewhole PrP^{Sc} even in classical TSEs such as sCJD, thus calling into question the rationale on which TSE diagnosis is currently based (i.e. demonstration of protease resistant PrP^{Sc}) [49,156,178]. It has been also suggested that $sPrP^{Sc}$ consists of aggregates of shorter size with respect to the protease-resistant PrP^{Sc} [137,179] and that, at least in some sCJD variants, the concentration and stability of these $sPrP^{Sc}$ conformers correlate with the duration of clinical disease [86]. In addition to its quaternary structure (i.e. the size of aggregates) [137,179], the basis for the degree of protease resistance of PrP^{Sc} has been also attributed to its tertiary structure [118,147]. Whatever the case, it remains unknown what drives PrP^{Sc} to adopt a certain conformation, that in turn, would lead to a specific disease phenotype.

PrP^{Sc} glycosylation, beside protein conformation and, possibly, other factors, may also contribute to the molecular basis of strain variation. Indeed, the PrP^{Sc} molecules associated with distinct subtypes of human prion disease often show distinct glycoform ratios [115,127,136]. A recent example of this phenomenon, which has been highly publicized, is represented by the PrP^{Sc} associated with vCJD, which has a type 2 migration pattern, like other subtypes of CJD [127,136] but shows a glycoform ratio which is distinct from that seen in sporadic and iatrogenic CJD or kuru [46,127,136]. Since glycosylation is a co-translational process that is known to differ among cell types [94], the different glycoform ratios may reflect the involvement or the targeting of distinct neuronal populations, as a consequence of a strain-specific cellular tropism [50]. Alternatively, different prion strains may preferentially convert certain PrP^{Sc} glycoforms.

Although altogether all of the above cited studies provide strong evidence in favour of the contention that different PrP^{Sc} conformers underlie the concept of prion "strains", the direct proof for this contention is not available as yet. Indeed, since these results are mainly indirect and the determination of the ultimate molecular structure of distinct PrP^{Sc} molecules is still lacking, questions and alternative interpretations of the data remain. For example, it is not known whether the distinctive properties of PrP^{Sc} directly reflect PrP^{Sc} conformation or are rather determined by interactions between PrP^{Sc} and other molecules. PrP^{Sc} is extracted from the brain in a highly aggregated state and the heterogeneity in size of the protein core may well reflect the quaternary rather than the secondary or tertiary structure of the molecule. For example, formation of PrP^{Sc} type 1 and type 2 may follow distinct ligand interactions of PrP^{Sc}. Similarly, the extent of conversion of each glycoform of PrP^{Sc}, which ultimately determines the glycoform ratio of PrP^{Sc}, at least in sporadic and acquired prion disease, may well represent a signature imparted by another informational molecule that interacts with PrP. Finally, the central question that still remains to be answered is how an identical primary sequence can drive different tertiary conformations in the prion protein, if no other informational molecule exists.

Given the analogy of some properties of prions with viruses and/or viroids and the overall need to fully clarify the well-known effects of co-factors on prion replication, attempts to demonstrate the PrP^{Sc} association with conventional nucleic acid and other molecules continue. In the most recent of such studies, Simoneau *et al*. [168] identified small RNA molecules as part of the TSE infectious particle. Previously, Safar *et al*. [157] also detected short nucleic acids (not exceeding 25 nucleotides) in association with PrP molecules, but ruled out the possibility that they could be prion specific. Irrespectively of whether or not they have a role in strain diversity, nucleic acids such as polyanions, but also polysaccharides, may well participate in amyloid formation, since amyloid plaques have been shown to contain sig-

nificant amounts of both these molecules. Nucleic acids have also been demonstrated to affect amyloid protein conformation, by driving the final three-dimensional structure in the protein aggregates [104]. Despite no conclusive results are available for the role of nucleic acids associated with PrP^{Sc}, novel evidence indicating that prions, like viruses, "mutate" and adapt to the environment in which they replicate has been recently published. It has been found that when grown in cell cultures in the presence of swainsonine, a chemical compound which inhibits protein glycosylation, some strains become drug-resistant while others do not [102]. According to these authors, the process of mutation and selection that characterizes the different strains suggests that prions naturally exist in a variety of conformers, constituting 'quasi-species', from which the one replicating most efficiently in a particular environment is selected [45,53,101,167]. The mechanisms underlying this presumed PrP capability of assuming different conformations remain, however, to be elucidated.

PrP^{Sc} types and the molecular basis of current sporadic human prion disease classification

In human prion disease, it has been convincingly shown that there is a strong correlation between two PrP^{Sc} types with distinct physicochemical properties, named types 1 and 2, and the genotype at the methionine (M) / valine (V) polymorphic codon 129 of *PRNP* on one hand, and the pathological and clinical disease phenotype on the other [133,135]. PrP^{Sc} types 1 and 2 have been originally described in sCJD by Parchi *et al*. in 1996 [129] as two alternative forms of the protease-resistant, unglycosylated C-terminal core of the PrP^{Sc} molecule (Fig. 1). PrP^{Sc} type 1 has a relative electrophoretic mobility of 21 kDa and a primary cleavage site at residue 82 while PrP^{Sc} type 2 has a relative molecular mass of 19 kDa and a primary cleavage site at residue 97 [127,136]. Interestingly, PrP^{Sc} types 1 and 2 characterize all subtypes of CJD, i.e. sporadic, inherited or acquired by infection [127,136], thus suggesting a common mechanism of PrP^{Sc} formation that is independent from the apparently distinct aetiology of the disease.

The reliability of the molecular classification proposed by Parchi *et al*., based on two PrP^{Sc} types and 3 *PRNP* codon 129 genotypes (i.e. MM, MV, and VV) has recently been demonstrated in a collaborative study between seven laboratories involved in prion disease surveillance [134]. Following a detailed and standardized protocol, the inter-institutional agreement between laboratories in the classification of blindly distributed samples was excellent, indicating that the general technique and in particular the classification system based on the distinction between types 1 and 2 are robust and represent a reliable basis for diagnostic and epidemiologic purposes.

The identification of an excess of pathological phenotypes and prion strains (see paragraph on transmission studies) with respect to PrP^{Sc} types 1 and 2 dichotomy has also led to the search for further distinctive PrP^{Sc} properties. The study of PrP^{Sc} in rigorously controlled experimental conditions using a highly sensitive gel electrophoresis technique led to the demonstration that PrP^{Sc} types 1 and 2 are indeed heterogeneous species (i.e. slightly differ depending on the codon 129 genotype), which can be further distinguished into molecular subtypes that correspond to the current histopathological classification of sCJD. Using standardized high buffer strength for brain homogenization, PK digestion at pH 6.9 with a high enzyme concentration, and long running gels, Notari *et al*. [120] showed that distinctive PrP^{Sc} properties can

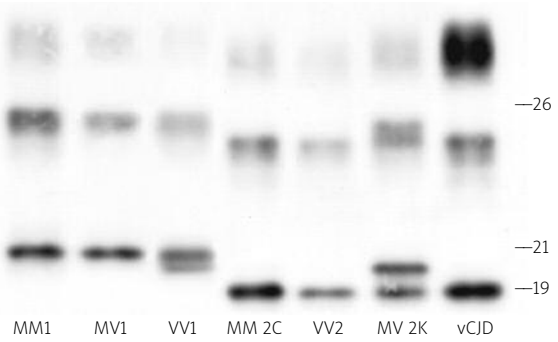

Fig. 1. Western blot analysis of PrP^{Sc} extracted from frontal cortex homogenates of sCJD variants and vCJD. Samples were digested with PK at pH 6.9 and run in a Tris-glycine PAGE 15% gel, 15 cm long. This electrophoresis setting, compared to standard 12% mini gels, allows for a better distinction of the two main PrP^{Sc} fragments (type 1 and type 2, with a molecular mass of 21 and 19 kDa) and shows additional variations of the PrP^{Sc} profile in some CJD variants. Note, for example, that the PK digestion of both VV1 and MV 2K samples at pH 6.9 generates a doublet (e.g. two PrP^{Sc} unglycosylated fragments of about 21 and 20 k for VV1 and of about 20 and 19 kDa for MV 2K). Membranes were incubated with mAb 3F4 as primary antibody. Approximate molecular masses are in kDa.

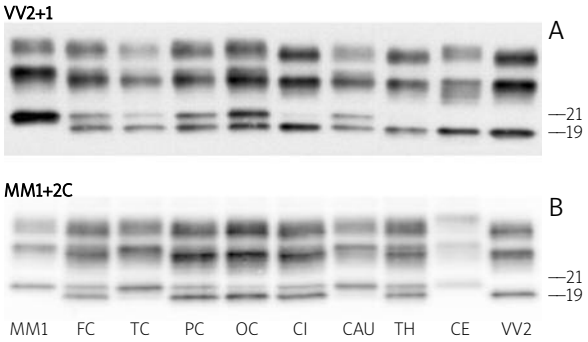

Fig. 2. Western blot analysis for PrP^{Sc} in tissue homogenates from different brain areas of individual sCJD cases showing a mixed VV 2+1 (**A**) or MM1+2C (**B**) phenotype. Both presence and relative amount of each PrP^{Sc} type varies according to the brain region analysed. Note that the samples with a "pure" molecular phenotype in the VV case show PrP^{Sc} type 2 (**A**), while those in the MM case show PrP^{Sc} type 1 (**B**). In the first and last lanes of both gels, control samples from a pure MM1 and a pure VV2 have been included for comparison. Membranes were incubated with mAb 3F4 as primary antibody. Approximate molecular masses are in kDa. *FC – frontal cortex, TC – temporal cortex, PC – parietal cortex, OC – occipital cortex, CI – cingulate gyrus, CAU – caudate nucleus, TH – medial thalamus, CE – cerebellum.*

be found in sCJD phenotypes sharing the same PrP^{Sc} type. For example, i) PrP^{Sc} type 2 from MV cases shows a unique doublet band that differs from PrP^{Sc} type 2 in MM and VV cases, and ii) type 1 PrP^{Sc} from VV cases migrates faster than type 1 PrP^{Sc} from MM1 and MV1 samples when PK digestion is performed at pH under 7.2 (Fig. 1). However, the recognition of these subtle differences between subtypes of type 1 and type 2 groups, respectively, was less satisfactory when tested among CJD surveillance laboratories, probably because it requires highly standardized gel electrophoresis protocols that are unsuitable for routine diagnostic needs [134].

Interestingly, PrP^{Sc} types 1 and 2 have also been found to co-occur in the same brain (Fig. 2). Following the original report of this phenomenon [133,151], a series of studies indicated that the prevalence of cases with co-occurrence of PrP^{Sc} types 1 and 2 varies between 12% and 44% [66,67,100,151,166,180]. To explain these findings, it has been proposed that the incidence of sCJD cases with both types 1 and 2 PrP^{Sc} reflects the extent of brain sampling and the sensitivity with which a minority type may be detected in the presence of larger amounts of the other protein fragment [66]. Pursuing this idea, subsequent studies, taking advantage of novel, putative type-1 [148,187] and type-2 [89] selective antibodies claimed that little amounts of type-1 PrP^{Sc} are found in all patients with sCJD type 2 and vice versa, that type-2 PrP^{Sc} is detected in all patients with sCJD MM1. However, the interpretation and relevance of these data for CJD classification and strain-typing was later contested, by showing that the PrP^{Sc}-selective antibodies recognize PrP fragments that do not match in physicochemical properties those detected by standard PrP^{Sc} typing [29,121]. For example, when relatively mild digestion conditions are used, the type 1 specific antibodies are incapable of distinguishing *bona fide* type 1 "core" fragment from the fragments obtained by an incomplete digestion of PrP^{Sc} type 2 N-terminus [29,121]. Similarly, as also discussed by the authors of the original study [89], the weak band recognized by the type 2-selective antibody may well represent a PrP^{C} fragment, truncated at residue 97, that remains embedded in the PrP^{Sc} type 1 aggregate, rather than *bona-fide* PrP^{Sc} type 2. Indeed, while the antibody recognizes a PrP^{Sc} type 2-like fragment in the cerebellum of MM1 subjects, the same fragment is not detected in subjects affected by sCJD MM 2C. Based on these considerations, we believe that the use of type 1 or type 2 selective antibodies alone is not recommended for strain typing studies in human prion disease.

The issues of incidence, diagnosis and phenotypic effects of the co-occurrence of PrP^{Sc} types have been recently re-examined comprehensively in a large series of sCJD cases [135]. To this aim, an extensive brain sampling and the most accurate methodology available for detection of the PrP^{Sc} type co-occurrence, which provides good sensitivity combined with absolute specificity (i.e. the distinction between *bona fide* types 1 and 2 "core" fragments from the fragments that are produced by incomplete protease digestion) has been applied [121]. The results obtained in a largely unselected series of 200 cases demonstrated an overall prevalence of PrP^{Sc} types 1 and 2 co-occurrence of 35% [135]. Although co-occurrence was detected in all codon 129 genotypes, PrP^{Sc} types 1 and 2 co-occurred more frequently in MM (43%) than in MV (23%) or VV (15%) subjects. The overall results indicated that the deposition of either type 1 or 2, when concurrent, is not random and always characterized by the coexistence of phenotypic features previously described for the "pure" subtypes. Indeed, most cases with a mixed phenotype, as defined in this

study [135], were also characterized by well identifiable distinctive histopathological traits indicative of the co-existence of two CJD variants within the same brain. Such a good correlation between biochemical and neuropathological features in our opinion, also supports the idea that the co-occurrence of PrP^{Sc} types in sCJD is not the rule, as the Western blot studies using the type 1 or type 2 selective antibodies would instead suggest, based on a rough evaluation. Given the importance of the recognition and correct classification of such cases for future epidemiological and transmission studies aimed to identify the aetiology and extent of strain variation in sCJD, a protocol of PrP^{Sc}typing has been proposed that is based on analysis of at least four critical regions such as the temporal, parietal and occipital neocortices, and medial thalamus [135].

Another aspect of molecular typing that has seen some developments in recent years concerns the characterization of novel truncated PrP^{Sc} species of lower molecular weight than PrP^{Sc} types 1 and 2. Unglycosylated PrP^{Sc} fragments of 7-8 kDa, truncated at both N- and C-terminal ends, were first identified in GSS [131,144,174]. Subsequently, in both sporadic and genetic forms of CJD, Capellari *et al*. [32] and Zou *et al*. [192] detected C-terminal fragments of about 12 and 13 kDa (PrP-CTF12/13), and showed that they are generated by a PrP^{Sc} cleavage at residues 162-167 and 154-156 respectively. Interestingly, the relative amount of PrP-CTF 13 varies between sCJD subtypes; in particular all PrP^{Sc} type 2-associated sCJD subtypes but the MM 2T show only traces of this fragment, whereas the peptide is present in a significant amount in MM1 cases and is particularly abundant in VV1 subjects [122].

Notari *et al*. [122] also identified a novel C-terminally-truncated PrP^{Sc} fragment showing an apparent molecular mass of either ~18.5 kDa (when associated with type 1) or ~17 kDa (when associated with type 2). This fragment shares the primary N-terminal sequence with either type 1 or type 2 but lacks the very end of the C-terminus together with the GPI anchor. Finally, a fragment with an apparent molecular mass of about 16 kDa, which is only generated in partially denaturing conditions, has been detected in sCJD MM1/MV1. Epitope mapping indicates that the fragment has an intact C-terminal end and is truncated in the region between residue 112 and residue 144. Taken together, these data suggest that each sCJD subtype can be associated with a specific profile of abnormal PrP fragments, possibly reflecting subtype-specific structural characteristics of the abnormal protein aggregate.

PrP^{Sc} heterogeneity also extends to the sugar moieties. As stated previously, the non-obligatory addition of one or two sugar chains results in three differently glycosylated isoforms (di-, mono-, or unglycosylated) in both PrP^{C} and PrP^{Sc}. It has been recognized for a long time that the PrP^{Sc} associated with different prion disease forms or subtypes may show distinct ratios of the three differently glycosylated form (the so-called glycoform ratio). These differences are widely used as a diagnostic tool for prion disease subtype recognition, especially between bovine spongiform encephalopathy (BSE) and natural scrapie in sheep, or between vCJD and sCJD or iCJD in humans [9,72,127, 172]. In the large majority of CJD cases, PrP^{Sc} glycosylation is characterized by an over-representation of the monoglycosylated form. A rather major distinction with undoubted diagnostic relevance has been introduced to distinguish the above described "pattern A" from "pattern B" characterized by a predominance of the fully glycosylated form, the latter being found in vCJD [46,127] or in gCJD and fatal familial insomnia (FFI) linked to the E200K or D178N mutations, respectively [115,136]. However, finer significant differences in PrP^{Sc} glycoform ratio have also been described among CJD subtypes with either "pattern A" or "pattern B" using mono- or two-dimensional gel electrophoresis [73, 126,127].

Subtypes of sCJD and their distinctive clinico-pathological features

Following the demonstration of the critical role of codon 129 genotype on sCJD susceptibility and phenotypic expression and the characterization of PrP^{Sc} types 1 and 2 [127], the clinical and pathological spectrum of sCJD has been completely re-examined. Based on a comprehensive and systematic analysis of molecular, clinical and pathological features, Parchi *et al*. [133] have originally described six molecular and corresponding clinico-pathological subtypes. Later studies have confirmed the basis of this classification [30,66, 99,134,161]. Furthermore, the spectrum of phenotypes with mixed pathological features and the co-occurrence of PrP^{Sc} types, which accounts for about one third of all sCJD cases, have been recently more rigorously defined [29,135]. The main clinical and pathological features of both pure and mixed sCJD subtypes are summarized in Tables I and II.

Table I. Relative frequency, clinical features, CSF tests and DW-MRI findings in sporadic prion disease variants

Disease variant	(%)*	Age at onset (years)*	Disease duration (months)*	Early clinical features*	CSF tests# and DW-MRI† findings
MM/MV1	40	70 (48-86)	4 (1-24)	Very rapidly progressive dementia with myoclonus. Ataxia in 50% of cases, visual signs and dysphasia in 25%.	• 14-3-3 and/or t-tau CSF tests are positive in almost all cases • Hyperintensity in striatum and cerebral cortex
VV2	15	65 (45-83)	6.5 (3-18)	Rapidly progressive ataxia sometimes preceded by transient diplopia or vertigo. Peripheral signs and fasciculation may occur	• 14-3-3 and/or t-tau CSF tests are positive in almost all cases • Hyperintensity in striatum and thalamus
MV 2K	8	65 (40-81)	16 (5-48)	Rapidly progressive ataxia and cognitive decline (either one may occur first)	• 14-3-3 and/or t-tau CSF tests are positive in about 70% of cases • Hyperintensity in cerebral cortex, striatum and thalamus
MM/MV 2C	< 1	68 (61-75)	20 (12-36)	Rapidly progressive cognitive decline	• 14-3-3 and t-tau CSF tests are positive in about 90% of cases • Hyperintensity in cerebral cortex
MM 2T	<1	50 (36-71)	15.5 (8-24)	Insomnia, psychomotor hyperactivity, postural instability, diplopia, psychiatric signs	• 14-3-3 and t-tau CSF tests are negative in most cases • Negative DW-MRI; MRS may indicate thalamic gliosis [105]
VV1	<1	40 (24-49)	15.5 (14-16)	Rapidly progressive cognitive decline	• 14-3-3 and/or t-tau CSF tests are positive in almost all cases • Hyperintensity in cerebral cortex
MM/MV 1 + 2C	28	69 (42-89)	4 (1-26)	Depends on the relative amount of PrP^{Sc} type 1 vs. type 2. Most cases are similar to MM/MV1	NA
MM/MV 2C + 1	~2	65 (48-86)	18	Depends on the relative amount of PrP^{Sc} type 2 vs. type 1. Most cases are similar to MM/MV 2C	NA
MV 2 K+C	~2	NA	NA	Most cases are similar to MV 2K but with more prominent dementia than ataxia	NA
MM 2 T+C	<1	NA	NA	NA	NA
VV 2 + 1	~2	69	6.5 (3.5-13)	Similar to VV2	NA
VPSPr	~2 in USA, <1% in Europe	129MM: 64,78 129MV: 72 (65-81) 129VV: 65 (48-72)	MM: 41,50 MV: 36 (7-72) VV: 24 (10-60)	Behavioural and mood changes (more prominent in VV), language deficits, cognitive impairment, and motor signs, especially parkinsonism and ataxia (more prominent in MM)	• Preliminary data indicate that 14-3-3 ant t-tau levels are not elevated in most cases [68,69,79,152,193] • Preliminary data indicate that DW-MRI is negative in most cases [68,69,79,152,193]

*NA – no published data available; *From [133,135], unless otherwise specified. #From [34,48,61,161], unless otherwise specified. †From [112], unless otherwise specified*

Table II. Neuropathological features, amplification *in vitro* and transmission properties of sporadic prion disease variants

Disease variant	Distinctive neuropathologic features*	Amplification *in vitro* (PMCA [83], QuIC [140])	Transmission properties (animal model)
MM/MV1	Spongiform change with small (2-10 μm) vacuoles in neocortex, striatum, thalamus and cerebellar molecular layer (often only focal). Hippocampus and brainstem are remarkably spared. IHC: synaptic type of PrP^{Sc} staining in the cerebral cortex, basal ganglia and cerebellum	• PMCA: ++++/+++ for HuMM, ++/+ for HuMV, +/0 for HuVV substrates in MM1/MV1 • QuIC: rapid and efficient amplification with maximum reached within 15 h; not affected by codon 129 of substrate	• Distinct from MM 2C (bank voles) [119] • Distinct from MV 2K, VV2, MM 2C, and VV1 (Tg mice expressing human PrP) [15] • Distinct from VV2 and MV 2K (non-human primates) [130]
VV2	Spongiform change with medium size vacuoles in striatum, hippocampus, limbic cortex, thalamus, cerebellum (molecular layer), and midbrain. In the neocortex the spongiform change are confined to or predominate in the deep layers. IHC: Diffuse plaque-like PrP^{Sc} deposits and perineuronal staining in cortical layers 4 to 6	• PMCA: 0 for HuMM, 0 for HuMV, ++++ for HuVV substrates • QuIC: rapid and efficient amplification with maximum reached within 15 h; not affected by codon 129 of substrate	• Less efficient than MM1/MV1 and MM 2C (bank voles) [119] • Distinct from MM1/MV1, MM 2C, and VV1, but indistinguishable from MV 2K (Tg humanized mice) [15] • Distinct from MM1/MV1 but indistinguishable from MV 2K (non-human primates) [130]
MV 2K	Unicentric amyloid plaques of kuru type in the granular layer of cerebellum. Other features are similar to VV2, but with more consistent plaque-like deposits	• PMCA: 0 for HuMM, + for HuMV, +++ for HuVV substrates • QuIC: rapid and efficient amplification with plateau phase within 15 h	• Less efficient than MM1/MV1 and MM 2C (bank voles) [119] • Distinct from MM1/MV1 but indistinguishable from VV 2 (non-human primates) [130] • Distinct from MM1/MV1, MM 2C, and VV1, but indistinguishable from VV2 (Tg humanized mice) [15]
MM/MV 2C	Spongiform change comprising relatively large confluent vacuoles associated to perivacuolar PrP deposition. Cortico-striatal distribution of pathology with relative sparing of brainstem and cerebellum	• PMCA: +++ for HuMM and HuMV, 0 for HuVV substrates • QuIC: long amplification times, with maximum reached within 60 h	• Distinct from MM1/MV1 (bank voles) [119] • Distinct from MM1/MV1, MV 2K, VV2, and VV1 (Tg humanized mice) [15]
MM 2T	Moderate to severe atrophy of medial thalamic and inferior olivary nuclei in absence of definite spongiform change. Inconsistent or weak PrP deposition	• PMCA: ++ for HuMM, + for HuMV and 0 for HuVV substrates • QuIC: NA	• Distinct from MM/MV1 and MM 2C (Tg mice expressing chimeric or human PrP) [109,114]
VV1	Spongiform change with medium-size vacuoles in the cerebral cortex and striatum and relative sparing of cerebellum. Faint, synaptic type of PrP deposition	• PMCA: 0 for HuMM and HuMV, +++ for HuVV substrates • QuIC: very long incubation times, with maximum not reached within 90 h	• Distinct from MM1/MV1, MM 2C, MV 2K, and VV2 (Tg humanized mice) [15]

Table II. Cont.

MM/MV 1 + 2C	As in MM/MV 1 but with clusters of large vacuoles associated to perivacuolar/coarse PrP deposits mainly in cerebral cortex or thalamus	NA	• Similar to MM1/MV1 (non-human primates) [130]
MM/MV 2C +1	As in MM/MV 2C but with synaptic-type PrP staining in the molecular layer of the cerebellum	NA	NA
MV 2 K+C	As in MV 2K but with clusters of large vacuoles associated to perivacuolar and coarse PrP deposition mainly in cerebral cortex	NA	NA
VV 2 + 1	Virtually indistinguishable from VV2	NA	NA
VPSPr	Spongiform change with medium-size vacuoles in the cerebral cortex, striatum and thalamus. Microplaques in molecular layer of cerebellum Target-like pattern of PrP deposition made of clusters of granules, which are often interspersed within background of fine granules [193]	NA	NA

**From [133,135], unless otherwise specified.*

sCJD types with "pure" molecular and clinico-pathological features

MM/MV 1 type

The most common phenotype is the so-called MM/MV 1 type, which comprises about 40% of all sCJD cases, and is seen in subjects who are homozygous for methionine or heterozygous at codon 129 and have PrP^{Sc} type 1 [133,135]. The mean age at onset of symptoms is about 65 years and the average clinical duration 4 months. Signs at onset, which are often combined, vary and include cognitive decline in most cases, ataxia in approximately half of them, language dysfunction, visual signs of central origin, myoclonus or other involuntary movements, each occurring in about one fourth of patients. Neurological signs are unilateral at onset in about 25% of cases. Diffusion weighted (DW) magnetic resonance imaging (MRI) shows a signal increase in the basal ganglia in about 70% of cases, whereas the involvement of the cerebral cortex in at least three different regions is detected in about half of the patients [112]. The absence of hippocampal and thalamic signal increase is a distinctive feature of this subtype [112]. Periodic sharp-wave complexes (PSWCs) are recorded in the electroencephalogram (EEG) in about 80% of cases, usually within the first three months of symptoms [48,131].

This phenotype shows the classical pathology of CJD: spongiform change with small (2-10 µm) round to oval vacuoles, predominantly located in the neuropil (Fig. 3A). In the cerebral neocortex, the vacuolation is seen in all layers and is often more prominent in the occipital lobe. The basal ganglia and thalamus are similarly affected, whereas the hippocampus and brainstem are remarkably spared. In the cerebellum, spongiform change is seen in the molecular layer, often in a focal distribution. Immunohistochemistry (IHC) shows a synaptic type of PrP^{Sc} staining, which is best seen in the cerebral cortex (Fig. 3B), and cerebellum, and less consistently, in the basal ganglia and thalamus [133,135].

The second most common phenotype comprises about 15% of sCJD cases and includes subjects that are homozygous for valine at codon 129 and show PrP^{Sc} type 2 (VV2) [133,135]. The mean age at onset and clinical duration are 60 years and 6.5 months, respectively. Clinically, VV2 patients show prominent gait ataxia at onset, while dementia often occurs later in the course of the illness. Prominent myoclonus is absent in about one third of the cases, and most patients also lack PSW discharges on EEG. DW-MRI shows involve-

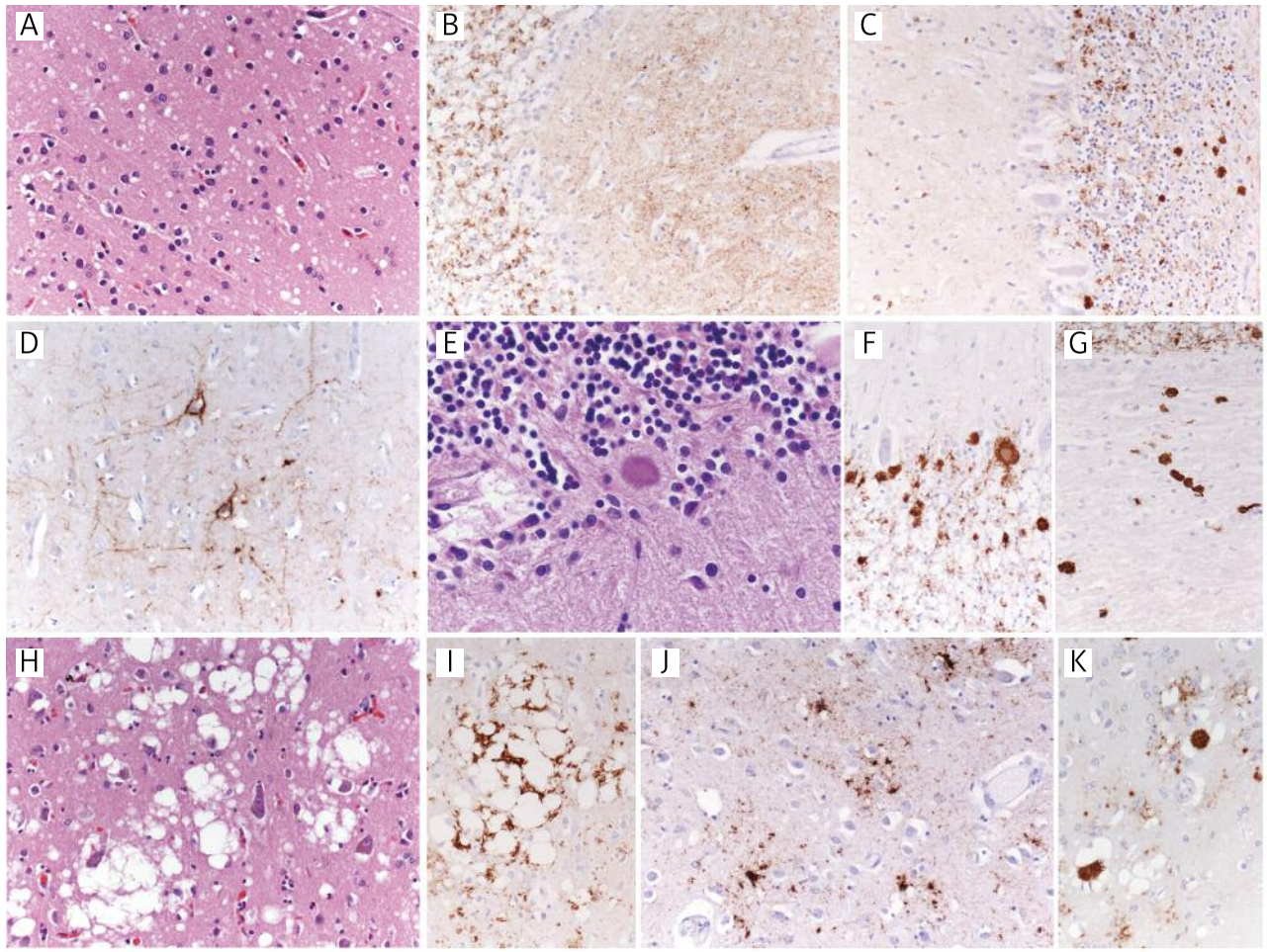

Fig. 3. Distinctive histopathological features of sporadic human prion disease subtypes. **A**) Typical spongiform change characterized by small, fine, micro vacuoles in the neuropil of sCJD MM1 (H&E stain, occipital cortex). **B**) Synaptic pattern of PrP deposition in cerebellum of sCJD MM1. A delicate diffuse staining is seen in the molecular layer while in the granular cell layers, the cerebellar glomeruli are stained. **C**) Plaque-like PrP deposits in the granular and molecular layers of the cerebellum in sCJD VV2. These plaque-like deposits are not visible in routine H&E or PAS-stained sections. **D**) Perineuronal PrP^{Sc} staining in the deep cortical layers of sCJD VV2. **E**) Unicentric amyloid plaque of the kuru-type in the cerebellar granular layer in sCJD MV 2K (H&E, stain). Plaque-like PrP deposits in the granular and molecular layers of cerebellum (**F**) and cerebellar white matter (**G**) in sCJD MV 2K. Spongiform change characterized by relatively large confluent vacuoles (**H**) (H&E stain), with perivacuolar PrP^{Sc} staining (**I**) as typically seen in MM 2C, and MM/MV 1 + 2C sCJD cases. Typically, dense, coarse deposits of PrP are seen surrounding confluent vacuoles. The cerebellum in MM 2C is either PrP negative or shows a focal patchy/coarse staining. **J**) Target-like pattern of PrP deposition in the caudate nucleus of a MV case with VPSPr. **K**) Florid plaques in the cerebral cortex of vCJD. All PrP immunostaining is with mAb 3F4. All pictures have the same magnification (200x), except for panel E (400x).

ment of the basal ganglia and thalamus, with the highest frequency among the sCJD subtypes. In contrast, the cerebral cortical signal increase is usually limited and most frequently found in the limbic cortex rather than in the neocortex. Pathologically, the VV2 subjects show moderate to severe spongiform change and gliosis with variable neuronal loss in the grey matter of limbic structures, striatum, thalamus, hypothalamus, cerebellum and brain stem. In contrast, the neocortex is often spared, particularly in cases with a relatively rapid course (i.e. less than 6 months). The spongiform change in the cortex is often laminar and mainly involves the deep layers. Immunostaining is characterized by the presence of plaque-like, focal PrP deposits (Fig. 3C) that are not visible with routine staining procedures and do not contain PrP amyloid, since they are Congo red and thioflavin S negative. Another distinctive immunostaining feature is the strong reaction around some neuronal perikarya (e.g. perineuronal staining) (Fig. 3D), while the neuropil shows the synaptic pattern. Moreover, cases with a less than one-year duration are characterized by laminar distribution of the immunostaining in deep cortical layers corresponding to the spongiform change. The distribution of PrP^{Sc}, like the distribution of the spongiform change, is affected by the duration of the disease. In cases with a less than 5-month duration, PrP^{Sc} is present in relatively large and similar amounts throughout the brain except for the neocortex where it is present to a limited extent. This distribution is quite different from that of MM1 and MV1 subjects in which PrP^{Sc} is most abundant in the neocortex. In cases with over 1-year duration, PrP^{Sc} also markedly increases in the neocortex so that it becomes homogeneously distributed in all grey matter regions [133,135].

The third most common phenotype affects about 8% of cases, and comprises the kuru plaque variant, which is linked to MV at codon 129 and PrP^{Sc} type 2 (MV 2K) [133,135]. The mean age at onset and clinical duration are 59 years and 18 months, respectively. This subtype shows striking similarities with the VV2 phenotype, but is characterized by a significantly lower progression rate. Indeed, a long clinical duration of disease exceeding two years is not infrequent in this type and may raise diagnostic difficulties at the clinical stage. Clinically both ataxia and dementia are prominent. Typically, there are no PSWCs on EEG recording. DW-MRI shows, like in the VV2 type, a quite constant involvement of basal ganglia and thalamus. In particular, the thalamic signal increase is most frequently observed in the pulvinar, sometimes giving rise to a typical "pulvinar sign" [190], followed by the mediodorsal nuclei and the anterolateral nuclei. Involvement of the cerebral cortex is less frequent and most often includes the frontal lobes and cingulate gyri. The distinctive features of this type are cerebellar kuru plaques (Fig. 3E), while other pathological changes are similar to VV2 with more consistent plaque-like deposits (Fig. 3F, G). Kuru plaques are round, dense, PrP^{Sc}-positive amyloid plaques, which, in contrast to plaque-like deposits, are visible on routine haematoxylin-eosin (HE) staining (Fig. 3E). They are regularly found in the molecular and granular layers of the cerebellum but are only rarely seen in other cerebral structures [133,135].

Two distinct phenotypes affect subjects who are homozygous for methionine at codon 129 and show PrP^{Sc} type 2 [133,135]. The first (MM2-cortical or MM 2C) comprises about 1% of the sCJD population. The mean age at onset is 65 years, and the average disease duration 17 months. Clinically, these subjects present with cognitive impairment, while sustained myoclonus, visual signs and typical EEG changes are usually absent. Cerebellar signs are mild or absent, even late in the course of the disease. A widespread DW-MRI cortical signal increase, which typically includes the temporal lobes, is characteristic of this subtype. Basal ganglia involvement is instead rather limited, whereas, compared to the MM1 type, a thalamic signal increase occurs more frequently in these cases. The lesion profile of MM 2C subjects is similar to that of the MM1 or MV1 group, with the exception of the cerebellum that lacks significant spongiform change despite the long duration of symptoms. Moreover, the spongiform change typically consists of large and coarse vacuoles (Fig. 3H). PrP immunohistochemistry shows a coarse pattern of staining (Fig. 3I), which is often called perivacuolar because of its prevalent localization at the rim of the large vacuoles. Recently, this phenotype was also described in at least one MV subject [135].

The second phenotype linked to PrP^{Sc} type 2 and methionine homozygosity also affects about 1% of the sporadic human prion disease population. This variant (MM2-thalamic or MM 2T) is virtually indistinguishable from FI [116,128], and there is now convincing evidence that both the sporadic MM 2T variant and FI are caused by the same prion strain (see below). Clinically, insomnia with inability to initiate and maintain sleep, frequent arousals, and enacted dreams is often an early and dominant symptom. Motor signs are also part of the clinical phenotype and may include diplopia, dysarthria, dysphagia, pyramidal signs and gait abnormalities. Myoclonus, spontaneous and evoked, is always found.

As a rule, there are no PSWCs on EEG. MM 2T patients usually show no signal alterations on DW-MRI [64] and this can be attributed to the absence or limited extent of spongiform changes in the basal ganglia, thalamus, and cerebral cortex [108]. However, two recent studies [63,105] have documented, by MR spectroscopy, a metabolic pattern indicating gliosis restricted to the thalamus, which may be quite specific to the MM 2T phenotype. The diagnostic sensitivity of 14-3-3 and total tau detection in the cerebrospinal fluid (CSF) is also unrewarding, since in most cases, the CSF levels of both proteins do not reach the threshold for a positive test. Neuropathologically, there is prominent atrophy of the thalamus and inferior olive with only minor pathologic changes in other areas of the brain. Spongiform change may be absent or focal and is virtually limited to the cerebral cortex. The relative preservation of the striatum is also unique among sCJD subtypes. PrP^{Sc} IHC may be positive only in the entorhinal cortex.

Finally, a rare phenotype that affects about 1% of the sCJD population is linked to PrP^{Sc} type 1 and homozygosity for valine at codon 129 [133,135]. The VV1 subjects show a mean age at onset of about 40 years, which is by far the youngest among sCJD subtypes, and a mean duration of 15 months. Although VV1 subjects identified to date are limited in number, they appear to show a quite homogeneous and consistent phenotype, both clinically [113] and pathologically [133,135]. Symptoms at onset include progressive dementia, often of the fronto-temporal type, which may evolve for some months without significant motor signs, such as ataxia or myoclonus. In addition, a typical EEG is absent. The pathology in these subjects predominantly affects the cortico-striatal regions, while other subcortical structures, including the cerebellum, are relatively spared. Routine PrP immunohistochemistry shows a faint punctuate staining in the cerebral cortex, despite the severe spongiform change.

sCJD types with "mixed" molecular and pathological features

MM/MV 1+2C type

The MM/MV 1+2C variant is by far the most common mixed type of sCJD. As many as 43% of all MM cases turned out to be mixed MM 1+2C cases on very close inspection [135]. This includes cases in which the large and confluent vacuoles are only found in very restricted regions of the cerebral cortex or the thalamus making the demonstration of PrP^{Sc} type 1 and 2 co-occurrence by Western blot very difficult. Given the rarity of the other mixed phenotypes, this is the only subtype in which clinical correlations were drawn. It was found that both disease duration and frequency of symptoms at onset in MM/MV 1+2C subjects vary according to the relative load of the two protein types. Thus, while the clinical phenotype in MM/MV 1+2C with a focal PrP^{Sc} type 2 deposition (up to 5 brain regions involved) do not significantly differ from those of the MM/MV 1 phenotype, the disease duration becomes significantly longer and cerebellar signs less frequent at onset with increasing type 2 load.

Similarly, the histopathology in this variant shows varying relative amounts of features previously described in the pure MM/MV 1 and MM/MV 2C subtypes. More precisely, this type is characterized by confluent foci of spongiform change with large vacuoles interspersed with the classic microvacuolation, and by a mixed synaptic/perivacuolar or coarse pattern of PrP deposition. There is a significant quantitative variation of these combined features from case to case which are overall more common in the cerebral cortex than in subcortical areas. The temporal, parietal and occipital lobes are the most affected cortical areas, whereas among subcortical nuclei, the thalamus most often shows the perivacuolar or coarse staining. Similarly, the lesion profile in MM/MV 1+2C largely overlaps with that of pure MM/MV 1 cases [135].

VV 2+1 type

At variance with patients carrying the M allele, the occurrence of mixed phenotypic features in VV cases is essentially based on the demonstration of PrP^{Sc} type 1 and 2 co-existence by immunoblotting. Indeed, the lesion profile and the pattern of PrP staining in the rare VV 2+1 type show features that are largely overlapping with those of typical VV2 sCJD cases. As the only distinctive feature, two cases with a significant type 1 accumulation showed a less consistent laminar pattern of spongiform change in the cerebral cortex and a milder cerebellar pathology when compared to VV2 cases with similar disease duration [135].

MV 2 K+C and MM 2 T+C types

The remaining two neuropathologic types with mixed features are also rare [135]. Furthermore, they are only identifiable histopathologically since they are characterized by the co-occurrence of two phenotypes

that are both associated with PrP^{Sc} type 2. The first of these mixed types comprises MV cases showing both kuru plaques in the cerebellum and confluent vacuoles associated with a perivacuolar and coarse focal PrP^{Sc} deposition in the cerebral cortex. According to the most recent nomenclature this type has been defined as MV 2 K+C [135].

The second of these mixed types has been named MM 2 T+C and has been to date reported in only 2 individuals carrying MM at codon 129. It is characterized by the co-occurrence of thalamic and inferior olivary atrophy coexisting with confluent vacuoles and the perivacuolar and coarse PrP^{Sc} pattern of deposition in the cerebral cortex [135].

"Atypical" sporadic human prion disease: Variably protease-sensitive prionopathy (VPSPr)

A few thousand cases of sporadic prion disease have been diagnosed to date by surveillance centres worldwide and the vast majority of them fit the classification system described above. Only a few exceptions were noted. Most of such "atypical" cases were diagnosed in the USA and are now recognized to belong to a novel form of human sporadic prion disease designated VPSPr [193], while the remaining ones stand at the moment as a small group or a case report awaiting further observations and characterizations [88,189].

To date, a total of 30 cases of VPSPr have been published [68,69,79,152,193]. The disease can apparently affect all 3 codon 129 genotypes, although the expression of valine seems to increase susceptibility. Among the reported cases, 19 were VV at codon 129, 8 MV, and 3 MM. Symptoms at onset included mental behavioural and mood changes, language deficits, cognitive impairment, and motor signs, especially Parkinsonism and ataxia. The mental signs are prominent in the codon 129 VV patients whereas motor signs are more common in the MV and MM patients. In the latter groups, myoclonus was also described. The analysis of protein 14-3-3 in the CSF is negative and cerebral DW-MRI shows no abnormalities in most cases [68,69,79,152, 193].

Pathologically, the spongiform change, which is especially seen in neocortical and subcortical regions of the cerebrum such as the striatum and thalamus, consists of relatively large vacuoles, which are more prominent in the codon 129 MV and VV genotypes than in the MM subjects. Amyloid microplaques containing PrP are often present in the cerebellar molecular layers in both VV and MV subjects. The pattern of PrP immunostaining in both VV and MV subjects consists of target-like rounded formations made of clusters of granules, which are often interspersed within a background of fine granules (Fig. 3J). In contrast, MM cases show a PrP immunostaining pattern that resembles the plaque-like pattern described in VV2 sCJD.

The Western blot profile of abnormal PrP is by far the most distinctive feature of VPSPr [193]. After treatment with proteases, PrP^{Sc} forms a striking, ladder-like, electrophoretic profile including at least 4 bands. Furthermore, the abnormal PrP shows a variable degree of protease resistance according to the codon 129 genotype. It is protease-sensitive in subjects with VV, whereas it shows a degree of resistance comparable to some sCJD types in MV or MM subjects at codon 129.

Characterization of human prion strains by experimental transmission

Prion strains, defined as isolates that cause distinct disease phenotypes upon transmission to syngeneic animals, which persist on serial transmission, are believed to be the main cause of phenotypic diversity and are therefore also the main candidates to explain the heterogeneity of human prion disease.

Compared to the number of strain typing transmission studies conducted with the scrapie agent since the early 1970s, the characterization of human prion strains has been significantly delayed. Early studies of non-human primates aimed to prove the transmissibility of human prion disease but lacked the characterization of the isolated strains [21]. Furthermore, transmission of sCJD to wild-type mice did not often result in clinical disease [23], whereas transmissions to bank voles have only recently proved more efficient in terms of clinical outcome, although mainly for sCJD isolates linked to the M allele (MM1, MV1, and MM 2C) [119]. To facilitate CJD transmission studies, various lines of transgenic mice have been produced that express full-length or chimerical human and mouse PrP genes [72,87,93].

In the late 1990s, most transmission studies were prompted by the BSE crisis. This has led to the demonstration that vCJD and the large majority of BSE cases are caused by the same prion strain, which is distinct from prions found in sCJD, iCJD and kuru [23,72,95]. At about the same time, the first characterization of human sporadic prion isolates was accomplished in transgenic mice. Inocula from a single sporadic FI (i.e. MM 2T) case

produced disease characteristics that differed from those induced by sCJD MM1 as well as gCJD E200K-129M, V210I-129M inocula [109].

Preliminary data concerning other sCJD subtypes became available later [93,119] but only very recently, the results obtained by a re-evaluation of the National Institutes of Health series of prion disease transmitted to non-human primates and by more comprehensive experimental transmissions to transgenic mice have significantly contributed to the issue of the extent of strain variation in sporadic human prion disease and provided answers to the crucial question of how the current classification relates to different strains of sCJD [15,90,130].

The results of these studies indicate that five out of six of the neuropathologic and molecular "pure" types of sCJD defined by Parchi *et al*. [133,135], behave indeed as different strains of the agent after transmission. Most importantly, sCJD MM1 and MV1 isolates have identical transmission properties, which significantly differ from those of sCJD VV2 or MV 2K. Furthermore, sCJD MM 2C, sCJD MM 2T and sCJD VV1 subtypes behave differently from each other and from the other isolates after transmission to Tg mice. However, at variance with the sCJD MM1/MV1 and VV2/MV 2K strains, only one or two cases of sCJD MM 2C, MM 2T, and VV 1 have been examined, with the assumption that transmission characteristics of a single case will be representative of the particular subgroup. Thus, the results obtained for these rare subtypes, although clear and somehow expected, await confirmation.

Very recently, preliminary results of the transmission of VPSPr of VV 129 genotype to Tg mice expressing human PrP129-V, have shown that VPSPr is indeed infectious, although VPSPr prions transmit less efficiently than sCJD VV2 prions [57]. Inoculated animals developed PrP plaques and the typical ladder-like PrP^{Sc} profile of VPSPr, but did not show signs of clinical disease or prominent spongiform change.

About the presumed origin of sporadic prion disease

The origin of prion strains in a naturally occurring disease such as sCJD, remains difficult to explain. According to the leading scientists who believe in the protein only hypothesis, sCJD results from the random occurrence of a somatic mutation in *PRNP* or a stochastic spontaneous conversion of PrP^{C} into PrP^{Sc} [124, 150,182]. The main argument in favour of this hypothesis is that CJD has a uniform worldwide occurrence, with only limited evidence [60] of changing incidence over the years, and no convincing geographic clustering, except for areas with large numbers of familial cases. Thus, epidemiological studies do not depict the characteristic of a transmissible disease. In addition, no environmental risk factors have been definitely proven, although surgical procedures, at least in a study, have been significantly associated with the development of sCJD [47].

What is less discussed and publicized, however, is the fact that there are also features of the sporadic prion disease that do not so easily fit the scenario defined for this disorder by the prion hypothesis. For example, if the origin of sCJD is indeed stochastic, one would expect to observe a high phenotypic heterogeneity of the disease with no tendency to produce well defined patterns with regard to type and distribution of lesions. In contrast, only two major types of PrP^{Sc}, and a limited number of clinico-pathological phenotypes that are highly consistent among subjects are found. This indicates that events leading to the formation of PrP^{Sc} in sCJD are few, and stereotyped rather than random or stochastic.

Genetic data concerning strain-specific susceptibility are also intriguing. It is well established that codon 129 of *PRNP* modulates the susceptibility to sCJD [124, 159,185]. This notion is based on the findings that codon 129 MM and, possibly VV genotypes are more prevalent among sCJD cases than in the general population, whereas MV heterozygotes are almost three times less prevalent. The relative protection given by the heterozygous status is usually explained within the protein only model of prion replication [124]. According to this model, PrP^{C} conversion into PrP^{Sc} would occur most favourably in individuals with two identical copies of the PrP^{C} (i.e. homozygotes MM or VV), rather than in heterozygotes [124]. It has been recently found, however, that there are at least two distinct groups of heterozygous sCJD subjects, showing distinct disease phenotypes [133,135]. Interestingly, the heterozygotes linked to PrP^{Sc} type 1 are less numerous than those with type 2, despite the fact that type 1 exceed type 2 by a ratio of 2 to 1 in the homozygotes [133]. Thus, the degree of protection given by the heterozygous genotype changes according to whether PrP^{C} converts to type 1 or to type 2. This observation appears difficult to elucidate in terms of a spontaneous PrP^{C} to PrP^{Sc} conversion, whereas it is more readily explained by differences in allelic interaction between

two distinct prion strains. It is also intriguing that codon 129 M and V alleles seem to have a quite different behaviour in terms of strain susceptibility and associated phenotypes. Indeed, at least 5 different strains have been linked to the M allele, whereas only 2 have been associated with the V allele to date. If indeed the prion strains are enciphered by differences in protein conformation or misfolding, how can a single amino acid change at codon 129 cause such a difference between the M and V allele in terms of protein misfolding?

Moving to a different issue, recent data have shown that the frequency distribution of age at onset in sCJD is not uniform [133], and certainly does not increase exponentially after a certain age, as it would be logical if a stochastic event, particularly a somatic mutation, would be the trigger of the disease. Instead, there are significant differences in age at onset among different sCJD variants [133,135], which more likely reflect age-related differences in the susceptibility to distinct strains of the CJD agent.

Finally, it appears also difficult to conciliate the spontaneous origin of sCJD with the notion that in all animal prion diseases a horizontal transmission of the disease has been demonstrated. This also, considering the clinico-pathologic variability of sporadic human prion diseases, mirrors that of animal diseases, since in both the basis of phenotypic heterogeneity has been shown to depend on both agent strain variation and the genetic variability of the host.

Taken together, all these characteristics of sporadic prion disease are difficult to explain by theories attributing the origin of the disease to random, stochastic events. As a consequence, the hypothesis that at least some variants of sporadic prion disease may represent acquired disorders should not be completely dismissed. It would be important, in particular, that further epidemiological studies are conducted not considering sCJD as a single entity, but rather distinguishing among the different variants, in order to search for possible spatial or geographic clustering and potential subtype-specific risk factors. In this respect, Jansen *et al*. [80] have recently reported a higher proportion of MV heterozygous sCJD patients compared to previous studies in Caucasian, whereas the VV homozygous patients were relatively underrepresented. Although not conclusive, this finding is overall intriguing and deserves consideration and future follow-up.

Laboratory test for the pre-mortem diagnosis of sporadic human prion disease

Detection of surrogate markers in the CSF

At present the definitive diagnosis of sCJD can only be obtained through the demonstration of PrP^{Sc} in affected tissues. Pre-mortem diagnostic criteria assessing diagnostic probability, originally introduced for epidemiological purposes, have been recently updated [191]. They rely on clinical features and the results of cerebral DW-MRI and laboratory assessment of surrogate markers, such as 14-3-3 [76] and total tau (t-tau) [123] proteins in CSF.

14-3-3 proteins are a group of cytosolic polypeptides with regulatory functions, which are released in the CSF during neuronal damage. As a consequence, not only CJD but also other pathological conditions characterized by a rapid course and neuronal damage, such as viral, paraneoplastic or Hashimoto encephalitis, a recent stroke, metabolic encephalopathy such as Wernicke, epilepsy, or even rapidly progressive AD or Lewy body dementia may lead to a (false) positive test [28,42,161]. Overall, the test specificity for sCJD has been reported to vary from 74% to 96%, depending on type of controls used for the comparison [28,42,161]. Indeed, the specificity of this test increases significantly if cases with a positive standard MRI and/or those showing an inflammatory pattern in the CSF are excluded. Thus, 14-3-3 CSF detection represents a significant supportive diagnostic tool only in the appropriate clinical context.

Recently, attempts have also been made to improve the sensitivity and/or the specificity of the test by modifying the methodology of protein detection. Gmitterová *et al*. [61] developed an improved ELISA technique allowing a better quantification of the protein compared to the analysis by immunoblotting, but did not obtain any improved sensitivity. In another study, Satoh *et al*. [165], by using a semi-quantitative Western blot and various antibodies to specifically detect distinct 14-3-3 isoforms, showed that the specific detection of the γ-isoform may increase the test specificity without influencing significantly its sensitivity.

Beside 14-3-3 the microtubule associated protein tau is also largely used as a surrogate CSF marker for the pre-mortem diagnosis of sCJD, having showed a comparable, or even higher, sensitivity and specificity than 14-3-3 [34,123,161,164]. Indeed, current routine CSF tests in most laboratories involved in CJD diagnosis include both 14-3-3 and t-tau detection since the com-

bination of the two results is thought to increase diagnostic sensitivity. The latter is still a critical issue for the clinical diagnosis of sporadic prion disease, given the high degree of heterogeneity of the disease. Unfortunately, the more the disease subtype is atypical and difficult to recognize clinically, and worse the CSF tests perform. Proteins 14-3-3 and t-tau are markedly elevated in sCJD MM1/MV1 and their measurement provides a diagnostic pre-mortem test with a reported sensitivity that ranges from 91% to 100% [34, 48,61,161]. Similarly, the reported diagnostic sensitivity of 14-3-3 and t-tau detection is quite high also for the VV2 (from 84% to 100%) [34,48,61,161] and the VV1 subtypes, although for the latter the data are based on a few cases only, given the rarity of this CJD type. The diagnostic sensitivity of the CSF tests is significantly lower for both the MM 2C (from 61% to 78%) and MV 2K (from 57% to 89%) subtypes [34,48, 61,161] and becomes inconsistent (lower than 50%) in sCJD MM 2T and VPSPr [56,161].

Other markers for neuronal damage (neuronal specific enolase, a glycolytic enzyme, and S-100 beta protein, a glial protein) have also been tested during the past years, but currently they provide no added value to the test based on 14-3-3 and/or t-tau detection [161].

Finally, concerning the differential diagnosis with other neurodegenerative dementias such as AD, which is the most frequent cause of a false positive test based on 14-3-3 or t-tau among the non-prion neurodegenerative dementia, the analyses of both t-tau/phosphorylated tau ratio and Aβ-amyloid levels have been reported to be useful [188].

In conclusion, despite the lack of specific tests to detect the disease-associated PrP in body fluids, the constellation of clinical symptoms and signs together with the results of EEG, cerebral DW-MRI, 14-3-3 and t-tau CSF and *PRNP* genotype examinations have significantly increased the overall specificity and sensitivity of sCJD *in vivo* diagnosis. The interpretation of the results of CSF investigations, however, requires significant attention and knowledge about the clinical conditions that may lead to false positive results, and most importantly, about the existence of atypical forms of sporadic prion disease that are often associated with a negative CSF or DW-MRI examination. Thus, it remains very important for epidemiological and public health issues that all cases with atypical dementia are followed to autopsy.

Protein misfolding cyclic amplification (PMCA)

In the early 1990s Kocisko *et al*. [91] developed the original *in vitro* prion protein conversion assay by showing that partially purified and GdnHCl denatured brain PrP^{Sc} is able to convert protease-sensitive PrP^{C} isolated from cultured cells in a test tube. Although instrumental in gaining a better understanding, the mechanisms by which PrP^{C} can be converted to PrP^{Sc}, this assay [91] suffered from a number of limitations, particularly the relative inefficiency of the reaction. To overcome this shortcoming, the ability of PrP^{Sc} to convert *in vitro* native PrP into PrP^{Sc} has been more recently exploited in a variety of detection techniques, where the signal from *in vitro* generated abnormal PrP is amplified through cycles of sonication or shaking in the presence of an excess of PrP^{C} or recombinant PrP (rec-PrP) as a substrate [6,171]. In the PMCA, a sonication step is alternated to an incubation step in a variable number of rounds to favour PrP^{Sc} disaggregation and increase the conversion rate [35,153]. In the serial PMCA (sPMCA), the reaction product is cyclically diluted into fresh substrate after each round, in order to maximize the conversion rate [14,36]. It has been shown that PMCA throughput varies considerably according to the reaction conditions, which implicates a role for molecular co-factors [35]. Recently, for example Yokoyama *et al*. [186] demonstrated that the amplification efficiency of cell-PMCA for vCJD PrP^{Sc} increased to more than 100-fold per round in the presence of heparin, whereas the same effect is not seen when sCJD or hamster-adapted 263K scrapie are used as seed. Thus, these results suggest that each prion strain requires a specific pool of co-factors for PrP^{C} conversion to be optimized.

Another important variable affecting PMCA efficiency concerns the similarity between the seed and the substrate. In sCJD, for example, PMCA efficiency tends to increase when the codon 129 genotype of the seed matches that of the substrate, although exceptions to this rule are seen. Indeed, 129VV PrP^{Sc} seems to exclusively convert 129VV PrP^{C}, while 129MM PrP^{Sc} converts, with decreasing efficiency, 129MM PrP^{C}, 129MV PrP^{C} or 129VV PrP^{C} (the latter, however, only with the MM1 PrP^{Sc} substrate). Finally, 129MV PrP^{Sc} appears to convert either 129MM or VV PrP^{C}, depending on the PrP^{Sc} type to which it is associated (type 1 or 2): PrP^{Sc} MV1 converts with the best efficiency PrP^{C} MM, while PrP^{Sc} MV2 converts with the best efficiency PrP^{C} VV [83]. Given that PrP^{Sc} type 1 in sCJD is preferentially found

to be associated with the MM 129 genotype, whereas type 2 is preferentially matched to the VV genotype, the converting activity of 129MV PrP^{Sc} seems to reproduce this peculiar association. It has also been shown that PMCA amplification reproduces with high fidelity the original PrP^{Sc} type of the seed both in terms of fragment size and glycosylation ratio [82-84].

The theory behind the so-called prion *in vitro* conversion on which PMCA is founded, postulates that both PrP^{Sc} and PrP^{C} are required for PrP^{Sc} amplification to occur. At variance with this scenario, however, it has been shown that PrP^{C} conversion may also occur spontaneously in the presence of poly-A^{+} RNA, and that this newly generated PrP^{Sc} is infectious like its natural counterpart [51]. Other authors have also underlined the lack of specificity of PMCA, thus confirming this consistent drawback of the technique. Despite this significant limitation, some scientists continue to use PMCA to increase PrP^{Sc} levels in order to make it detectable in body fluids such as CSF and blood that are accessible for premortem, non-invasive diagnostic assays.

The basic PMCA format has also been changed over the years by introducing technical changes that would simplify the technique or make it more efficient. rPrP-PMCA [3], for example, employed a recombinant PrP substrate expressed in bacterial cells to overcome the difficulties and high expenses related to PrP^{C} recovery from human or transgenic mice brain tissue or from cell cultures. Furthermore, PMCA with teflon beads (PMCAb) has been shown to increase consistently the reaction efficiency with respect to the original format [62]. Beads are thought to help sonication in breaking PrP^{Sc} fibrils into smaller fragments, thus increasing the PrP^{C} amount that undergoes conversion from 10% up to 100% in a single 24-hour round.

Quaking-induced conversion (QuIC)

In this technique, as in rPrP-PMCA, recombinant human PrP expressed by E. Coli is used as the substrate to be seeded by PrP^{Sc}, although sonication is replaced by shaking at a relatively high temperature.

QuIC has ultimately gained more interest than PMCA as a detection technique with diagnostic applicability since it is apparently free from the potential drawbacks of the PMCA approach like the time taken, the complexity of the substrate and reliance on sonication, which is difficult to standardise [4].

Real-time (RT) QuIC is a recent adaptation of QuIC in which the seeded conversion of hamster recombinant PrP (rPrP) into aggregates of PrP^{Sc} is monitored in real time [183]. Thioflavin T (ThT) is included in the reaction and binds to the aggregated PrP^{Sc} causing a change in the ThT emission spectrum which is monitored using fluorescence spectroscopy. The reaction is performed in multiwell microplates, thus allowing the analysis of 96 samples at a time.

This improved QuIC format was inspired by the so-called amyloid seeding assay [44] in which polymerization of rPrP into amyloid fibrils is "seeded" by partially purified preparations of prions without any shaking or sonication and detected by monitoring the changes in the fluorescence emission of thioflavin T, when binds amyloid fibrils.

RT-QuIC seems to be a very promising tool for PrP^{Sc} detection in biological fluids, In this regard, two recent studies [5,110] have shown that CSF samples from patients with neuropathologically confirmed sCJD, at variance with non-CJD controls, can "seed" the RT-QuIC reaction to produce a sustained increase in ThT fluorescence. The first study reported that positive RT-QuIC responses were seen in 29 out of 34 (85%) CSF samples from patients with neuropathologically confirmed sCJD and in none of 14 control patients (100% specificity). Noteworthy, RT-QuIC was able to discriminate sCJD affected patients carrying all three *PRNP* codon 129 genotypes [5]. The second study involved 56 CSF samples from neuropathologically confirmed sCJD patients and 53 from CJD suspected patients that were subsequently found to have an alternative diagnosis. In this study, RT-QuIC showed 91% sensitivity and 98% specificity, compared to 91% sensitivity and 55% specificity for 14-3-3 detection performed on the same samples, thus demonstrating a relevant increase in specificity, without loss in sensitivity, for RT-QuIC with respect to the 14-3-3 test [110]. Both these studies suggest that RT-QuIC may lead to a significant improvement in the pre-mortem sCJD diagnosis and encourage further investigation upon larger patients' cohorts and across a larger number of laboratories. RT-QuIC analysis appears to have similar sensitivity but markedly more specificity than those of both CSF 14-3-3 and tau protein detection. However, the numbers are too small to draw any firm conclusions and further prospective studies are needed to determine whether these figures hold true when larger numbers of patients are investigated. Furthermore, issues such as reproducibility and specificity must be particularly investigated, also in the interlaboratory setting, given the strict dependency of the RT-QuIC on the recombinant substrate. The use of the latter should be carefully investigated in regard to

the potential problem of spontaneous generation of PrP aggregates, an issue that has been disclosed with PMCA but not as yet fully explored in the RT-QuIC.

In another study, Peden *et al*. [140] have addressed the question of whether PrP^{Sc} derived from different CJD variants (including both sCJD and vCJD) could affect the amplification efficiency of RT-QuIC, as it was demonstrated with PMCA. They found no substantial decrease in the assay throughput when the 129 genotype between seed and substrate did not match. Moreover, similar amplification efficiency for hamster rec-PrP was observed for all sCJD phenotypes with the only exception of sCJD VV1 and MM2, which appeared slightly less permissive to amplification. At variance with sCJD, vCJD PrP^{Sc} showed a poor amplification, even using a homologous 129M human rec-PrP substrate. This diversity was apparently not determined by inhibiting factors, but rather by conformational characteristics of vCJD PrP^{Sc}. Overall, these data suggest that RT-QuIC may be better standardized than PMCA, at least for sCJD variants, which makes RT-QuIC the current best candidate technique for the development of a pre-mortem CSF diagnostic test for sCJD with better sensitivity for all variants. In addition, a better understanding of the factors differentially affecting PMCA and RT-QuIC could also help to better elucidate the PrP^{Sc} amplification process *in vivo*.

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References

1. Aguzzi A, Baumann F, Bremer J. The prion's elusive reason for being. Ann Rev Neurosci 2008; 31: 439-477.
2. Aguzzi A, Heikenwalder M, Polymenidou M. Insights into prion strains and neurotoxicity. Nat Rev Mol Cell Biol 2007; 8: 552-561.
3. Atarashi R, Moore RA, Sim VL, Hughson AG, Dorward DW, Onwubiko HA, Priola SA, Caughey B. Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. Nat Methods 2007; 4: 645-650.
4. Atarashi R, Sano K, Satoh K, Nishida N. Real-time quaking-induced conversion: A highly sensitive assay for prion detection. Prion 2011; 5: 150-153.
5. Atarashi R, Satoh K, Sano K, Fuse T, Yamaguchi N, Ishibashi D, Matsubara T, Nakagaki T, Yamanaka H, Shirabe S, Yamada M, Mizusawa H, Kitamoto T, Klug G, McGlade A, Collins SJ, Nishida N. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. Nat Med 2011; 17: 175-178.
6. Atarashi R, Wilham JM, Christensen L, Hughson AG, Moore RA, Johnson LM, Onwubiko HA, Priola SA, Caughey B. Simplified ultrasensitive prion detection by recombinant PrP conversion with shaking. Nat Methods 2008; 5: 211-212.
7. Baron GS, Caughey B. Effect of glycosylphosphatidylinositol anchor-dependent and -independent prion protein association with model raft membranes on conversion to the protease-resistant isoform. J Biol Chem 2003; 278: 14883-14892.
8. Baron GS, Wehrly K, Dorward DW, Chesebro B, Caughey B. Conversion of raft associated prion protein to the protease-resistant state requires insertion of PrP^{res} (PrP^{Sc}) into contiguous membranes. EMBO J 2002; 21: 1031-1040.
9. Baron TG, Madec JY, Calavas D, Richard Y, Barillet F. Comparison of French natural scrapie isolates with bovine spongiform encephalopathy and experimental scrapie infected sheep. Neurosci Lett 2000; 284: 175-178
10. Benestad SL, Arsac JN, Goldmann W, Nöremark M. Atypical/Nor98 scrapie: properties of the agent, genetics, and epidemiology. Vet Res. 2008; 39: 19.
11. Béranger F, Mangé A, Goud B, Lehmann S. Stimulation of PrP^{C} retrograde transport toward the endoplasmic reticulum increases accumulation of PrP^{Sc} in prion-infected cells. J Biol Chem 2002; 27: 38972-38977.
12. Bessen RA, Kocisko DA, Raymond GJ, Nandan S, Lansbury PT, Caughey B. Non-genetic propagation of strain-specific properties of scrapie prion protein. Nature 1995; 375: 698-700.
13. Bessen RA, Marsh RF. Distinct PrP properties suggest the molecular basis of strain variation in transmissible mink encephalopathy. J Virol 1994; 68: 7859-7868.
14. Bieschke J, Weber P, Sarafoff N, Beekes M, Giese A, Kretzschmar H. Autocatalytic self-propagation of misfolded prion protein. Proc Natl Acad Sci U S A 2004; 10: 12207-12211.
15. Bishop MT, Will RG, Manson JC. Defining sporadic Creutzfeldt-Jakob disease strains and their transmission properties. Proc Natl Acad Sci U S A 2010; 107: 12005-12010.
16. Borchelt DR, Taraboulos A, Prusiner SB. Evidence for synthesis of scrapie prion proteins in the endocytic pathway. J Biol Chem 1992; 267: 16188-16199.
17. Botto L, Masserini M, Cassetti A, Palestini P. Immunoseparation of prion protein-enriched domains from other detergent-resistant membrane fractions, isolated from neuronal cells. FEBS Lett 2004; 557: 143-147.
18. Brown DR, Qin K, Herms JW, Madlung A, Manson J, Strome R, Fraser PE, Kruck T, von Bohlen A, Schulz-Schaeffer W, Giese A, Westaway D, Kretzschmar H. The cellular prion protein binds copper in vivo. Nature 1997; 390: 684-687
19. Brown LR, Harris DA. Copper and zinc cause delivery of the prion protein from the plasma membrane to a subset of early endosomes and the Golgi. J Neurochem 2003; 87: 353-363.
20. Brown P, Brandel JP, Preece M, Sato T. Iatrogenic Creutzfeldt-Jakob disease: the waning of an era. Neurology 2006; 6: 389-393.
21. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, Asher DM, Sulima MP, Bacote A, Goldfarb LG, Gajdusek DC. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. Ann Neurol 1994; 35: 513-529.
22. Bruce ME, McConnell I, Fraser H, Dickinson AG. The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. J Gen Virol 1991; 72: 595-603.

23. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 1997; 389: 498-501.
24. Bruce ME. Strain typing studies on scrapie and BSE. In: Baker H, Ridley RM (eds.). Methods in Molecular Medicine: Prion Diseases. Humana Press Inc. 1996; p. 223.
25. Budka H, Head MW, Ironside JW. Sporadic Creutzfeldt-Jacob disease. In: Dickson DW and Weller RO (eds.). Neurodegeneration. Wiley-Blackwell, Chichester 2011; pp. 322-335
26. Büeler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, Weissmann C. Mice devoid of PrP are resistant to scrapie. Cell 1993; 73: 1339-1347.
27. Büeler H, Fischer M, Lang Y, Bluethmann H, Lipp H, Lipp P, DeArmond SJ, Prusiner SB, Aguet M and Weissman C. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. Nature 1992; 356: 577-582.
28. Burkhard PR, Sanchez JC, Landis T, Hochstrasser DF. CSF detection of the 14-3-3 protein in unselected patients with dementia. Neurology. 2001; 56: 1528-1533.
29. Cali I, Castellani R, Alshekhlee A, Cohen Y, Blevins J, Yuan J, Langeveld JP, Parchi P, Safar JG, Zou WQ, Gambetti P. Co-existence of scrapie prion protein types 1 and 2 in sporadic Creutzfeldt-Jakob disease: its effect on the phenotype and prion-type characteristics. Brain 2009; 132: 2643-2658.
30. Cali I, Castellani R, Yuan J, Al-Shekhlee A, Cohen ML, Xiao X, Moleres FJ, Parchi P, Zou WQ, Gambetti P. Classification of sporadic Creutzfeldt-Jakob disease revisited. Brain 2006; 129: 2266-2277.
31. Campana V, Sarnataro D, Zurzolo C. The highways and byways of prion protein trafficking. Trends Cell Biol 2005; 15: 102-111.
32. Capellari S, Parchi P, Russo CM, Sanford J, Sy MS, Gambetti P, Petersen RB Effect of the E200K mutation on prion protein metabolism. Comparative study of a cell model and human brain. Am J Pathol 2000; 157: 613-622.
33. Capellari S, Strammiello R, Saverioni D, Kretzschmar H, Parchi P. Genetic Creutzfeldt-Jakob disease and fatal familial insomnia: insights into phenotypic variability and disease pathogenesis. Acta Neuropathol 2011; 121: 21-37.
34. Castellani RJ, Colucci M, Xie Z, Zou W, Li C, Parchi P, Capellari S, Pastore M, Rahbar MH, Chen SG, Gambetti P. Sensitivity of 14-3-3 protein test varies in subtypes of sporadic Creutzfeldt-Jakob disease. Neurology 2004; 63: 436-442.
35. Castilla J, Saá P, Morales R, Abid K, Maundrell K, Soto C. Protein misfolding cyclic amplification for diagnosis and prion propagation studies. Methods Enzymol 2006; 412: 3-21.
36. Castilla J, Saá P, Soto C. Detection of prions in blood. Nat Med 2005; 11: 982-985.
37. Caughey B, Raymond GJ, Bessen RA. Strain-dependent differences in beta-sheet conformations of abnormal prion protein. J Biol Chem 1998; 273: 32230-32235.
38. Caughey B, Raymond GJ, Kocisko DA, Lansbury PT, Jr. Scrapie infectivity correlates with converting activity, protease resistance, and aggregation of scrapie-associated prion protein in guanidine denaturation studies. J Virol 1997; 71: 4107-4110.
39. Caughey B, Raymond GJ. The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. J Biol Chem 1991; 266: 18217-18223.
40. Caughey BW, Dong A, Bhat KS, Ernst D, Hayes SF, Caughey WS. Secondary structure analysis of the scrapie-associated protein PrP 27-30 in water by infrared spectroscopy. Biochemistry 1991; 30: 7672-7680.
41. Chen SG, Teplow DB, Parchi P, Teller JK, Gambetti P, Autilio-Gambetti L. Truncated forms of the human prion protein in normal brain and in prion diseases. J Biol Chem 1995; 270: 19173-19180.
42. Chohan G, Pennington C, Mackenzie JM, Andrews M, Everington D, Will RG, Knight RS, Green AJ. The role of cerebrospinal fluid 14-3-3 and other proteins in the diagnosis of sporadic Creutzfeldt-Jakob disease in the UK: a 10-year review. J Neurol Neurosurg Psychiatry 2010; 81: 1243-1248.
43. Colby DW, Prusiner SB. Prions. Cold Spring Harb Perspect Biol 2011; 3: a006833.
44. Colby DW, Zhang Q, Wang S, Groth D, Legname G, Riesner D, Prusiner SB. Prion detection by an amyloid seeding assay. Proc Natl Acad Sci U S A 2007; 104: 20914-20919.
45. Collinge J, Clarke AR. A general model of prion strains and their pathogenicity. Science 2007; 318: 930-936.
46. Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 1996; 383: 685-690.
47. Collins S, Law MG, Fletcher, Boyd A, Kaldor J, Masters CL. Surgical treatment and risk of sporadic Creutzfeldt-Jakob disease: a case control study. Lancet 1999; 353: 693-697.
48. Collins SJ, Sanchez-Juan P, Masters CL, Klug GM, van Duijn C, Poleggi A, Pocchiari M, Almonti S, Cuadrado-Corrales N, de Pedro-Cuesta J, Budka H, Gelpi E, Glatzel M, Tolnay M, Hewer E, Zerr I, Heinemann U, Kretszchmar HA, Jansen GH, Olsen E, Mitrova E, Alpérovitch A, Brandel JP, Mackenzie J, Murray K, Will RG. Determinants of diagnostic investigation sensitivities across the clinical spectrum of sporadic Creutzfeldt-Jakob disease. Brain 2006; 129: 2278-2287.
49. Cronier S, Gros N, Tattum MH, Jackson GS, Clarke AR, Collinge J, Wadsworth JD. Detection and characterization of proteinase K-sensitive disease-related prion protein with thermolysin. Biochem J. 2008; 416: 297-305.
50. DeArmond SJ, Sanchez H, Yehiely F, Qiu Y, Ninchak-Casey A, Daggett V, Camerino AP, Cayetano J, Rogers M, Groth D, Torchia M, Tremblay P, Scott MR, Cohen FE, Prusiner SB. Selective neuronal targeting in prion disease. Neuron 1997; 19: 1337-1348.
51. Deleault NR, Harris BT, Rees JR, Supattapone S. Formation of native prions from minimal components in vitro. Proc Natl Acad Sci U S A 2007; 104: 9741-9746.
52. Dobson CM. Protein misfolding, evolution and disease. Trends Biochem Sci 1999; 24: 329-332.
53. Eigen M. Prionics or the kinetic basis of prion diseases. Biophys Chem 1996; 63: A1-18.
54. Farquhar CF, Somerville RA, Bruce ME. Straining the prion hypothesis. Nature 1998; 391: 345-346.
55. Fraser H, Dickinson AG. Scrapie in mice. Agent-strain differences in the distribution and intensity of grey matter vacuolation. J Comp Pathol 1973; 83: 29-40.
56. Gambetti P, Dong Z, Yuan J, Xiao X, Zheng M, Alshekhlee A, Castellani R, Cohen M, Barria MA, Gonzalez-Romero D, Belay ED, Schonberger LB, Marder K, Harris C, Burke JR, Montine T, Wisniewski T, Dickson DW, Soto C, Hulette CM, Mastrianni JA, Kong Q, Zou

WQ. A novel human disease with abnormal prion protein sensitive to protease. Ann Neurol. 2008; 63: 697-708.
57. Gambetti P, Zou WQ, Torres JM, Soto C, Notari S, Espinosa JC, Xiao X. Variably protease-sensitive prionopathy: transmissibility and PMCA studies. Prion 2011; 5: 14.
58. Gao JM, Zhou XB, Xiao XL, Zhang J, Chen L, Gao C, Zhang BY, Dong XP. Influence of guanidine on proteinase K resistance in vitro and infectivity of scrapie prion protein PrP^{Sc}. Acta Virol 2006; 50: 25-32.
59. Ghetti B, Tagliavini F, Kovacs GG, Piccardo P. Gertsmann- Sträussler-Scheinker disease. In: Dickson DW and Weller RO (eds.). Neurodegeneration. Wiley-Blackwell, Chichester 2011; pp. 364-377.
60. Glatzel M, Rogivue C, Ghani A, Streffer JR, Amsler L, Aguzzi A. Incidence of Creutzfeldt-Jakob disease in Switzerland. Lancet 2002; 360: 139-141.
61. Gmitterová K, Heinemann U, Bodemer M, Krasnianski A, Meissner B, Kretzschmar HA, Zerr I. 14-3-3 CSF levels in sporadic Creutzfeldt-Jakob disease differ across molecular subtypes. Neurobiol Aging 2009; 30: 1842-1850.
62. Gonzalez-Montalban N, Makarava N, Ostapchenko VG, Savtchenk R, Alexeeva I, Rohwer RG, Baskakov IV. Highly efficient protein misfolding cyclic amplification. PLoS Pathog 2011; 7: e1001277.
63. Haik S, Galanaud D, Linguraru MG, Peoc'h K, Privat N, Faucheux BA, Ayache N, Hauw JJ, Dormont D, Brandel JP. In vivo detection of thalamic gliosis: a pathoradiologic demonstration in familial fatal insomnia. Arch Neurol 2008; 65: 545-549.
64. Hamaguchi T, Kitamoto T, Sato T, Mizusawa H, Nakamura Y, Noguchi M, Furukawa Y, Ishida C, Kuji I, Mitani K, Murayama S, Kohriyama T, Katayama S, Yamashita M, Yamamoto T, Udaka F, Kawakami A, Ihara Y, Nishinaka T, Kuroda S, Suzuki N, Shiga Y, Arai H, Maruyama M, Yamada M. Clinical diagnosis of MM2-type sporadic Creutzfeldt-Jakob disease. Neurology 2005; 64: 643-648.
65. Harris DA. Trafficking, turnover and membrane topology of PrP. Br Med Bull 2003; 66: 71-85.
66. Head MW, Bunn TJ, Bishop MT, McLoughlin V, Lowrie S, McKimmie CS, Williams MC, McCardle L, MacKenzie J, Knight R, Will RG, Ironside JW). Prion protein heterogeneity in sporadic but not variant Creutzfeldt-Jakob disease: UK cases 1991-2002. Ann Neurol 2004; 55: 851-859.
67. Head MW, Ironside JW. Sporadic Creutzfeldt-Jakob disease: discrete subtypes or a spectrum of disease? Brain 2009; 132: 2627-2629.
68. Head MW, Knight R, Zeidler M, Yull H, Barlow A, Ironside JW. A case of protease sensitive prionopathy in a patient in the UK. Neuropathol Appl Neurobiol 2009; 35: 628-632.
69. Head MW, Lowrie S, Chohan G, Knight R, Scoones DJ, Ironside JW. Variably protease-sensitive prionopathy in a PRNP codon 129 heterozygous UK patient with co-existing tau, α synuclein and $A\beta$ pathology. Acta Neuropathol 2010; 120: 821-823.
70. Hecker R, Taraboulos A, Scott M, Pan KM, Yang SL, Torchia M, Jendroska K, DeArmond SJ, Prusiner SB. Replication of distinct scrapie prion isolates is region specific in brains of transgenic mice and hamsters. Genes Dev 1992; 6: 1213-1228.
71. Herms J, Tings T, Gall S, Madlung A, Giese A, Siebert H, Schurmann P, Windl O, Brose N, Kretzschmar H. Evidence of presynaptic location and function of the prion protein. J Neurosci 1999; 19: 8866-8875.
72. Hill AF, Desbruslais M, Joiner S, Sidle KC, Gowland I, Collinge J, Doey LJ, Lantos P. The same prion strain causes vCJD and BSE. Nature 1997; 389: 448-450, 526.
73. Hill AF, Joiner S, Beck JA, Campbell TA, Dickinson A, Poulter M, Wadsworth JD, Collinge J. Distinct glycoform ratios of protease resistant prion protein associated with PRNP point mutations. Brain 2006; 129: 676-685.
74. Horiuchi M, Caughey B. Specific binding of normal prion protein to the scrapie form via a localized domain initiates its conversion to the protease-resistant state. EMBO J 1999; 18: 3193-3203.
75. Hornshaw MP, McDermott JR, Candy JM, Lakey JH. Copper binding to the N-terminal tandem repeat region of mammalian and avian prion protein: structural studies using synthetic peptides. Biochem Biophys Res Commun 1995; 214: 993-999.
76. Hsich G, Kenney K, Gibbs CJ, Lee KH, Harrington MG. The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. N Engl J Med 1996; 335: 924-930.
77. Ironside JW, Head MW, Will RG. Variant Creutzfeldt-Jacob disease. In: Dickson DW and Weller RO (eds.). Neurodegeneration. Wiley-Blackwell, Chichester 2011; pp. 354-363.
78. Ironside JW, Knight RSG, Head MW. Iatrogenic Creutzfeldt-Jacob disease. In: Dickson DW and Weller RO (eds.). Neurodegeneration. Wiley-Blackwell, Chichester 2011; pp. 381-388.
79. Jansen C, Head MW, van Gool WA, Baas F, Yull H, Ironside JW, Rozemuller AJ. The first case of protease-sensitive prionopathy (PSPr) in The Netherlands: a patient with an unusual GSS-like clinical phenotype. J Neurol Neurosurg Psychiatry 2010; 81: 1052-1055.
80. Jansen C, Parchi P, Capellari S, Ibrahim-Verbaas CA, Schuur M, Strammiello R, Corrado P, Bishop MT, van Gool VA, Verbeek MM, Baas F, van Saane V, Spliet WGM, Jansen GH, van Duijn CM, Rozemuller AJM. Human prion diseases in The Netherlands (1998-2009): Clinical, genetic and molecular aspects. PloS One 2012 (in press).
81. Jansen C, Parchi P, Capellari S, Strammiello R, Dopper EG, van Swieten JC, Kamphorst W, Rozemuller AJ. A second case of Gerstmann-Sträussler-Scheinker disease linked to the G131V mutation in the prion protein gene in a Dutch patient. J Neuropathol Exp Neurol 2011; 70: 698-702.
82. Jones M, Peden AH, Prowse CV, Gröner A, Manson JC, Turner ML, Ironside JW, MacGregor IR, Head MW. in vitro amplification and detection of variant Creutzfeldt-Jakob disease PrP^{Sc}. J Pathol 2007; 213: 21-26.
83. Jones M, Peden AH, Wight D, Prowse C, Macgregor I, Manson J, Turner M, Ironside JW, Head MW. Effects of human PrP^{Sc} type and PRNP genotype in an in-vitro conversion assay. Neuroreport 2008; 19: 1783-1786.
84. Jones M, Wight D, Barron R, Jeffrey M, Manson J, Prowse C, Ironside JW, Head MW. Molecular model of prion transmission to humans. Emerg Infect Dis 2009; 15: 2013-2016.
85. Kaneko K, Vey M, Scott M, Pilkuhn S, Cohen FE, Prusiner SB. COOH-terminal sequence of the cellular prion protein directs subcellular trafficking and controls conversion into the scrapie isoform. Proc Natl Acad Sci U S A 1997; 94: 2333-2338.
86. Kim C, Haldiman T, Cohen Y, Chen W, Blevins J, Sy MS, Cohen M, Safar JG. Protease-sensitive conformers in broad spectrum of distinct PrP^{Sc} structures in sporadic Creutzfeldt-Jakob disease are indicator of progression rate. PLoS Pathog 2011; 7: e1002242.

87. Kitamoto T, Mohri S, Ironside JW, Miyoshi I, Tanaka T, Kitamoto N, Itohara S, Kasai N, Katsuki M, Higuchi J, Muramoto T, Shin RW. Follicular dendritic cell of the knock-in mouse provides a new bioassay for human prions. Biochem Biophys Res Commun 2002; 294: 280-286.
88. Kobayashi A, Arima K, Ogawa M, Murata M, Fukuda T, Kitamoto T. Plaque-type deposition of prion protein in the damaged white matter of sporadic Creutzfeldt-Jakob disease MM1 patients. Acta Neuropathol 2008; 116: 561-566.
89. Kobayashi A, Mizukoshi K, Iwasaki Y, Miyata H, Yoshida Y, Kitamoto T. Co-occurrence of types 1 and 2 PrP^{res} in sporadic Creutzfeldt-Jakob disease MM1. Am J Pathol 2011; 178: 1309-1315.
90. Kobayashi A, Sakuma N, Matsuura Y, Mohri S, Aguzzi A, Kitamoto T. Experimental verification of a traceback phenomenon in prion infection. J Virol 2010; 84: 3230-3238.
91. Kocisko DA, Come, JH, Priola SA, Chesebro B, Raymond GJ, Lansbury PT, Caughey B. Cell-free formation of protease-resistant prion protein. Nature 1994; 370: 471-474.
92. Kocisko DA, Lansbury PT Jr, Caughey B. Partial unfolding and refolding of scrapie-associated prion protein: evidence for a critical 16-kDa C-terminal domain. Biochemistry 1996; 35: 13434-13442.
93. Korth C, Kaneko K, Groth D, Heye N, Telling G, Mastrianni J, Parchi P, Gambetti P, Will R, Ironside J, Heinrich C, Tremblay P, DeArmond SJ, Prusiner SB. Abbreviated incubation times for human prions in mice expressing a chimeric mouse-human prion protein transgene. Proc Natl Acad Sci U S A 2003; 100: 4784-4789.
94. Kuczius T, Koch R, Keyvani K, Karch H, Grassi J, Groschup MH. Regional and phenotype heterogeneity of cellular prion proteins in the human brain. Eur J Neurosci 2007; 25: 2649-2655.
95. Lasmezas CI, Fournier JG, Nouvel V, Boe H, Marce D, Lamoury F, Kopp N, Hauw JJ, Ironside J, Bruce M, Dormont D, Deslys JP Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health. Proc Natl Acad Sci U S A 2001; 98: 4142-4147.
96. Lee KS, Magalhães AC, Zanata SM, Brentani RR, Martins VR, Prado MA. Internalization of mammalian fluorescent cellular prion protein and N-terminal deletion mutants in living cells. J Neurochem 2001; 79: 79-87.
97. Legname G, Nguyen HO, Baskakov IV, Cohen FE, Dearmond SJ, Prusiner SB. Strain-specified characteristics of mouse synthetic prions. Proc Natl Acad Sci U S A 2005; 102: 2168-2173.
98. Legname G, Nguyen HO, Peretz D, Cohen FE, DeArmond SJ, Prusiner SB. Continuum of prion protein structures enciphers a multitude of prion isolate-specified phenotypes. Proc Natl Acad Sci U S A 2006; 103: 19105-19110.
99. Levavasseur E, Laffont-Proust I, Morain E, Faucheux BA, Privat N, Peoc'h K, Sazdovitch V, Brandel JP, Hauw JJ, Haik S. Regulating factors of PrP glycosylation in Creutzfeldt-Jakob disease--implications for the dissemination and the diagnosis of human prion strains. PLoS One 2008; 3: e2786.
100. Lewis V, Hill AF, Klug GM, Boyd A, Masters CL, Collins SJ. Australian sporadic CJD analysis supports endogenous determinants of molecular-clinical profiles. Neurology 2005; 65: 113-118.
101. Li J, Browning S, Mahal SP, Oelschlegel AM, Weissmann C. Darwinian evolution of prions in cell culture. Science. 2010; 327: 869-872.
102. Li J, Mahal SP, Demczyk CA, Weissmann C. Mutability of prions. EMBO Rep 2011; 12: 1243-1250.
103. Linden R, Martins VR, Prado MA, Cammarota M, Izquierdo I, Brentani RR. Physiology of the prion protein. Physiol Rev 2008; 88: 673-728.
104. Liu C, Zhang Y. Nucleic acid-mediated protein aggregation and assembly. Adv Protein Chem Struct Biol 2011; 84: 1-40.
105. Lodi R, Parchi P, Tonon C, Manners D, Capellari S, Strammiello R, Rinaldi R, Testa C, Malucelli E, Mostacci B, Rizzo G, Pierangeli G, Cortelli P, Montagna P, Barbiroli B. Magnetic resonance diagnostic markers in clinically sporadic prion disease: a combined brain magnetic resonance imaging and spectroscopy study. Brain 2009; 132: 2669-2679.
106. Mallucci G, Dickinson A, Linehan J, Klöhn PC, Brandner S, Collinge J. Depleting neuronal PrP in prion infection prevents disease and reverses spongiosis. Science 2003; 302: 871-874.
107. Mallucci GR, Ratté S, Asante EA, Linehan J, Gowland I, Jefferys JG, Collinge J. Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. EMBO J 2002; 21: 202-210.
108. Manners DN, Parchi P, Tonon C, Capellari S, Strammiello R, Testa C, Tani G, Malucelli E, Spagnolo C, Cortelli P, Montagna P, Lodi R, Barbiroli B. Pathologic correlates of diffusion MRI changes in Creutzfeldt-Jakob disease. Neurology 2009; 72: 1425-1431.
109. Mastrianni JA, Nixon R, Layzer R, Telling GC, Han D, DeArmond SJ, Prusiner SB. Prion protein conformation in a patient with sporadic fatal insomnia. N Engl J Med 1999; 340: 1630-1638.
110. McGuire LI, Peden AH, Appleford N, Mallinson G, Orrů C, Wilham J, Raymond G, Andrews M, Head MW, Caughey B, Will R, Knight R, Green A. Prion seeding activity in cerebrospinal fluid from sporadic Creutzfeldt-Jacob disease patients using real-time QuIC analysis: a potential new diafgnostic test? Prion 2011; 5: 18.
111. McLean CA. Kuru. In: Dickson DW and Weller RO (ed). Neurodegeneration. Wiley-Blackwell, Chichester 2011, pp. 354-363.
112. Meissner B, Kallenberg K, Sanchez-Juan P, Collie D, Summers DM, Almonti S, Collins SJ, Smith P, Cras P, Jansen GH, Brandel JP, Coulthart MB, Roberts H, Van Everbroeck B, Galanaud D, Mellina V, Will RG, Zerr I. MRI lesion profiles in sporadic Creutzfeldt-Jakob disease. Neurology 2009; 72: 1994-2001.
113. Meissner B, Westner IM, Kallenberg K, Krasnianski A, Bartl M, Varges D, Bösenberg C, Kretzschmar HA, Knauth M, Schulz-Schaeffer WJ, Zerr I. Sporadic Creutzfeldt-Jakob disease: clinical and diagnostic characteristics of the rare VV1 type. Neurology 2005; 65: 1544-1550.
114. Moda F, Suardi S, Di Fede G, Indaco A, Limido L, Vimercati C, Ruggerone M, Campagnani I, Langeveld J, Terruzzi A, Brambilla A, Zerbi P, Fociani P, Bishop MT, Will RG, Manson JC, Giaccone G, Tagliavini F. MM2-thalamic Creutzfeldt-Jakob disease: neuropathological, biochemical and transmission studies identify a distinctive prion strain. Brain Pathol 2012 Jan 30 [Epub ahead of print]
115. Monari L, Chen SG, Brown P, Parchi P, Petersen RB, Mikol J, Gray F, Cortelli P, Montagna P, Ghetti B, Goldfarb LG, Gajdusek DC, Lugaresi E, Gambetti P, Autilio-Gambetti L. Fatal familial insomnia and familial Creutzfeldt-Jakob disease: different prion proteins determined by a DNA polymorphism. Proc Natl Acad Sci U S A 1994; 91: 2839-2842.
116. Montagna P, Gambetti P, Cortelli P, Lugaresi E. Familial and sporadic fatal insomnia. Lancet Neurol 2003; 2: 167-176.

117. Naslavsky N, Stein R, Yanai A, Friedlander G, Taraboulos A. Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J Biol Chem 1997; 272: 6324-6331.
118. Nazor KE, Kuhn F, Seward T, Green M, Zwald D, Pürro M, Schmid J, Biffiger K, Power AM, Oesch B, Raeber AJ, Telling GC. Immunodetection of disease-associated mutant PrP, which accelerates disease in GSS transgenic mice. EMBO J 2005; 24: 2472-2480.
119. Nonno R, Di Bari MA, Cardone F, Vaccari G, Fazzi P, Dell'Omo G, Cartoni C, Ingrosso L, Boyle A, Galeno R, Sbriccoli M, Lipp HP, Bruce M, Pocchiari M, Agrimi U. Efficient transmission and characterization of Creutzfeldt-Jakob disease strains in bank voles. PLoS Pathog 2006; 2: e12.
120. Notari S, Capellari S, Giese A, Westner I, Baruzzi A, Ghetti B, Gambetti P, Kretzschmar HA, Parchi P Effects of different experimental conditions on the PrP^{Sc} core generated by protease digestion: implications for strain typing and molecular classification of CJD. J Biol Chem 2004; 279: 16797-16804.
121. Notari S, Capellari S, Langeveld J, Giese A, Strammiello R, Gambetti P, Kretzschmar HA, Parchi P. A refined method for molecular typing reveals that co-occurrence of PrP^{Sc} types in Creutzfeldt-Jakob disease is not the rule. Lab Invest 2007; 87: 1103-1112.
122. Notari S, Strammiello R, Capellari S, Giese A, Cescatti M, Grassi J, Ghetti B, Langeveld JP, Zou WQ, Gambetti P, Kretzschmar HA, Parchi P. Characterization of truncated forms of abnormal prion protein in Creutzfeldt-Jakob disease. J Biol Chem 2008; 283: 30557-30565.
123. Otto M, Wiltfang J, Cepek L, Neumann M, Mollenhauer B, Steinacker P, Ciesielczyk B, Schulz-Schaeffer W, Kretzschmar HA, Poser S. Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology 2002; 58: 192-197.
124. Palmer MS, Dryden AJ, Hughes JT, Collinge J. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. Nature 1991; 352: 340-342.
125. Pan KM, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, Prusiner SB Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci U S A 1993; 90: 10962-10966.
126. Pan T, Colucci M, Wong BS, Li R, Liu T, Petersen RB, Chen S, Gambetti P, Sy MS. Novel differences between two human prion strains revealed by two-dimensional gel electrophoresis. J Biol Chem 2001; 276: 37284-37288.
127. Parchi P, Capellari S, Chen SG, Petersen RB, Gambetti P, Kopp N, Brown P, Kitamoto T, Tateishi J, Giese A, Kretzschmar H. Typing prion isoforms. Nature 1997; 386: 232-234.
128. Parchi P, Capellari S, Chin S, Schwarz HB, Schecter NP, Butts JD, Hudkins P, Burns DK, Powers JM, Gambetti P. A subtype of sporadic prion disease mimicking fatal familial insomnia. Neurology 1999; 52: 1757-1763.
129. Parchi P, Castellani R, Capellari S, Ghetti B, Young K, Chen SG, Farlow M, Dickson DW, Sima AA, Trojanowski JQ, Petersen RB, Gambetti P. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol 1996; 39: 767-778.
130. Parchi P, Cescatti M, Notari S, Schulz-Schaeffer WJ, Capellari S, Giese A, Zou WQ, Kretzschmar H, Ghetti B, Brown P. Agent strain variation in human prion disease: insights from a molecular and pathological review of the National Institutes of Health series of experimentally transmitted disease. Brain 2010; 133: 3030-3042.
131. Parchi P, Chen SG, Brown P, Zou W, Capellari S, Budka H, Hainfellner J, Reyes PF, Golden GT, Hauw JJ, Gajdusek DC, Gambetti P Different patterns of truncated prion protein fragments correlate with distinct phenotypes in P102L Gerstmann-Straussler-Scheinker disease. Proc Natl Acad Sci U S A 1998; 95: 8322-8327.
132. Parchi P, Gambetti P, Capellari S. Genetic Creutfeldt-Jacob disease. In: Dickson DW and Weller RO (eds.). Neurodegeneration. Wiley-Blackwell, Chichester 2011; pp. 336-345.
133. Parchi P, Giese A, Capellari S, Brown P, Schulz-Schaeffer W, Windl O, Zerr I, Budka H, Kopp N, Piccardo P, Poser S, Rojiani A, Streichemberger N, Julien J, Vital C, Ghetti B, Gambetti P, Kretzschmar H. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann Neurol 1999; 46: 224-233
134. Parchi P, Notari S, Weber P, Schimmel H, Budka H, Ferrer I, Haik S, Hauw JJ, Head MW, Ironside JW, Limido L, Rodriguez A, Strobel T, Tagliavini F, Kretzschmar HA. Inter-laboratory assessment of PrP^{Sc} typing in Creutzfeldt-Jakob disease: a Western blot study within the NeuroPrion Consortium. Brain Pathol 2009; 19: 384-391.
135. Parchi P, Strammiello R, Notari S, Giese A, Langeveld JP, Ladogana A, Zerr I, Roncaroli F, Cras P, Ghetti B, Pocchiari M, Kretzschmar H, Capellari S Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrP^{Sc} types: an updated classification. Acta Neuropathol 2009; 118: 659-671.
136. Parchi P, Zou W, Wang W, Brown P, Capellari S, Ghetti B, Kopp N, Schulz-Schaeffer WJ, Kretzschmar HA, Head MW, Ironside JW, Gambetti P, Chen SG. Genetic influence on the structural variations of the abnormal prion protein. Proc Natl Acad Sci U S A 2000; 97: 10168-10172.
137. Pastrana MA, Sajnani G, Onisko B, Castilla J, Morales R, Soto C, Requena JR. Isolation and characterization of a proteinase K-sensitive PrP^{Sc} fraction. Biochemistry 2006; 45: 15710-15717.
138. Pattison IH, Millson GC. Scrapie produced experimentally in goats with special reference to the clinical syndrome. J Comp Pathol 1961; 71: 101-109.
139. Pauly PC, Harris DA. Copper stimulates endocytosis of the prion protein. J Biol Chem 1998; 273: 33107-33110.
140. Peden AH, McGuire LI, Appleford NE, Mallinson G, Wilham JM, Orrú CD, Caughey B, Ironside JW, Knight RS, Will RG, Green AJ, Head MW. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. J Gen Virol 2012; 93: 438-449.
141. Perera WS, Hooper NM. Ablation of the metal ion-induced endocytosis of the prion protein by disease-associated mutation of the octarepeat region. Curr Biol 2001; 11: 519-523.
142. Peretz D, Scott MR, Groth D, Williamson RA, Burton DR, Cohen FE, Prusiner SB. Strain-specified relative conformational stability of the scrapie prion protein. Protein Sci 2001; 10: 854-863.
143. Peters PJ, Mironov A Jr, Peretz D, van Donselaar E, Leclerc E, Erpel S, DeArmond SJ, Burton DR, Williamson RA, Vey M, Prusiner SB. Trafficking of prion proteins through a caveolae-mediated endosomal pathway. J Cell Biol 2003; 162: 703-717.

144. Piccardo P, Dlouhy SR, Lievens PM, Young K, Bird TD, Nochlin D, Dickson DW, Vinters HV, Zimmerman TR, Mackenzie IR, Kish SJ, Ang LC, De Carli C, Pocchiari M, Brown P, Gibbs CJ, Jr., Gajdusek DC, Bugiani O, Ironside J, Tagliavini F, Ghetti B. Phenotypic variability of Gerstmann-Sträussler-Scheinker disease is associated with prion protein heterogeneity. J Neuropathol Exp Neurol 1998; 57: 979-988.
145. Pinheiro TJ. The role of rafts in the fibrillization and aggregation of prions. Chem Phys Lipids 2006; 141: 66-71.
146. Pirisinu L, Di Bari M, Marcon S, Vaccari G, D'Agostino C, Fazzi P, Esposito E, Galeno R, Langeveld J, Agrimi U, Nonno R. A new method for the characterization of strain-specific conformational stability of protease-sensitive and protease-resistant PrP. PLoS One 2010; 5: e12723.
147. Polymenidou M, Prokop S, Jung HH, Hewer E, Peretz D, Moos R, Tolnay M, Aguzzi A. Atypical prion protein conformation in familial prion disease with PRNP P105T mutation. Brain Pathol 2011; 21: 209-214.
148. Polymenidou M, Stoeck K, Glatzel M, Vey M, Bellon A, Aguzzi A Coexistence of multiple PrP^{Sc} types in individuals with Creutzfeldt-Jakob disease. Lancet Neurol 2005; 4: 805-814.
149. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982; 21: 136-144.
150. Prusiner SB. Prions. Proc Natl Acad Sci U S A 1998; 95: 13363-13383.
151. Puoti G, Giaccone G, Rossi G, Canciani B, Bugiani O, Tagliavini F. Sporadic Creutzfeldt-Jakob disease: co-occurrence of different types of PrP^{Sc} in the same brain. Neurology 1999; 53: 2173-2176.
152. Rodríguez-Martínez AB, Garrido JM, Zarranz JJ, Arteagoitia JM, de Pancorbo MM, Atarés B, Bilbao MJ, Ferrer I, Juste RA. A novel form of human disease with a protease-sensitive prion protein and heterozygosity methionine/valine at codon 129: Case report. BMC Neurol 2010; 10: 99.
153. Saborio GP, Permanne B, Soto C. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 2001; 411: 810-813.
154. Safar J, Roller PP, Gajdusek DC, Gibbs CJ Jr. Conformational transitions, dissociation, and unfolding of scrapie amyloid (prion) protein. J Biol Chem 1993; 268: 20276-20284.
155. Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen FE, Prusiner SB. Eight prion strains have PrP^{Sc} molecules with different conformations. Nat Med 1998; 4: 1157-1165.
156. Safar JG, Geschwind MD, Deering C, Didorenko S, Sattavat M, Sanchez H, Serban A, Vey M, Baron H, Giles K, Miller BL, Dearmond SJ, Prusiner SB. Diagnosis of human prion disease. Proc Natl Acad Sci U S A 2005; 102: 3501-3506.
157. Safar JG, Kellings K, Serban A, Groth D, Cleaver JE, Prusiner SB, Riesner D. Search for a prion-specific nucleic acid. J Virol 2005; 79: 10796-10806.
158. Sakaguchi S, Katamine S, Nishida N, Moriuchi R, Shigematsu K, Sugimoto T, Nakatani A, Kataoka Y, Houtani T, Shirabe S, Okada H, Hasegawa S, Miyamoto T, Noda T. Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. Nature 1996; 380: 528-531.
159. Salvatore M, Genuardi M, Petraroli R, Masullo C, D'Alessandro M, Pocchiari M. Polymorphisms of the prion protein gene in Italian patients with Creutzfeldt-Jakob disease. Hum Genet 1994; 94: 375-379.
160. Samaia HB, Brentani RR. Can loss-of-function prion-related diseases exist? Mol Psychiatry 1998; 3: 196-197.
161. Sanchez-Juan P, Green A, Ladogana A, Cuadrado-Corrales N, Saanchez-Valle R, Mitrovaa E, Stoeck K, Sklaviadis T, Kulczycki J, Hess K, Bodemer M, Slivarichova D, Saiz A, Calero M, Ingrosso L, Knight R, Janssens AC, van Duijn CM, Zerr I. CSF tests in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology 2006; 67: 637-643.
162. Sarnataro D, Campana V, Paladino S, Stornaiuolo M, Nitsch L, Zurzolo C. PrP^{C} association with lipid rafts in the early secretory pathway stabilizes its cellular conformation. Mol Biol Cell 2004; 15: 4031-4042.
163. Sarnataro D, Paladino S, Campana V, Grassi J, Nitsch L, Zurzolo C. PrP^{C} is sorted to the basolateral membrane of epithelial cells independently of its association with rafts. Traffic 2002; 3: 810-821.
164. Satoh K, Shirabe S, Tsujino A, Eguchi H, Motomura M, Honda H, Tomita I, Satoh A, Tsujihata M, Matsuo H, Nakagawa M, Eguchi K. Total Tau protein in cerebrospinal fluid and diffusion-weighted MRI as an early diagnostic marker for Creutzfeldt-Jakob disease. Dement Geriatr Cogn Disord 2007; 24: 207-212.
165. Satoh K, Tobiume M, Matsui Y, Mutsukura K, Nishida N, Shiga Y, Eguhchi K, Shirabe S, Sata T. Establishment of a standard 14-3-3 protein assay of cerebrospinal fluid as a diagnostic tool for Creutzfeldt-Jakob disease. Lab Invest 2010; 90: 1637-1644.
166. Schoch G, Seeger H, Bogousslavsky J, Tolnay M, Janzer RC, Aguzzi A, Glatzel M. Analysis of prion strains by PrP^{Sc} profiling in sporadic Creutzfeldt-Jakob disease. PLoS 2006; Med 3: e14.
167. Shorter J. Emergence and natural selection of drug-resistant prions. Mol Biosyst 2010; 6: 1115-1130.
168. Simoneau S, Ruchoux MM., Vignier N, Lebon P, Freire S, Comoy E, Deslys JP, Fournier JG. Small critical RNAs in the scrapie agent. Nature Precedings 2009; Available at http://hdl.handle.net/10101/npre.2009.3344.1.
169. Somerville RA, Chong A, Mulqueen OU, Birkett CR, Wood SC, Hope J. Biochemical typing of scrapie strains. Nature 1997; 386: 564.
170. Somerville RA, Hamilton S, Fernie K. Transmissible spongiform encephalopathy strain, PrP genotype and brain region all affect the degree of glycosylation of PrP^{Sc}. J Gen Virol 2005; 86: 241-246.
171. Soto C, Saborio GP, Anderes L. Cyclic amplification of protein misfolding: application to prion-related disorders and beyond. Trends Neurosci 2002; 25: 390-394.
172. Stack MJ, Chaplin MJ, Clark J. Differentiation of prion protein glycoforms from naturally occurring sheep scrapie, sheep-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform encephalopathy (BSE) cases and Romney and Cheviot breed sheep experimentally inoculated with BSE using two monoclonal antibodies. Acta Neuropathol 2002; 104: 279-286.
173. Sunyach C, Jen A, Deng J, Fitzgerald KT, Frobert Y, Grassi J, McCaffrey MW, Morris R. The mechanism of internalization of glycosylphosphatidylinositol-anchored prion protein. EMBO J 2003; 22: 3591-3601.
174. Tagliavini F, Prelli F, Ghiso J, Bugiani O, Serban D, Prusiner SB, Farlow MR, Ghetti B, Frangione B. Amyloid protein of Gerstmann-Straussler-Scheinker disease (Indiana kindred) is an 11 kd fragment of prion protein with an N-terminal glycine at codon 58. EMBO J 1991; 10: 513-519.

175. Taraboulos A, Raeber AJ, Borchelt DR, Serban D, Prusiner SB. Synthesis and trafficking of prion proteins in cultured cells. Mol Biol Cell 1992; 3: 851-863.
176. Taraboulos A, Scott M, Semenov A, Avrahami D, Laszlo L, Prusiner SB. Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. J Cell Biol 1995; 129: 121-132.
177. Telling GC, Parchi P, DeArmond SJ, Cortelli P, Montagna P, Gabizon R, Mastrianni J, Lugaresi E, Gambetti P, Prusiner SB. Evidence for the conformation of the pathologic isoform of the prion protein enciphering and propagating prion diversity. Science 1996; 274: 2079-2082.
178. Thackray AM, Hopkins L, Klein MA, Bujdoso R. Mouse-adapted ovine scrapie prion strains are characterized by different conformers of PrP^{Sc}. J Virol 2007; 81: 12119-12127.
179. Tzaban S, Friedlander G, Schonberger O, Horonchik L, Yedidia Y, Shaked G, Gabizon R, Taraboulos A. Protease-sensitive scrapie prion protein in aggregates of heterogeneous sizes. Biochemistry 2002; 41: 12868-12875.
180. Uro-Coste E, Cassard H, Simon S, Lugan S, Bilheude JM, Perret-Liaudet A, Ironside JW, Haik S, Basset-Leobon C, Lacroux C, Peoch K, Streichenberger N, Langeveld J, Head MW, Grassi J, Hauw JJ, Schelcher F, Delisle MB, Andreoletti O. Beyond PrP^{res} type 1/type 2 dichotomy in Creutzfeldt-Jakob disease. PLoS Pathog 2008; 4: e1000029.
181. Vey M, Pilkuhn S, Wille H, Nixon R, DeArmond SJ, Smart EJ, Anderson RG, Taraboulos A, Prusiner SB. Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. Proc Natl Acad Sci U S A 1996; 93: 14945-14949.
182. Weissmann C. Molecular genetics of transmissible spongiform encephalopathies. J Biol Chem 1999; 274: 3-6.
183. Wilham JM, Orrú CD, Bessen RA, Atarashi R, Sano K, Race B, Meade-White KD, Taubner LM, Timmes A, Caughey B. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. PLoS Pathog 2010; 6: e1001217.
184. Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996; 347: 921-925.
185. Windl O, Dempster M, Estibeiro JP, Lathe R, de Silva R, Esmonde T, Will R, Springbett A, Campbell TA, Sidle KC, Palmer MS, Collinge J. Genetic basis of Creutzfeldt-Jakob disease in the United Kingdom: a systematic analysis of predisposing mutations and allelic variation in the PRNP gene. Hum Genet 1996; 98: 259-264.
186. Yokoyama T, Takeuchi A, Yamamoto M, Kitamoto T, Ironside JW, Morita M. Heparin enhances the cell-protein misfolding cyclic amplification efficiency of variant Creutzfeldt-Jakob disease. Neurosci Lett 2011; 498: 119-123.
187. Yull HM, Ritchie DL, Langeveld JP, van Zijderveld FG, Bruce ME, Ironside JW, Head MW. Detection of type 1 prion protein in variant Creutzfeldt-Jakob disease. Am J Pathol 2006; 168: 151-157.
188. Zanusso G, Fiorini M, Ferrari S, Gajofatto A, Cagnin A, Galassi A, Richelli S, Monaco S. Cerebrospinal fluid markers in sporadic creutzfeldt-jakob disease. Int J Mol Sci 2011; 12: 6281-6292.
189. Zanusso G, Polo A, Farinazzo A, Nonno R, Cardone F, Di Bari M, Ferrari S, Principe S, Gelati M, Fasoli E, Fiorini M, Prelli F, Frangione B, Tridente G, Bentivoglio M, Giorgi A, Schininŕ ME, Maras B, Agrimi U, Rizzuto N, Pocchiari M, Monaco S. Novel prion protein conformation and glycotype in Creutzfeldt-Jakob disease. Arch Neurol 2007; 64: 595-599.
190. Zeidler M, Sellar RJ, Collie DA, Knight R, Stewart G, Macleod MA, Ironside JW, Cousens S, Colchester AC, Hadley DM, Will RG. The pulvinar sign on magnetic resonance imaging in variant Creutzfeldt-Jakob disease. Lancet 2000; 355: 1412-1418.
191. Zerr I, Kallenberg K, Summers DM, Romero C, Taratuto A, Heinemann U, Breithaupt M, Varges D, Meissner B, Ladogana A, Schuur M, Haik S, Collins SJ, Jansen GH, Stokin GB, Pimentel J, Hewer E, Collie D, Smith P, Roberts H, Brandel JP, van Duijn C, Pocchiari M, Begue C, Cras P, Will RG, Sanchez-Juan P. Updated clinical diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Brain 2009; 132: 2659-2668.
192. Zou WQ, Capellari S, Parchi P, Sy MS, Gambetti P, Chen SG. Identification of novel proteinase K-resistant C-terminal fragments of PrP in Creutzfeldt-Jakob disease. J Biol Chem 2003; 278: 40429-40436.
193. Zou WQ, Puoti G, Xiao X, Yuan J, Qing L, Cali I, Shimoji M, Langeveld JP, Castellani R, Notari S, Crain B, Schmidt RE, Geschwind M, Dearmond SJ, Cairns NJ, Dickson D, Honig L, Torres JM, Mastrianni J, Capellari S, Giaccone G, Belay ED, Schonberger LB, Cohen M, Perry G, Kong Q, Parchi P, Tagliavini F, Gambetti P. Variably protease-sensitive prionopathy: A new sporadic disease of the prion protein. Ann Neurol 2010; 68: 162-172.