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Review: Creutzfeldt–Jakob disease: prion protein type, disease phenotype and agent strain

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The human transmissible spongiform encephalopathies or human prion diseases are one of the most intensively investigated groups of rare human neurodegenerative conditions. They are generally held to be unique in terms of their complex epidemiology and phenotypic variability, but they may also serve as a paradigm with which other more common protein misfolding disorders might be compared and contrasted. The clinico-pathological phenotype of human prion diseases appears to depend on a complex interaction between the prion protein genotype of the affected individual and the physico-chemical properties of the neurotoxic and transmissible agent, thought to comprise of misfolded prion protein. A major focus of research in recent years has been to define the phenotypic heterogeneity of the recognized human prion diseases, correlate this with molecular-genetic features and then determine whether this molecular-genetic classification of human prion disease defines the biological properties of the agent as determined by animal transmission studies. This review seeks to survey the field as it currently stands, summarize what has been learned, and explore what remains to be investigated in order to obtain a more complete scientific understanding of prion diseases and to protect public health.

Keywords: agent strain, Creutzfeldt–Jakob disease, neuropathology, prion protein, *PRNP* gene, protein misfolding disease

Transmissible spongiform encephalopathies and prion diseases

What we now term Creutzfeldt–Jakob disease (CJD) was first recognized in the 1920s [1,2]. During the intervening period the nosology and aetiology of the disorder have been subjects of a debate that is not yet fully resolved. Nevertheless, four major themes characterize the last half-century of research into CJD. First, the recognition that CJD (in its sporadic, genetic, iatrogenic and variant forms) belongs to a family of human neurodegenerative conditions, currently comprising Gerstmann–Straussler–Scheinker disease (GSS), kuru, fatal familial insomnia (FFI), prion protein cerebral amyloid angiopathy (PrP-CAA) and variably protease-sensitive prionopathy (VPSPr) (Table 1). Second, that these human disorders share features with (and in one case can be directly linked to) a group of animals diseases, collectively known as the transmissible spongiform encephalopathies (TSE) (Table 2). Third, that the epidemiology of these diseases is complex, perhaps even unique, as they occur in genetic and in spontaneously occurring forms, but they can also be acquired (Tables 1,2). Last, that if a transmissible agent is involved (as the existence of acquired forms implies), then that agent is highly atypical, based on its biological behaviour and its inactivation characteristics. These peculiarities could have rendered CJD a biological curiosity among human neurodegenerative diseases were it not for the epidemic of bovine spongiform encephalopathy (BSE) in UK cattle in the 1980s, the appearance of a new form of

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Table 1. The human prion diseases, their acronyms and their probable aetiology

Human prion disease	*Probable aetiology*
Sporadic CJD (sCJD) and its subtypes	Idiopathic
Sporadic fatal insomnia (sFI)	Idiopathic
Variably protease sensitive prionopathy (VPSPr)	Idiopathic
Kuru	Acquired (sCJD)
Iatrogenic CJD (iCJD)	Acquired (sCJD)
Variant CJD (vCJD)	Acquired (BSE)
Familial or genetic CJD (fCJD or gCJD)	Genetic (*PRNP* mutations)
Gerstmann–Straussler–Scheinker disease (GSS)	Genetic (*PRNP* mutations)
Fatal familial insomnia (FFI)	Genetic (*PRNP* mutations)
Prion protein cerebral amyloid angiopathy (PrP-CAA)	Genetic (*PRNP* mutations)

Note: The probable source of infectivity in the acquired forms is shown in parentheses.
BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease.

Table 2. Animal transmissible spongiform encephalopathies, their acronyms and their probable aetiology

Animal transmissible spongiform encephalopathies	*Probable aetiology*
Scrapie (in sheep and goats)	Acquired
Transmissible mink encephalopathy (TME, in mink)	Acquired
Chronic wasting disease (CWD, in deer and elk)	Acquired
Bovine spongiform encephalopathy (C-type BSE)	Acquired
Feline spongiform encephalopathy (FSE, in cats)	Acquired
H-type and L-type BSE	Idiopathic
Atypical scrapie (for example, Nor98 in sheep)	Idiopathic

BSE, bovine spongiform encephalopathy.

CJD [variant CJD (vCJD)] as a consequence in the UK in the 1990s, and the subsequent realization that the long clinically silent incubation period involved in primary vCJD cases allows for the secondary spread of the disease from asymptomatic infected individuals to others by routes such as blood transfusion. These public health concerns have prompted renewed surveillance for, and research into CJD in the UK and elsewhere.

CJD epidemiology

CJD occurs world-wide with an incidence of between one and two cases per million of population per annum and the majority of these cases are idiopathic and termed sporadic CJD (sCJD) [3]. A minority are familial or genetic (fCJD or gCJD) and are found in association with a growing list of mutations (including point mutations, insertions and deletions) all of which occur in the open reading frame of the prion protein gene, *PRNP* [4]. The remainder are acquired in the form of iatrogenic CJD (iCJD) and vCJD. iCJD has been acquired through contaminated cadaveric-derived growth hormone (over 200 cases, largely in France), dura mater grafting (over 200 cases, largely in Japan) in addition to limited numbers of cases attributed to neurosurgical instruments, stereotactic EEG electrodes and corneal transplantation [5]. vCJD has largely affected the UK resulting in 176 cases (as of January 2012), three of which have been attributed to secondary transmission via blood transfusion [6]. Kuru achieved epidemic proportions in the middle years of the last century in the Fore tribe of Papua New Guinea and was acquired orally through the cultural practice of ritual endocannibalism [7]. Like gCJD, GSS, FFI and PrP-CAA are found in association with *PRNP* mutations [8–10].

PRNP genetic variability

In addition to highly penetrant disease-associated mutations, the *PRNP* gene has several polymorphic codons, the most important of which is at codon 129 and codes for either methionine (M) or valine (V). Heterozygosity with respect to codon 129 (MV) is the most frequently occurring genotype in normal Caucasian populations (approximately 51%), with methionine homozygosity next most frequent (approximately 37%) and valine homozygosity comparatively rare (approximately 12%). These genotype frequencies are modified in nearly all forms of CJD, in which *PRNP* codon 129 homozygosity, especially methionine homozygosity, tends to predominate. In addition to being a risk factor for CJD, the codon 129 genotype can substantially modify the clinico-pathological phenotype of CJD [2].

Phenotypic variability

The neuropathological phenotype of CJD involves neuronal loss, gliosis and spongiform change and in some cases the formation of 'kuru type' amyloid plaques. The severity and targeting of specific neuroanatomical regions is characteristic in different forms of CJD and this presumably underlies the clinical picture. vCJD typically has an early age at onset, long disease duration, is characterized by behavioural or psychiatric signs at onset followed by

sensory abnormalities, ataxia and dementia later during the disease course. Neuropathologically the condition is characterized by multiple florid plaques in the cerebral and cerebellar cortex and numerous cluster plaques, amorphous pericellular and perivascular prion protein deposits in the same areas. There is severe spongiform change and perineuronal and axonal prion protein accumulations in the caudate nucleus and putamen, marked astrocytosis and neuronal loss in the posterior thalamus and reticular and perineuronal accumulation in the brain stem. Unlike other forms of CJD, the lymphoreticular and peripheral nervous systems show prominent and probably early involvement during the course of vCJD [2,6]. Thus far, only those of the MM *PRNP* codon 129 genotype have developed definite clinical vCJD, but there is evidence that the other genotypes can also become infected [11–14].

In comparison to vCJD, sCJD is clinically and neuropathologically heterogeneous, generally affecting older age groups with a very much shorter duration of illness in most cases. The pathological phenotypic heterogeneity (including the morphology and distribution of spongiform change, the severity and distribution on neuronal loss and the presence or absence of amyloid plaques) all depend in part on the *PRNP* codon 129 genotype of the patient [3]. Dura mater graft-associated iCJD resembles sCJD and involves dementia, whereas human growth hormone therapy-associated iCJD is usually characterized by a progressive cerebellar syndrome. The regional severity of pathology in dura mater graft-associated iCJD can reflect the site of grafting and involves the presence of florid plaques in some cases. In contrast, human growth hormone therapy-associated iCJD often involves severe cerebellar pathology and in some cases the presence of kuru plaques [5]. Kuru also presents as a cerebellar syndrome with spongiform change in the cerebellum and cerebral cortex and the presence of amyloid or 'kuru' plaques [2,7]. The phenotypes of individual acquired forms may reflect the phenotypic forms of CJD to which that affected individual had been exposed [2].

Phenotypic diversity within gCJD is complex, but appears to depend primarily on the *PRNP* mutation itself and secondarily on the codon 129 polymorphism found on the mutated allele, which can be further modified by the codon 129 polymorphism of the wild-type allele [4,9,10]. Most forms of gCJD appear to share features with specific subtypes of sCJD. For example, the neuropathology of gCJD E200K resembles that of the most common form of sCJD [15], FFI (*PRNP* D178N-129V) resembles the rare thalamic variant of sCJD (also known as sporadic fatal insomnia or sFI) [16], and VPSPr has been proposed to share significant similarities with GSS [17]. It is therefore possible that the neuropathological variability of the idiopathic forms (sCJD, sFI, VPSPr) represent individual phenocopies of specific genetic forms (gCJD, FFI, GSS), perhaps resulting from equivalent somatic mutations, although this hypothesis is intrinsically difficult to find supporting evidence for, as the implicated (presumably neuronal lineage) cells would be among the first to be lost during pathogenesis.

Molecular basis

Understanding the molecular basis of CJD pathogenesis is key to determining the reasons for the observed phenotypic variability of the disease. The prion hypothesis as originally proposed, unified the human and animal TSE renaming them prion diseases and proposing a fundamentally different aetiology for these diseases from other neurodegenerative and other infectious diseases, in which the conformational switch of a single gene product (the prion protein or PrP) was sufficient to account for the neurotoxicity and transmissibility of CJD and the animal TSE [18,19]. A 'protein only' and therefore epigenetic pathogen was proposed to be the cause of the disease and to behave essentially as a transmissible amyloidosis. In this scenario a significant barrier to the conversion to the disease-associated isoform (PrP^{Sc}) prevents prion toxicity under normal circumstances. Rarely under normal conditions, inevitably where a *PRNP* mutation exists, or predictably in response to exogenous PrP^{Sc} exposure, a cascade of conversion of the normal cellular isoform (PrP^{C}) to the pathological isoform occurs, resulting in neurodegeneration and the generation of further prion infectivity. PrP^{Sc} is characterized by relative insolubility and partial protease resistance dependent on a refolded secondary structure and its propensity to self-aggregate. A considerable body of evidence has accumulated to indicate that the prion hypothesis is substantially correct, including; (i) the demonstration that PrP^{C} expression is required for pathological change in animal models of prion disease; (ii) the development of cell-free model systems in which PrP^{Sc} seeds the production of PrP^{Sc} from PrP^{C} substrate; and (iii) the production of infectious prion preparations from refolded recombinant PrP^{C} [20–22].

Relationship to agent strain

The proposal that TSE agents or prions occur in a series of stable and definable strains comes directly from paradigms established in scrapie research. Individual brain isolates from sheep with scrapie can be used to derive lines of rodent adapted scrapie that can be serially passaged in hamsters, guinea pigs or mice. When different lines are compared (in the same host) they can differ in terms of incubation period and the precise pattern of spongiform change across specified neuroanatomical regions (lesion profile), suggesting a series of distinct stable scrapie strains. Different strains, as so defined, can be derived from the same isolate, and the polymorphisms in the murine prion protein gene play an important role in specifying incubation period and the strain derived from an individual isolate [23]. If prions were conventional agents then the most obvious explanation for these phenomena would be based on neo-Darwinian principles, but if the agent is essentially epigenetic, as the prion hypothesis suggests, then another explanation must be sought.

The prion protein as a carrier of heritable information

The first indications of phenotype-associated differences in prion protein structure came in the early 1990s. The 'hyper' and 'drowsy' phenotypes of hamster-adapted transmissible mink encephalopathy were found to have differently sized prion protein products of approximately 21 kDa and 19 kDa after proteinase K digestion of brain homogenates [24]. Similarly gCJD (D178N-129V) and FFI (D178N-129M) were found to correlate with approximately 21 kDa and 19 kDa protease resistant prion protein (PrP^{res}) fragments respectively [25]. gCJD E200K-129M also gave a 21 kDa PrP^{res} fragment. Moreover the ratio of the 21 kDa or 19 kDa non-glycosylated PrP^{res} to its mono- and diglycosylated counterparts differed between gCJD (D178N-129V) and FFI (D178N-129M) [25]. Taken together with a previous observation that the multicentric plaques that characterize GSS are composed of an 11 kDa PrP^{res} fragment [26], these findings established a precedent that different human prion diseases are characterized by different conformations and glycosylation ratios of PrP^{res} as defined by the availability of different regions of the prion protein polypeptide chain to degradation by proteinase K. The idea that these different conformers and glycotypes might encipher or encrypt heritable strain-like phenotypic properties of human prions was proposed in two landmark publications in 1996 [27,28]. Collinge *et al.* [27] demonstrated a glycoform ratio signature common to cattle, humans, macaques and mice, naturally or experimentally infected with BSE. In Telling *et al.* [28] the biochemical and biological properties of gCJD E200K-129M and FFI (D178N-129M) were compared by transmission to transgenic mice expressing a human-mouse chimeric PrP. The biochemical properties (21 or 19 kDa fragment size) of the human PrP^{res} of inocula were conserved in the abnormal PrP that accumulated in the brains of infected mice, and more remarkably the targeting of different human brain regions was recapitulated in the mice: heavy PrP^{res} accumulation in the thalamus in the FFI inoculated mice and gCJD E200K inoculated mice showing greater PrP^{res} accumulation in the cortex [28]. These papers provided a conceptual framework within which to examine the relationships between prion protein biochemistry and disease phenotype and between prion disease phenotype and agent strain.

Prion protein biochemistry and disease phenotype

Over the past 15 years a considerable body of literature has accumulated on what has come to be known as PrP^{res} typing or 'molecular strain typing' in CJD and other human prion diseases. Initial difficulties over nomenclature having been largely resolved, the majority of researchers use a PrP^{res} typing system originally described by Gambetti and colleagues in which the two major differentially N-terminally truncated 21 kDa and 19 kDa protease-resistant fragments are termed type 1 and type 2 PrP^{res} respectively [29]. PrP^{res} type and glycoform ratio are considered separately in this nomenclature. Glycotypes are best described as a ratio (% diglycosylated: % monoglycosylated: % nonglycosylated), but a shorthand has been developed in which examples where the diglycosylated band predominates are given the suffix B and those in which the monoglycosylated band predominates are given the suffix A [30]. Those in which both mono- and diglycosylated bands predominate at the expense of the nonglycosylated may be termed A/B type. When proteinase K is used to produce these fragments, sequencing studies show a molecular population is generated in which glycine 82 is the most frequently occurring N-terminal amino acid in type 1 PrP^{res} and serine 97 the most frequently occurring N-terminal amino acid in type 2, but

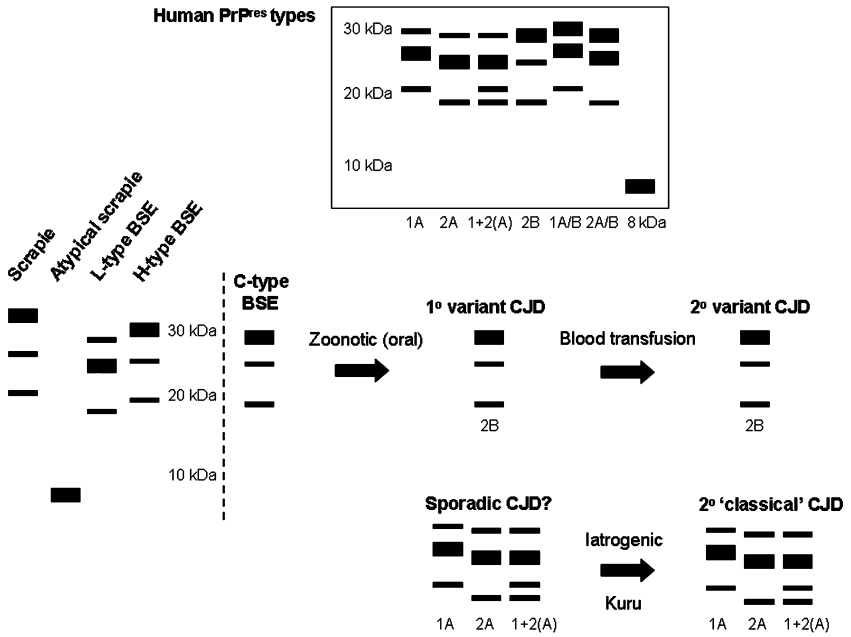

Figure 1. Schematic representation of the abnormal prion protein types as defined by the mobility and relative abundance of protease-resistant (PrP^{res}) core fragments demonstrated by Western blotting. Human PrP^{res} types 1A, 2A, 1 + 2(A), 2B, 1A/B and 2A/B and the 8 kDa are shown with the approximate relative positions of molecular weight standards marked in kilo Daltons (kDa). The appearance of the PrP^{res} type found in typical and atypical sheep scrapie and C-, H-, and L-type bovine spongiform encephalopathy (BSE) are shown for comparison. The PrP^{res} type found in classical BSE (C-type BSE) is conserved when C-type BSE is transmitted by the oral route to humans in the form of primary (1°) variant Creutzfeldt–Jakob disease (CJD) and conserved again in secondary (2°) variant CJD, acquired by blood transfusion from cases of primary variant CJD. In contrast, the range of PrP^{res} types found in iatrogenic CJD and kuru resemble those found in sporadic CJD, which is thought to be the original source of infectivity in these diseases (types 1A and/or 2A).

there are also additional N-terminally truncated species around these major N-termini [31]. Type 1 and type 2 have intact C-termini in their protease-resistant cores; however in some forms of GSS and in VPSPr smaller, approximately 8 kDa fragments, are found that result from both N- and C-terminal truncation by proteinase K [8,17]. The appearance of the different Western blot PrP^{res} types and their nomenclature is summarized in schematic form in Figure 1.

Table 3 shows how these molecular variables (*PRNP* mutations and codon 129 genotype, and PrP^{res} fragment size and glycosylation ratio) relate to different human prion diseases. It is interesting to note that where examples of secondary transmission are known, the findings are consistent with both the PrP^{res} type and the neuropathological phenotype being conserved during human-to-human spread. The PrP^{res} types found in kuru and iCJD resemble those found in sCJD (Figure 1), from which they were most likely derived [32]. The PrP^{res} type found in transfusion-associated secondary vCJD is the same as that found in primary vCJD [33,34] (Figure 1) and the neuropathological phenotypes of primary and secondary (transfusion-associated) vCJD are also closely similar (Figure 2). While there is insufficient known molecular variation overall to fully distinguish all phenotypic forms, there is a reasonable correlation between molecular-genetic types and clinico-pathological phenotypes to at least consider this a good working model for exploring the underlying mechanisms.

Sporadic CJD is a particular case in point. The sCJD classification system of Parchi *et al.* describes six phenotypes designated by their molecular-genetic correlates as; MM1/MV1, VV1, MM2c, MM2t, MV2, VV2 [35]. It is noteworthy that two combinations produce indistinguishable phenotypes (MM1 and MV1) and that one combination (MM2) is associated with two distinct phenotypes (sCJD MM2c for cortical, and MM2t – the thalamic variant or sFI).

Although transferable and reproducible as a typing system [36] and generally accepted internationally as a means by which to compare data, this classification system fails to accommodate the demonstrable fact that

Table 3. Summary of the main molecular-genetic correlates in human prion diseases

Disease	*PRNP genotypes affected*	*Main PrP^{res} types*
sCJD	NMD, 129MM > VV > MV	1A and/or 2A
sFI	NMD, 129MM	2A
VPSPr	NMD, 129VV > MV > MM	Approximately 8 kDa [1A, 2A]
Kuru	NMD, 129MM, MV, VV	2A
iCJD	NMD, 129MM, MV, VV	1A and/or 2A
vCJD	NMD, 129MM (MV)	2B [1B]
gCJD/fCJD	Point mutations and insertions, -129M or V	1A/B and/or 2A/B
GSS	Point mutations (and insertions), -129M	1A/B, approximately 8 kDa
FFI	Point mutation, -129M	2A/B
PrP-CAA	Stop mutation, -129M	Approximately 8 kDa

Notes: >, relative prevalence of affected genotypes; -129, the codon 129 polymorphism physically linked to the causative mutation; ( ), indicates a finding in a minority of cases or a provisional finding; [ ], indicates a minor molecular component.
CJD, Creutzfeldt–Jakob disease; FFI, Fatal familial insomnia; gCJD/fCJD, genetic or familial CJD; GSS, Gerstmann–Straussler–Scheinker disease; iCJD, iatrogenic CJD; NMD, no mutations detected; PrP-CAA, prion protein cerebral amyloid angiopathy; sCJD, sporadic CJD; sFI, sporadic fatal insomnia; vCJD, variant CJD; VPSPr, variably protease sensitive prionopathy.

large numbers of cases of sCJD can be found to contain both type 1 and type 2 PrP^{res} when sampling is extended to multiple regions and Western blot separation optimized (first shown [37]). Proposals to revise the sCJD classification scheme have recently been offered by Cali *et al.* [38] and by Parchi *et al.* [39]. Both proposals are predicated on there being cases in which co-occurrence exists, and others in which it does not; a point that has been disputed by others who suggest that the situation may be quantitative rather than qualitative [40–42]. An alternative point of view might be that phenotypic variation in sCJD is better considered as a spectrum in which the balance and distribution of the two PrP^{res} types is partially affected by codon 129 genotype and partly stochastic, but determines the regional pathology, which in turn determines the clinical features [43]. This could help to explain the undisputed influence of PrP^{res} type and codon 129 genotype, but also the partially overlapping nature of the clinico-pathological phenotypes. The situation in vCJD is perhaps more simple, with all parties agreeing that, irrespective of tissue and brain region sampled, the PrP^{res} type always has a major 19 kDa fragment with the diglycosylated form predominating, that is, it is type 2B [27,43–47].

Infectivity in CJD

Even within the theoretical confines of the prion hypothesis it quite possible to regard the infectivity associated with prion diseases as an epiphenomenon, especially when one considers the human prion diseases. The vast majority of cases of CJD world-wide either occur in a sporadic pattern or are associated with mutations. Although it remains possible that mutations act as susceptibility factors for an unrecognized, near-ubiquitous agent, there is little evidence to support this view. This epidemiological pattern (majority sporadic, minority genetic) is shared with several major neurodegenerative diseases. It is possible to view sporadic and genetic forms of CJD as disorders of prion protein metabolism. In this scenario PrP^{Sc} might be a minor or transitory normal catabolite. If, however, the flux through its degradative pathway is disturbed then a threshold may be reached at which pathogenic PrP^{Sc} formation becomes a self-sustaining process and one that is independent of normal metabolic pathways. The as yet unconfirmed detection of low levels of a PrP^{Sc}-like molecule in normal human brains is entirely consistent with this hypothesis [48]. Assuming that the initial formation of PrP^{Sc} does not occur synchronously in a cell-autonomous manner in gCJD and sCJD, it follows that changes must originate at a given location and the pathological process spreads throughout the brain during the course of the disease. This idea is commonplace in other neurodegenerative diseases and is the basis of staging the progress or severity of disease [49]. The ability for molecular changes to spread between cells in the nervous system can be viewed as a form of infectivity, and there is much speculation that 'prionoids' or the 'prion paradigm' could be usefully invoked as an explanation of inter-cellular spread for neurodegenerative diseases other than CJD

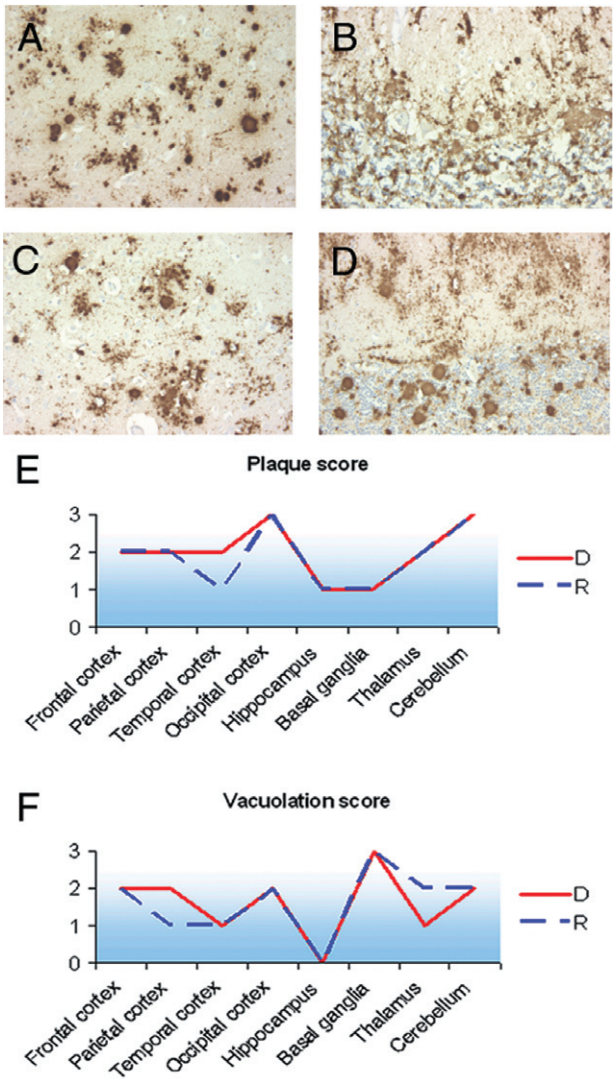

Figure 2. Similarity of the neuropathological profile of two cases of variant Creutzfeldt–Jakob disease (CJD) (primary and secondary variant CJD) linked by blood transfusion. (**A**–**D**) show immunohistochemistry for abnormal prion protein in the brain of the blood donor (**A**, **B**) and transfusion recipient (**C**, **D**). (**A**) and (**C**) show cerebral cortex and (**B**) and (**D**) show cerebellum. Lesion profiles of amyloid plaques and vacuolar pathology in specified brain regions are compared between the blood donor (D) and transfusion recipient (R) in (**E**) and (**F**). Regions were scored as mildly (1), moderately (2) or severely (3) affected, with a score of 0 indicating an unaffected region. Figure adapted from Head *et al.* [34].

[50–56]. More controversially, direct experimental evidence for the transmissibility of Aβ, amyloid A and apolipoprotein AII molecular pathology have also been presented [57–60]. Therefore, the question may be asked, why might CJD be demonstrably acquired in humans and yet other analogous protein misfolding diseases apparently not? The answer to this question is not obvious at present, but it may be important to consider the nature of the conditions under which CJD has been transmitted between people. In kuru individuals consumed brain tissue directly from other individuals who died with clinical prion disease within an increasingly high-risk population as the epidemic proceeded. In dura mater associated iCJD contaminated material was permanently transplanted in close proximity to the target organ. In blood transfusion-associated vCJD unit (approximately 0.4 kg) quantities of biological materials were introduced into patients in a live form which could be expected to target that material to peripheral tissues (such as spleen) that are known to be able to support prion replication. These examples argue for the need for very particular conditions to be in place before person-to-person transmission becomes likely and probably also depend in part upon the unusual nature of prions that enable them to retain infectivity in the face of harsh conditions *in vivo* and *ex vivo*. The processes of trying to quantify risk and minimize further transmissions of a transmissible fatal neurodegenerative condition have been major components of the public health response to CJD.

How exactly to detect and measure prion infectivity is not as straightforward a question to answer as it might first appear. With the exception of emerging cell culture methods (which are currently restricted to rodent adapted scrapie strains), infectivity assays are nearly always conducted in living animals. Historically, non-human primates have been used for this purpose. Nowadays rodents are employed routinely, specifically panels of wild-type mice or more commonly transgenic mice (either expressing randomly inserted relevant prion protein transgenes or produced by targeted gene replacement). The outcomes of such studies are not always as might be anticipated. For example, intracerebral inoculation of wild-type mouse panels (expressing murine PrP) are generally susceptible to vCJD (and BSE), but sCJD (even 129MM sCJD) transmits poorly to these mice rarely producing clinical disease [61,62], whereas humanized transgenic mice propagate sCJD and vCJD in a codon 129 genotype-dependent manner [63,64], but fail to become infected with BSE under similar conditions [63]. As vCJD is thought to be BSE in humans and both sCJD and vCJD manifestly do transmit between people (in the form of iCJD), care must be exercised when extrapolating from such animal models to issues of public health.

Transmission studies of CJD: BSE and vCJD

Transmission studies to rodents (and to some extent to non-human primates) played an important role in establishing a causal link between BSE and vCJD when it was first identified. Macaques intracerebrally inoculated with BSE reproduce some of the neuropathological hallmarks of vCJD in humans, which were reportedly absent from similar transmissions of sCJD [65]. Strain typing by incubation period and lesion profile using wild-type panels of mice differentiated BSE from scrapie, and vCJD from sCJD, but implicated the same strain in BSE and vCJD [61,62,66]. All natural and experimental BSE-related transmissions (including BSE in cattle, feline spongiform encephalopathy in cats, BSE in wild-type mice, and vCJD in humans and transmitted to non-human primate) were shown to have a PrP^{res} type dominated by the diglycosylated band (the BSE glycoform signature) [27]. The results of experimental transmissions of sCJD, vCJD and BSE to wild-type mice and to transgenic mice expressing the human prion protein were also interpreted as supporting the BSE/vCJD link [67]. Finally, in 1999 transmissions of BSE, vCJD and scrapie to transgenic mice over-expressing the bovine prion protein were said to have produced the most compelling evidence yet that vCJD was BSE in humans [68].

Nothing that has been published since these initial studies has cast doubt on the link between BSE and vCJD, but the publication of full study results from different laboratories has produced a much less clear-cut picture of the transmission properties of the BSE/vCJD agent, in which transmission characteristics appear to depend to a surprising degree on the murine model used: BSE has been reported to transmit in the absence of detectable PrP^{res} in C57BL/6 wild-type mice [69]. Some humanized transgenic mouse lines were found to be largely refractory to BSE infection, irrespective of *PRNP* codon 129 genotype [63,70], whereas others (over-expressing the 129V allele for example) were found to be susceptible [67]. Passage of the BSE agent through sheep was found to confer transmissibility of the BSE agent to the previously resistant humanized (PrP codon 129M) transgenic mice [71,72]. BSE has been reported to diverge into vCJD-like or sCJD-like phenotypes in one (Tg35), but not another (Tg45) transgenic mouse line expressing human 129M PrP [73]. A potentially related phenomenon has recently been reported in human/mouse chimeric PrP expressing transgenic mice (Tg1014), inoculated with vCJD [74]. Further distinct molecular and pathological phenotypes arose in transgenic mice lines expressing 129V (Tg152) [75] and in transgenic mice expressing both 129M and 129V (Tg45/152) when challenged with BSE [76]. Transmission of vCJD to these same lines [76] suggests increased virulence associated with adaptation of the BSE agent to humans, an observation common to several mouse models [63,70,76].

Gene-targeted transgenic mice differing only in their *PRNP* codon 129 genotypes (HuMM, HuMV, HuVV) showed genotype-specific differences in susceptibility, incubation period and neuropathology in response to vCJD, in which the heterozygotes were as susceptible as the methionine homozygotes, but with longer incubation periods [63]. Secondary transmission of vCJD by blood transfusion conferred no discernable changes in transmission characteristics compared to primary vCJD when the gene-targeted HuMM, HuMV and HuVV transgenic or wild-type mice panels were used [77]. The characteristic vCJD PrP^{res} type (2B) was maintained in the gene-targeted humanized transgenic mice, whether HuMM, HuMV or HuVV and whether transmission was performed from primary (BSE-related) or from secondary (blood transfusion-associated) vCJD [63,77].

It is important to note that all of the above experiments relate to the transmission of disease by intracerebral inoculation of brain tissue from affected individuals and subsequent analysis of the brain of the recipient animals. However, primary and secondary vCJD in humans is acquired peripherally, and the route of administration and tissues analysed may be relevant to the outcome and conclusions drawn from experiments in animal models. Transgenic mice over-expressing *PRNP* 129M human PrP (tg650) inoculated intracerebrally with vCJD brain tissue propagated either vCJD-like or sCJD-like agents [78], reminiscent of transmissions of the BSE/vCJD agent to Tg35 and Tg1014 lines described above [70,73]. Lymphotropism appeared to be an intrinsic feature of the vCJD agent in this model and lymphoid tissue involvement was always found in association with type 2B PrP^{res}. When peripheral inoculation was used instead of intracerebral inoculation in the tg650 model a stable peripheral infection was established without evidence of neuroinvasion or clinical symptoms [78].

Taken together these data indicate a meta-stable BSE/vCJD agent associated with a predominant PrP^{res} type (2B) and characteristic neuropathology, the latter of which can be modified by the species and *PRNP* genotype of the host

[61–63,77]. However, the data also indicate that under specific circumstances, including the use of particular transgenic mouse constructs and transmission conditions, strain selection or mutation can occur resulting in alternative phenotypes and PrP^{res} types more usually associated with sCJD [73,74,78]. It is tempting to propose that these apparently abrupt changes in biochemical and biological properties are related to the observation that vCJD and BSE, although dominated by type 2B PrP^{res} also contain low, but detectable levels of type 1 PrP^{res} [40,41,79]. Indeed, PrP^{Sc} existing as mixed molecular populations is consistent with (if not a prerequisite for) the conformational selection model proposed by Collinge and Clarke [80] to explain transmission barriers and their potential effects on prion disease phenotype [81,82].

In recent years two additional forms of bovine prion disease have been reported, termed H-type BSE and L-type BSE (formerly bovine amyloidotic spongiform encephalopathy or BASE) to distinguish them from 'classical' or C-type BSE. The PrP^{res} types found in C-, H- and L-type BSE and those of typical and atypical scrapie are shown in Figure 1. The epidemiology of H- and L-type BSE are consistent with them being sporadic bovine TSE, but they are transmissible by intracerebral inoculation in cattle [83]. Their pathogenicity for humans is not known, but comparative transmission studies in the tg650 human PrP 129M over-expression transgenic mouse line shows that L-type BSE transmits more readily than C-type BSE [84].

Transmission studies of CJD: sporadic, genetic and iatrogenic CJD

All reports appear to agree that the prion strain that causes vCJD can be distinguished from that causing sCJD, as determined by incubation period, neuropathological features, lesion profile or PrP^{res} type following transmission to non-human primates, wild-type mice, bovinized transgenic mice, a range of humanized transgenic mice lines, bank voles and guinea pigs [32,61,74,78,85–92]. Where the phenotypic characteristics of the sCJD cases used in the above studies were specified, they were of the most common MM1/MV1 or VV2 subtypes, and therefore these studies did not directly address whether the phenotypic variability known to occur in sCJD, correlates with different transmission characteristics and implies different strains of agent. This has to our present knowledge only been systematically investigated and reported on one occasion [64]. In this study, a brain specimen from individual cases of six sCJD cases (MM1, MM2, MV1, MV2, VV1 and VV2) was inoculated into gene-targeted HuMM, HuMV and HuVV transgenic mice and the biological properties of the transmissible agent characterized by incubation period, lesion profile, PrP immunohistochemistry and Western blotting for PrP^{res} [64]. The combined results from all three humanized transgenic mouse lines indicated four distinct transmission patterns from the six sCJD isolates. This was interpreted as evidence for the existence of four discrete sCJD strains; MM1/MV1 (termed M1), MV2/VV2 (termed V2), MM2 (termed M2) and VV1 (termed V1). Given that MM1/MV1 sCJD comprises a single clinico-pathological phenotypic group in humans, the outstanding issue in relation to the Parchi *et al*. classification system [35] is the failure of this transgenic mouse panel to distinguish between the MV2 and VV2 sCJD subtypes (which have quite distinct phenotypes in humans) suggesting that the determinants of these distinguishing features of the natural human disease do not operate in this animal model. Among the interesting aspects of the proposed M1, V2, V1, M2 sCJD strain classification is that the analysis of sCJD PrP^{Sc} by a novel biochemical stability-related method and by behaviour in cell-free conversion assays had previously come to similar conclusions [93,94]. A retrospective analysis of examples from the NIH non-human primate transmission series is also consistent with this view point [32]. The authors suggest that one human prion strain comprises cases with type 1 PrP^{res} and at least one 129M allele (whether they were derived from cases of sCJD, iCJD or gCJD E200K) and a second human prion strain comprises cases with type 2 PrP^{res} and at least one 129V allele, whether they were from sCJD or kuru [32]. Transmission of a range of sCJD and acquired CJD cases (iCJD and kuru) to wild-type and humanized 129V expressing Tg152 transgenic mice concluded that sCJD and these acquired forms also had similar transmission properties indicative of common origins and strain characteristics [91].

There are dangers inherent in generalizing from transmissions of single samples from individual cases, and this is especially true when it involves conflating results from primary transmissions of sometimes incompletely described human tissue specimens using different transgenic and other animal models. However, at this point in time there is evidence to suggest the existence of at least six different human prion strains on the basis of their biological behaviour (Table 4). These are: the common M1 strain of sCJD, iCJD and gCJD E200K; the less common V2

Table 4. Provisional classification of human prion strains based on evidence from experimental transmission studies

Strain	*Human prion disease*	*Key references*
M1	sCJD (MM1/MV1)	[64]
	iCJD (MM1)	[32]
	gCJD (E200K-MM1)	[28,32]
V2	sCJD (MV2, VV2)	[64,95]
	Kuru	[32,89]
M2	sCJD (MM2c)	[64]
V1	sCJD (VV1)	[64]
FI	FFI, sCJD (MM2t)/sFI	[16,28]
BSE	vCJD	[61,67]
ne	*GSS*	[93,94]
ne	*VPSPr*	[17]
ne	*PrP-CAA*	[10]

Note: Distinct human prion disease phenotypes that may represent additional strains, but for which comparable data are lacking are shown in italics and classified as not established (ne).
BSE, bovine spongiform encephalopathy; CJD, Creutzfeldt–Jakob disease; FFI, Fatal familial insomnia; gCJD, genetic CJD; GSS, Gerstmann–Straussler–Scheinker disease; iCJD, iatrogenic CJD; PrP-CAA, prion protein cerebral amyloid angiopathy; sCJD, sporadic CJD; sFI, sporadic fatal insomnia; vCJD, variant CJD; VPSPr, variably protease sensitive prionopathy.

strain of sCJD, iCJD and kuru; the M2 strain of sCJD MM2c; the FI strain associated with sFI (sCJD MM2t) and FFI; the very rare V1 strain associated with sCJD VV1, and the strain of agent (BSE) associated with vCJD. Whether or not the two different forms of GSS isolated in gene-targeted murine P101L homozygous mice [95,96] represent two further human prion strains or one additional strain and how these might relate to VPSPr (which is yet to be successfully transmitted) await experimental verification in the humanized transgenic mouse lines used to establish the transmission properties of the six strains described above. One possibility is that the form of GSS characterized by 8 kDa PrP^{res} fragments and VPSPr (which has a similar biochemical profile) will constitute an additional definable human prion strain characterized by a transmissible amyloidosis without a pronounced spongiform encephalopathy.

Key missing data

The exact relationship between disease phenotype and agent strain in CJD is both important and difficult to address for all of the above reasons, but a relatively circumscribed experiment with profound implications has been possible since the first description of the PrP^{res} type co-occurrence phenomenon in sCJD over 10 years ago [35,37]. This is the comparison of the transmission properties (biological strain type) of brain regions that have different PrP^{res} types (molecular strain types) from an individual case. There are two main possible outcomes to such an experiment: First, the two samples may transmit with different properties and ones that are characteristic of their molecular type. If this is so then we could conclude that molecular strain type and biological strain types are at the very least related, and most likely the biological properties depend upon the molecular type. However, we would then also be forced to conclude that the brain contained two distinct prion agents, which would necessitate a thorough re-examination of what we mean when we use the terms disease phenotype and agent strain in relation to prions, especially in those diseases that seem to arise in a sporadic fashion. Alternatively, the two regions might transmit with similar properties, in which case we would be forced to conclude either that the molecular strain type is a molecular epiphenomenon, or that animal transmission studies can select out subdetectable variants from molecular mixtures. The second possibility would be a particularly interesting outcome when seen in the light of the conformation selection model [80] and the recent evidence from Kitamoto and co-workers that the codon 129 genotype of a transgenic mouse host can effectively (and reversibly) modify the quantitative balance of PrP^{res} types able to replicate efficiently [97].

Concluding remarks

In some respects the concept of agent strain is problematic in CJD, particularly when applied to sCJD, for which there is very little evidence of an infecting agent (although sCJD itself is transmissible). It is however vitally important to understand sCJD and the deep phenotyping of CJD cases (including, where appropriate, transmission studies) is currently the most direct route to resolving our uncertainties about this perplexing condition. Understanding phenotypic variation is an important scientific objective, but it also has a public health dimension: It is likely that any new acquired human prion disease would be first recognized and classified as an atypical form of sCJD, until such times as sufficient numbers of cases accumulated and molecular, pathological, clinical and epidemiological studies pointed towards a new condition and a likely cause. It may be informative to look back at vCJD at this point. In retrospect the identification of vCJD is obvious, but human BSE

need not have targeted the young, need not have presented with behavioural or psychiatric signs, nor involved such a distinct and characteristic neuropathology, in which case its identification could have depended or more subtle differences from the spectrum of sCJD and on the detection of an excess of MM cases of CJD in the UK. However devastating vCJD has been for the 176 UK patients and their families, the animal and public health measures adopted seem to have been effective, resulting in the near eradication of BSE in the UK and as a consequence, a primary vCJD epidemic much smaller than might have otherwise been. The most effective form of insurance against any such future threat is continued clinico-pathologico-molecular surveillance, not just in the UK, but in an international surveillance network, such that any national changes in prion disease incidence and phenotype can be used to identify specific local risk factors, and allow for effect measures to be taken promptly.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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