

Variant Creutzfeldt-Jakob disease: an update

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Abstract

Variant Creutzfeldt-Jakob disease (vCJD) is a novel human prion disease caused by the bovine spongiform encephalopathy agent. Most cases have occurred in the UK, with smaller numbers in 11 other countries. All definite vCJD cases have occurred in methionine homozygotes at codon 129 in the prion protein gene. Following oral infection, the vCJD agent appears to replicate in lymphoid tissues during the asymptomatic phase of the incubation period. At present, four probable cases of vCJD infection have been identified following transfusion of red blood cells from asymptomatic donors who subsequently died from vCJD. Recently, one case of likely transmission of vCJD infection by UK Factor VIII concentrates has been reported in an elderly haemophilic patient in the UK. The recent report of a blood test that may be used to detect vCJD has raised the possibility of a new way to identify infected individuals, perhaps even before the onset of clinical symptoms.

Key words: variant CJD, prion, prion protein, blood, plasma, transfusion.

Introduction

Prion diseases (otherwise known as transmissible spongiform encephalopathies) [18,25] are a group of rare fatal neurodegenerative disorders that occur in humans and animals. The human prion diseases include sporadic, acquired and genetic disorders (Table I); examples of prion diseases in animals include bovine spongiform encephalopathy (BSE) in cattle and scrapie, a widespread disorder in sheep. Prion diseases are transmissible experimentally and naturally, and for many years the nature of the transmissible agent was uncertain, with suggestions of a “slow virus” or virino being responsible. In 1982, Stanley Prusiner proposed

the prion hypothesis, which stated that the transmissible agent was composed entirely of a modified host protein, the prion protein (PrP), which had no nucleic acid component and was partially resistant to proteolytic degradation [26].

The normal form of the prion protein (PrP^C) is expressed in many tissues in the body, but occurs at highest levels in neurones within the brain [25]. In prion diseases, PrP^C misfolds into an abnormal isoform (designated PrP^{Sc}), which accumulates in the brain. The site and precise mechanism of this misfolding process remains uncertain. PrP^{Sc} has an increased beta-sheet content that renders it relatively

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resistant to proteolytic digestion in comparison to PrP^C [25]. PrP^{Sc} is closely associated with infectivity and appears to be the major, if not the only, constituent of the transmissible agent. The high beta sheet content of PrP^{Sc} confers stability and relative resistance to conventional means of bacterial and viral decontamination. Furthermore, the current measures recommended for decontamination of prions are not necessarily capable of removing all infectivity [32], and none of these measures are applicable to blood or plasma products.

Variant Creutzfeldt-Jakob disease

Sporadic Creutzfeldt-Jakob disease (sCJD) is the commonest form of human prion disease (Table I) with an annual incidence of around 1.5 per million of the population, and a worldwide distribution [4]. There is evidence of genetic predisposition to sCJD, although the precise cause of this disorder is unknown. The naturally occurring polymorphism at codon 129 in the human prion protein gene (*PRNP*) located on chromosome 20 can encode either methionine or valine (Table II) [1]. A predominance of homozygotes in sporadic CJD, particularly methionine homozygotes, has been reported in contrast to the normal population (Table II).

In 1990, surveillance of CJD in the UK was reinstated in order to identify any possible effect of BSE in humans. BSE occurred as an epidemic in UK cattle following its identification in 1985, with over 180,000 clinical cases of BSE identified to date. However, when allowances are made for asymptomatic infections, the total number of UK cattle infected by BSE may be as high as 3 million, many of which may have entered the UK human food chain [28]. The UK National CJD Surveillance Unit in Edinburgh reported a new form of human prion disease in 1996, now known as variant CJD (vCJD) [34]. vCJD has a clinical and pathological phenotype that is distinct from sporadic CJD and other forms of human prion disease [19], with a young age at onset and a characteristic neuropathology, with florid plaques in the brain, widespread accumulation of abnormal prion protein (PrP) and thalamic gliosis (Figs. 1A-C). Western blot analysis of the brain shows a predominance of the diglycosylated form of PrP^{Sc}, in contrast to sCJD (Fig. 2). All definite vCJD patients who have undergone genetic testing are methionine homozygotes at codon 129 in the *PRNP* gene, suggesting

Table I. Classification of human prion diseases

Sporadic	Sporadic Creutzfeldt-Jakob disease
	Sporadic fatal insomnia
	Variably protease-sensitive prionopathy
Acquired	
from humans:	Kuru
	Iatrogenic Creutzfeldt-Jakob disease
from bovines:	Variant Creutzfeldt-Jakob disease
Genetic	
	Familial Creutzfeldt-Jakob disease
	Gerstmann-Sträussler-Scheinker syndrome and variants
	Fatal familial insomnia
	Prion protein congophilic angiopathy

a susceptibility to vCJD in this genetic subset. However, a possible case of vCJD has been reported recently in a *PRNP* codon 129 heterozygote (methionine/valine) [20].

Epidemiological evidence suggested that the outbreak of BSE in the UK was responsible for the emergence of vCJD, and this was subsequently supported by experimental transmission studies of vCJD and BSE to inbred and transgenic mice. These experiments demonstrated that the vCJD agent had closely similar biological properties to the BSE agent, which were different from those of sCJD [6,27]. This confirmed vCJD as a novel disorder that represents the only example of a human prion disease acquired from another species (Table I). Epidemiological studies performed by the UK National CJD Surveillance Unit indicated that the most likely source of human exposure to BSE is the consumption of infected meat

Table II. Prion protein gene polymorphisms in the normal population and in prion diseases

	Prion protein gene codon 129 polymorphisms (%)		
	MM	MV	VV
Normal population	39	50	11
Sporadic CJD	65	17	18
Variant CJD	100	–	–

M – methionine, V – valine

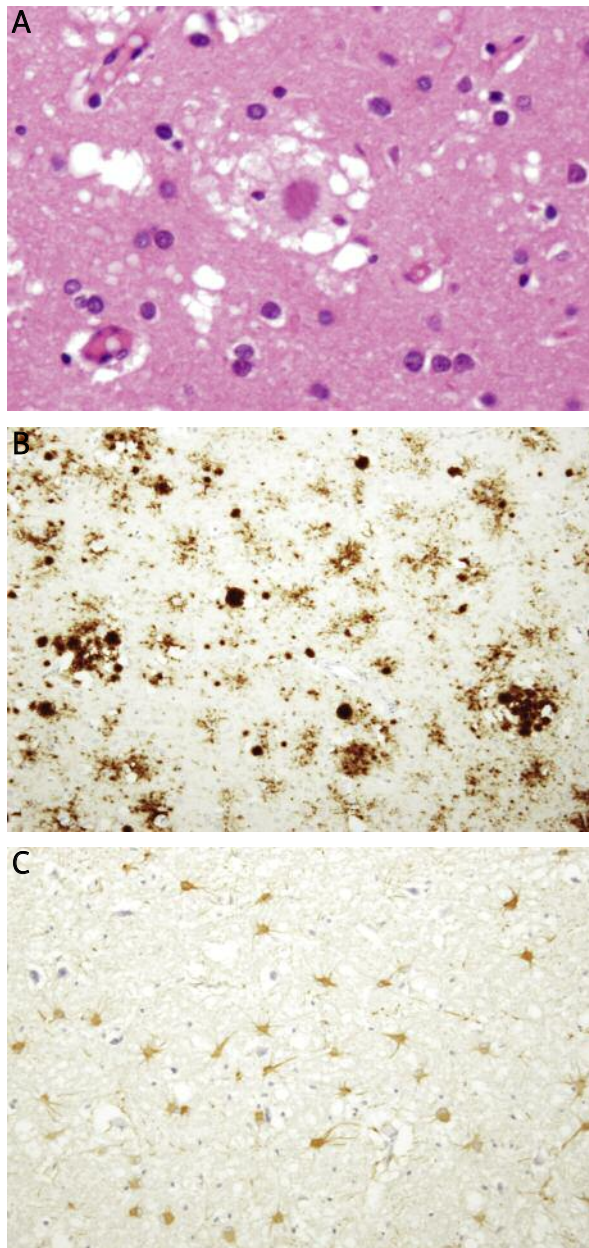


Fig. 1 **A)** A florid plaque in the frontal cortex in vCJD is composed of a dense amyloid core with a paler periphery, and surrounded by spongiform change (haematoxylin and eosin stain). **B)** Immunohistochemistry for abnormal prion protein in the frontal cortex in vCJD shows intense labelling of large florid plaques, with multiple smaller plaques and amorphous accumulations around neurones and blood vessels also labelled (anti-PrP antibody (KG9) with haematoxylin counterstain). **C)** The pulvinar in vCJD shows severe neuronal loss without marked spongiform change or plaque deposition, but accompanied by marked astrocytosis (anti-gial fibrillary acidic protein antibody).

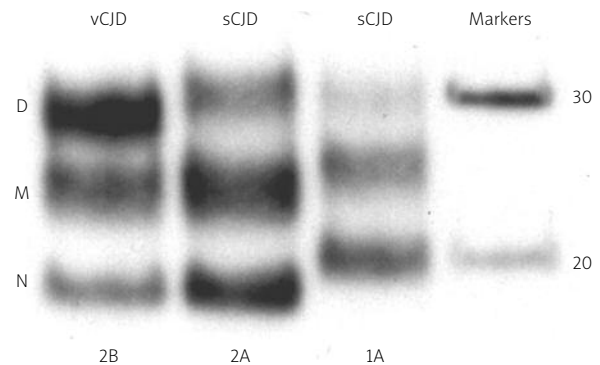


Fig. 2. Western blot analysis of protease-resistant prion protein (PrP^{res}) in frontal cortex samples from a case of variant CJD (vCJD) and two cases of sporadic CJD (sCJD). The three PrP^{res} glycoforms are labelled as D (diglycosylated), M (monoglycosylated) and N (nonglycosylated). PrP^{res} type 1A (nonglycosylated band ~21 kDa, monoglycosylated band predominating) and type 2A (nonglycosylated band ~19 kDa and monoglycosylated band predominating) are typical of different forms of sporadic CJD. In contrast, the PrP^{res} found in all cases of variant CJD tested has a nonglycosylated band of ~19 kDa and is characterised by a predominance of the diglycosylated form and termed type 2B. The size of the molecular weight markers (M) are given in kDa.

products [33]. After oral exposure to BSE, the transmissible agent replicates within lymphoid tissues, including the spleen, tonsil, lymph nodes, and gut-associated lymphoid tissue [19] (Fig. 3). Levels of infectivity in lymphoid tissues in vCJD are approximately 2-3 logs lower than in brain tissue [5]. Retrospective case analysis showed abnormal PrP in gut-associated lymphoid tissue within the appendix in 2 patients who had undergone appendectomy up to 2 years before the onset of vCJD by immunohistochemistry [16]. These findings lead to the suggestion that infectivity might be present in blood during the incubation period for vCJD [29], reinforced by the demonstration of infectivity in the blood of sheep experimentally infected with BSE before the recipient animals developed clinical signs and symptoms [17].

By February 2012, 176 definite and probable cases of vCJD have been confirmed in the UK, with 49 additional cases in 11 other countries (Table III). The incidence of vCJD has declined in the UK since

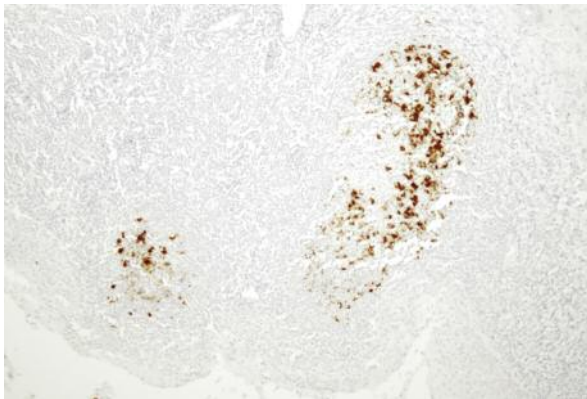


Fig. 3. Abnormal prion protein accumulates within germinal centres in the tonsil in vCJD, with dense labelling of follicular dendritic cells and occasional macrophages (anti-PrP antibody (KG9) with haematoxylin counterstain).

1999-2000 and at present there are no patients alive with vCJD in the UK. However, the number of asymptomatic infections in the UK remains uncertain; the results of earlier studies to detect abnormal prion protein in tonsil and appendix tissues suggest a prevalence of around 1 per 10,000 of the UK population [7,16,24]. This figure is higher than the current numbers of vCJD cases in the UK would suggest, indicating that some vCJD cases may have a prolonged asymptomatic carrier state, which perhaps does not result in clinical disease in all cases. A larger study is currently underway to examine the prevalence of abnormal prion protein accumulation in a larger series of surgically removed appendix specimens from across the UK. A recent analysis of the declining vCJD epidemic in the UK has suggested that the tail of the epidemic could be potentially long, with a peak annual incidence of around 11 cases, representing both primary oral infections from BSE and secondary transmission associated with blood transfusions in all *PRNP* genotypes [11].

Infectivity in blood in vCJD

At present, three cases of vCJD have occurred in individuals in the UK who received non-leucodepleted red blood cells from asymptomatic UK donors who subsequently died from vCJD after donation [15,35]. All 3 recipients were methionine homozygotes at codon 129 in the *PRNP* gene, with incubation periods ranging from 6.5 to 7.8 years between the date of the implicated transfusion and the onset

Table III. Variant CJD cases worldwide (February 2010)

Country	Number of primary cases	Number of secondary cases: transmission by blood transfusion
UK	171	3
France	25	–
Spain	5	–
Ireland	4	–
USA	3	–
Netherlands	3	–
Portugal	2	–
Italy	2	–
Canada	2	–
Japan	1	–
Saudi Arabia	1	–
Taiwan	1	–

of clinical disease. The clinical and neuropathological phenotypes of the disease in the recipients was similar to other cases of vCJD [14,15]. However, asymptomatic vCJD infection was identified in an elderly patient who had undergone transfusion of one unit of non-leucodepleted red blood cells from another asymptomatic donor who subsequently developed vCJD [23]. The recipient had no signs or symptoms of vCJD or any other neurological disorder and died of an unrelated illness 5 years after the transfusion. Analysis of the codon 129 polymorphism in the *PRNP* gene found that this recipient was heterozygous (methionine/valine). No neuropathological evidence of vCJD was found in the brain and Western blot for PrP^{Sc} in the brain was negative. However, immunohistochemistry for PrP^{Sc} was positive in the spleen (Fig. 4) and a cervical lymph node, but not in the tonsil or the appendix, and Western blot analysis confirmed the presence of PrP^{Sc} in the spleen [23].

The identification of vCJD infection in 4 individuals who had received red cell transfusions from vCJD-infected donors strongly suggests that blood is infectious during the incubation period for vCJD. Since donations from asymptomatic donors who

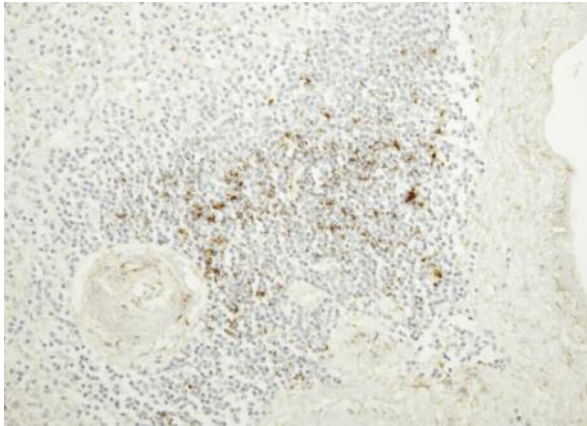


Fig. 4. Immunohistochemistry for abnormal PrP in the spleen in a case of asymptomatic variant CJD infection following blood transfusion shows labelling of follicular dendritic cells and macrophages within germinal centres (anti-PrP antibody (KG9) with haematoxylin counterstain).

subsequently died from vCJD were also used for plasma processing in the UK [15], these findings renewed concerns that vCJD might be transmissible by plasma products. In anticipation of and response to these concerns, a range of precautionary measures was introduced in the UK to reduce the likelihood of transmission of vCJD by blood and plasma products [22,30]. None of the measures are likely to remove all risks, but it appears that leucodepletion may reduce levels of infectivity in blood [13].

A risk assessment was commissioned by the Department of Health in the UK in order to address the possible transmission of vCJD by blood and blood products [9]. The conclusions of this exercise were based on generally pessimistic assumptions made concerning the likely levels of infectivity in blood in vCJD, and the potential consequences of the processing steps used in the manufacture of plasma products. Accordingly, recipients of concentrates Factor VIII and IX were deemed likely to be at a sufficiently increased risk of vCJD to require additional public health measures in order to minimise any risk of secondary transmission. Patients treated with UK-sourced pooled clotting factor concentrates between 1980 and 2001, including most of the adult haemophilia patients, were therefore informed that they had been assessed as being at an increased risk of infection with vCJD [31]. This approach was taken on the advice of the UK Haemophilia Centre

Doctors Organisation (UKHCDO) and was endorsed by the UK Haemophilia Society.

vCJD infection in a haemophilic patient in the UK

Around 4,000 patients with bleeding disorders who have been treated with UK-sourced pooled clotting factor concentrates are registered in the National Haemophilia Database in the UK [22]. A retrospective pathological review of autopsies performed in 22 UK haemophilic patients who died before 1998 using immunohistochemistry on fixed tissue samples found no evidence of vCJD infection [21]. A prospective surveillance study to detect vCJD infection in patients with haemophilia was established by UKHCDO and the National CJD Surveillance Unit in 2000 [22], which included Western blot analysis to detect PrP^{Sc} in unfixed biopsy and autopsy lymphoid or brain tissue samples when appropriate consent had been obtained. By 2009, 17 patients (10 autopsy cases and 7 biopsy cases) had tissue samples submitted for analysis, ranging from a single biopsy sample to a full range of autopsy tissues. In the autopsy cases, the spleen of a single autopsy case gave a strong positive result in one region on repeated testing for PrP^{Sc} [24]. The patient was a 73-year-old male who was a UK resident with no history of neurological disease; analysis of the codon 129 polymorphism in the *PRNP* gene showed that this patient was heterozygous (methionine/valine). He had received over 9,000 units of Factor VIII prepared from UK plasma pools that included donations from a UK donor who subsequently died from vCJD. He had also received blood transfusions of 14 units of red blood cells and had undergone several surgical procedures and invasive endoscopy. Estimates of the relative levels of risk of vCJD infection through diet, surgery/endoscopy, blood transfusion and receipt of plasma products in this patient, suggested that the most likely route of infection was the receipt of contaminated plasma products [24]. This case represents the first demonstration of vCJD infection in a haemophilic patient in the UK; no clinical cases of vCJD have so far been identified in any patient treated with UK-sourced clotting factor concentrates.

Future developments

Many of the uncertainties over the incidence of asymptomatic vCJD infection in the UK and the risks of infection with vCJD by blood and plasma compo-

nents could be addressed if a screening test for vCJD was available. A number of different approaches are currently underway, although the difficulties of developing a method of sufficient sensitivity and specificity are formidable [3,30]. Although one test has been reported to be able to detect vCJD prions in the blood of non-human primates experimentally infected with BSE [2], this research has now been terminated [8]. Details of another blood-based assay to detect prion infection in vCJD has recently been published, which has given positive results in vCJD blood samples, but not in blood samples from sCJD and controls [10]. Further investigations are required to assess the sensitivity and specificity of this test, and to determine whether it could be implemented as a large-scale screening assay for vCJD. However, even if a suitable screening test for vCJD was available, there will be a pressing need for a further confirmatory test, to address issues over the sensitivity and specificity of any screening test [12,30]. The lack of any effective treatment or prophylaxis for vCJD and other prion diseases has raised the question of the benefit of testing to any individual, particularly those in "at-risk" groups. Continuing surveillance for vCJD is required in order to assess more fully the risks to these patients and to obtain more information about the prevalence of this disorder, particularly in relation to *PRNP* genotypes in which definite cases of vCJD have not yet been identified.

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