REVIEW

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Variant CJD
18 years of research and surveillance

Abigail B Diack[1,†], Mark W Head[2,†], Sandra McCutcheon[1], Aileen Boyle[1], Richard Knight[2], James W Ironside[2], Jean C Manson[1,†,*], and Robert G Will[2,†]

[1]The Roslin Institute and R(D)SVS; University of Edinburgh; Easter Bush; Midlothian, Scotland, UK; [2]National CJD Research & Surveillance Unit; School of Clinical Sciences; University of Edinburgh; Western General Hospital; Edinburgh, Scotland, UK

[†]These authors contributed equally to this work.

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Abbreviations: BSE, bovine spongiform encephalopathy; CWD, chronic wasting disease; GSS, Gerstman–Sträussler–Scheinker disease; M, methionine; PPS, pentosan polysulphate; PrP^{res}, protease-resistant prion protein; PrP^{Sc}, abnormal prion protein; QuIC, quaking-induced conversion; TSE, transmissible spongiform encephalopathy; V, valine; vCJD, variant Creutzfeldt–Jakob disease; VPSPr, variably protease-sensitive prionopathy

It is now 18 years since the first identification of a case of vCJD in the UK. Since that time, there has been much speculation over how vCJD might impact human health. To date there have been 177 case reports in the UK and a further 51 cases worldwide in 11 different countries. Since establishing that BSE and vCJD are of the same strain of agent, we have also shown that there is broad similarity between UK and non-UK vCJD cases on first passage to mice. Transgenic mouse studies have indicated that all codon 129 genotypes are susceptible to vCJD and that genotype may influence whether disease appears in a clinical or asymptomatic form, supported by the appearance of the first case of potential asymptomatic vCJD infection in a *PRNP* 129MV patient. Following evidence of blood transfusion as a route of transmission, we have ascertained that all blood components and leucoreduced blood in a sheep model of vCJD have the ability to transmit disease. Importantly, we recently established that a *PRNP* 129MV patient blood recipient with an asymptomatic infection and limited PrP^{Sc} deposition in the spleen could readily transmit disease into mice, demonstrating the potential for peripheral infection in the absence of clinical disease. This, along with the recent appendix survey which identified 16 positive appendices in a study of 32 441 cases, underlines the importance of continued CJD surveillance and maintaining control measures already in place to protect human health.

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*Correspondence to: Jean C Manson; Email: jean.manson@roslin.ed.ac.uk
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Introduction

Transmissible spongiform encephalopathies (TSE) or prion diseases are a unique group of fatal neurodegenerative diseases occurring in humans and mammals. Prion diseases can be sporadic, heritable, or acquired, they can be transmitted both naturally and experimentally, and as yet, there is no known cure. In 1996, an acquired human prion disease, variant Creutzfeldt–Jakob disease (vCJD), was described in the United Kingdom (UK) leading to a flurry of news reports, changes in government policies regarding the beef industry, a ban on exports of meat, restrictions on blood donations and a widespread fear that anyone could be infected. Since that first report, researchers and health professionals have endeavored to try and understand the disease, identify the infectious agent, assess transmission risks and ultimately improve diagnosis and find a cure. This review will summarize 18 y of research from identification of disease strain, epidemiology, and genetics, to assessing risks of transmission, diagnosis, and therapeutics, and finally the current issues of subclinical disease and ongoing surveillance.

BSE and vCJD: The Same Strain of Agent

In 1985, a novel neurodegenerative disease of cattle was recognized in the UK. Pathological examination of the brain material from these cattle suggested that this was a new TSE subsequently named bovine spongiform encephalopathy (BSE).[1] A further examination of these cases was performed following transmission of brain material to a panel of wild-type mice. The RIII, C57BL, and VM mice gave similar incubation periods, rankings, and vacuolation profiles for each isolate. Vacuolation distribution in the *Prnp*-a mice (RIII and C57BL) show distinctive profiles with higher levels or "peak" of vacuolation in the dorsal medulla, hypothalamus, and septum (**Fig. 1**) whereas *Prnp*-b mice (VM) show peaks in the dorsal medulla, superior colliculus, thalamus, and septum.[2] This BSE signature was confirmed in a number of cases of BSE and was observed in a number of similar transmissions from cats,[3] kudu, and nyala.[4]

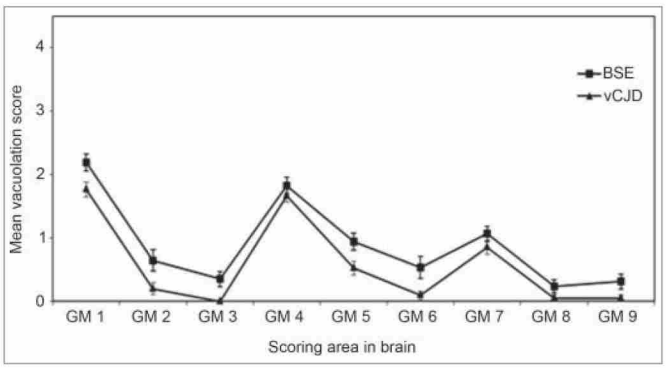

Figure 1. Vacuolation scoring in the mouse brain. Lesion profile comparison of vCJD and BSE following transmission to RIII mice. Data shows mean lesion profile ± standard error of the mean (n ≥ 6). G1-G9, gray matter scoring regions; (G1) dorsal medulla, (G2) cerebellar cortex, (G3) superior colliculus, (G4) hypothalamus, (G5) thalamus, (G6) hippocampus, (G7) septum, (G8) retrosplenial and adjacent motor cortex, (G9) cingulate and adjacent motor cortex.

Thus confirming a single agent was responsible for these new TSE cases in each species. Furthermore this agent was experimentally transmitted to sheep and goats.[5]

Ten years after BSE was recognized, the first case of an atypical form of Creutzfeldt–Jakob disease, termed "new variant CJD" (vCJD) in humans was identified;[6] both diseases were recognized to be prion diseases, raising serious concerns that BSE had now spread to humans via consumption of infected meat products.[7] Following the same protocols that had been used for the animal transmissions, a series of vCJD transmissions were set up. Initial results published in 1997 using RIII mice indicated that vCJD was indeed caused by the BSE agent.[8] At the same time, Hill et al.[9] showed similar results using FVB mice. Further studies have shown that incubation period rankings, lesion profiles and abnormal prion protein (PrP^{Sc}) deposition patterns are all identical to the BSE agent (**Figs. 1 and 2**).[10,11] The definitive evidence that BSE and vCJD were the same strain came with the characterization of primary and secondary transmission of 10 cases of vCJD to mice. In this study both CNS and peripheral material was transmitted and showed that in all cases transmission characteristics were similar to BSE.[12] To date, all cases of UK 129MM (methionine homozygous) vCJD that have been characterized have shown similar strain characteristics to BSE.

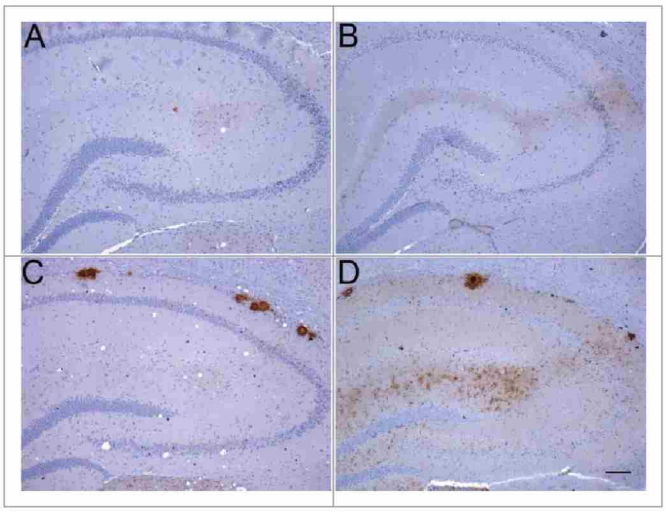

Figure 2. Abnormal PrP deposition in hippocampus of RIII and VM mice following inoculation with BSE or vCJD. (**A**) BSE in RIII mouse; (**B**) vCJD in RIII mouse; (**C**) BSE in VM mouse; (**D**) vCJD in VM mouse (bar, 100 μm; anti-PrP antibody: 6H4).

In addition to epidemiological, neuropathological, and biological evidence, the biochemical analysis of PrP^{Sc} deposited in both the BSE brain and vCJD brain also supports a link between the agents responsible. Western blotting of partially protease-resistant prion protein provides a surrogate for conformation and/or aggregation state and also reflects glycosylation site occupancy in the form of (generally 3) bands of protease-resistant prion protein (PrP^{res}) of defined mobility (determined by N-terminal truncation) and relative abundance (determined by glycosylation). Using this method BSE can be differentiated from most forms of sheep scrapie.[13] Similarly vCJD can be distinguished from other human prion diseases, in particular sporadic CJD (sCJD),[14,15] whereas BSE and vCJD share both mobility type and have a similar glycoform ratio.[14] This BSE/vCJD PrP^{res} type (referred to a type 2B or type 4) appears to be closely associated with the agent since (1) it also characterizes the PrP^{res} that accumulates in peripheral tissues in clinical vCJD,[16] (2) it is maintained following secondary transmission of vCJD by blood transfusion both in the brain of clinical cases,[17] and in the spleen of asymptomatic or preclinical individuals,[18,19] (3) it is largely stable on transmission to wild-type and humanised transgenic mice[12,20] and (4) it is maintained in cell-free conversion systems in which either BSE or vCJD brain homogenates are used to seed conversion of normal human prion protein.[21]

While type 2B PrP^{res} provides a convenient additional diagnostic tool for human BSE identification,[22] it does not provide a complete description of PrP^{Sc} in cases of vCJD, nor does it provide a biochemical definition of the agent. The largest amount of PrP^{Sc} in the vCJD brain is actually protease sensitive[23] and therefore does not figure in conventional PrP^{res} typing. Even within the protease-resistant fraction of vCJD PrP^{Sc} there is evidence of a minority PrP^{res} type.[24] The use of assays that do not depend upon protease-resistance as a definition of PrP^{Sc} show that aggregation state and stability are additional biochemical parameters that may be relevant to neurotoxicity and agent replication.[25,26]

vCJD in the UK and Beyond

Since the first recorded case of vCJD in 1996, 177 cases of definite or probable vCJD have been reported in the UK (as of April 2014). The annual number of deaths reached a peak in 2000 with 28 deaths but since 2006, deaths from vCJD have levelled off at 2–5 per year with none reported in 2012 and only one in 2013[27] (**Fig. 3**). Originally restricted to the UK, 51 cases have now been reported in 11 other countries with a worldwide

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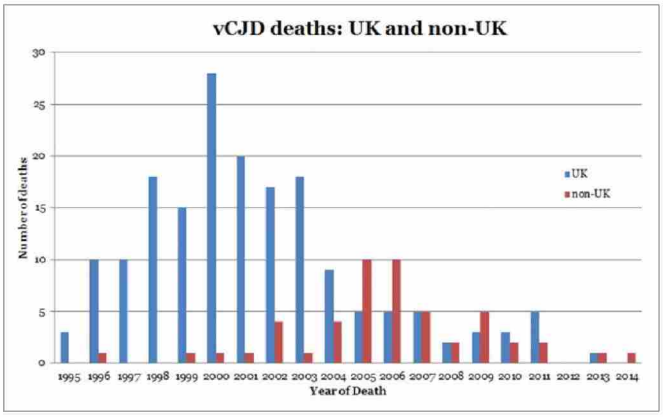

Figure 3. Reported incidence of vCJD deaths in the UK and in non-UK countries.

total of 228 (**Fig. 3**). Outside of the UK, most cases have occurred in France (27 cases to date) and are thought to be related to the peak in volume of beef imports originating from the UK during 1985–1995.[28] This potential relationship is shown in the peak in number of deaths from vCJD in France in 2005, 5 y after a similar peak in the UK.[28,29] Further evidence of the link between UK and French vCJD has arisen from comparative studies comparing epidemiologic, clinical, pathological, and biochemical analyses of vCJD cases from both countries indicating that the same strain of agent could be responsible.[30] The type 2B PrP^{res} that characterizes UK vCJD cases[15] is also present in vCJD patients from France[30] and cases from Holland, Portugal, Spain, and Italy (Head and Ironside, unpublished information), consistent with the same strain of agent being involved in these different countries.

Exports of UK meat or cattle are assumed to have played a major role in the incidence of vCJD cases in other countries;[31] however there is the possibility that indigenous BSE or another strain of agent is responsible. In order to assess whether the same strain of agent is responsible for all vCJD cases worldwide, Diack et al.[32] performed strain typing of French, Italian, Dutch, and American cases of vCJD. These were all of the 129MM genotype and with limited exposure to UK BSE. Analysis of the transmission properties showed that the non-UK cases shared the same characteristics as UK cases of vCJD, however small differences were apparent in the incubation period rankings which are currently being studied.[32] The similar characteristics between UK and non-UK cases of vCJD suggest that current diagnostic criteria are sufficient to detect cases in all countries at this time. However these studies characterized "typical" cases of vCJD and do not take into account atypical cases or those occurring in genotypes other than 129MM.

All Codon 129 Genotypes are Susceptible to vCJD

Mutations and polymorphisms in the prion protein gene (*PRNP*) can influence or be associated with disease, i.e., E200K-129M in genetic CJD or A117V-129V in Gerstman–Sträussler–Scheinker disease (GSS). The codon 129 polymorphism (methionine (M)-valine (V)) of *PRNP* is known to be associated with susceptibility to CJD[33] with evidence from studies of kuru suggesting that heterozygosity is associated with increased survival times.[34] As stated, all definite and probable cases of clinical vCJD have been of the 129MM genotype which is in contrast to the normal distribution of genotypes in the general UK population; 42% 129MM, 47% 129MV, and 11% 129VV[35] and is suggestive of an association between vCJD susceptibility and genotype.

Experimental transmission studies have utilized gene targeted mice expressing human PrP^{C} at physiological levels and overexpression models carrying each of the codon 129 genotypes to reveal that human-to-human transmission of vCJD is possible and that all genotypes have the potential to be affected.[9,20,36] Bishop et al.[20] used gene targeted models allowing direct comparison between mouse lines; these studies showed that transmission efficiency varied in the order MM > MV > VV with different pathological characteristics for each genotype. Mice expressing 129MM (HuMM) showed the greatest transmission efficiency and the earliest onset of both clinical disease and TSE related pathology. Although fewer HuMV mice were clinically affected and showed an extended incubation period, similar numbers demonstrated evidence of PrP^{Sc} compared with HuMM mice. In contrast only one HuVV mouse showed evidence of PrP^{Sc}.[20] This pattern of susceptibility has been repeated in a series of vCJD transmissions both from UK and non-UK material. This data suggests that in humans not only do all genotypes have the potential to be affected but that the different genotypes may manifest disease in different ways and indeed 129MV and 129VV individuals may have long asymptomatic incubation periods.

The evidence from the mouse studies has been shown in humans by the discovery of PrP^{Sc} in an asymptomatic PrP codon 129 heterozygote individual who died of a non-neurological disorder 5 y after receiving blood from an individual who later went onto develop clinical vCJD. In this individual evidence of PrP^{Sc} was found in the spleen and a cervical lymph node.[19] Transmission studies have now shown the spleen from this individual to be infectious.[37] A possible case of vCJD in a 129MV individual was reported in 2009, however vCJD was not confirmed since no autopsy was undertaken.[38] Additionally, retrospective studies of anonymised tonsil and appendix samples have shown evidence of PrP^{Sc} in all 3 genotypes giving further support to the evidence that all genotypes are susceptible to vCJD.[39,40]

Modeling human genetic susceptibility to BSE using cell-free assays confirms the importance of methionine at codon 129 of the *PRNP* gene as a susceptibility factor, and shows the conversion efficiency to be MM > MV > VV, irrespective of whether the brain homogenate used to seed the reaction is vCJD, cattle BSE or experimental sheep BSE.[21]

Blood as a Route of Transmission of vCJD

The UK shows the highest incidence of vCJD in the world.[27] At early stages of the epidemic, it was largely accepted that there was a minimal risk of transmission of vCJD from donations of

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peripheral blood/tissue from affected individuals to others via iatrogenic routes. That said great efforts were made to trace and track the fate of blood components used for transfusion from donors known to have vCJD.[41] Concomitantly, the likelihood of transmission of prion infection through blood, either by inoculation or transfusion and the distribution of prion-associated infectivity in blood components was being assessed using a range of animal models, typically small animal models.[42-48] These produced estimates of ~10 infectious doses (ID)/ml in hamster blood.[47,49] Rodent models do not however represent accurately the procedures used in a clinical setting for blood transfusion. Large animal models such as sheep or deer infected with BSE or scrapie provide a suitable alternative to better assess the likelihood of transmission of prion disease following exposure by blood transfusion.[50-52] Larger volumes of blood can be collected from such animals and processed into components with similar specifications as those used for transfusion to humans.[53] Furthermore the peripheral pathogenesis of scrapie and BSE in sheep closely resembles that of humans affected with vCJD.[54,55] Over a decade ago, transfusion studies in sheep first demonstrated that all clinically-relevant blood components collected at both preclinical and clinical time points contained sufficient titers of disease-associated infectivity and could be transmitted to recipients after a single transfusion event. Moreover, the number of recipients that developed disease was suggestive that blood transfusion was a highly efficient route by which prion diseases could be transmitted.[56] Of note, in this and other studies, was the finding that the process of leucoreduction alone did not prevent the transmission of prion disease following blood transfusion.[49,53] The relevance of the latter point being that all components used for blood transfusion in humans are subject to universal leucoreduction. Data showing that blood from prion-infected animals was infectious was confirmed by other research groups using sheep[57,58] and deer blood transfusion models.[59-61]

Since the late 1990s a number of risk reduction strategies were implemented to safeguard the UK blood (and blood product) supply. This included donor deferral and exclusion, importation of plasma from the USA for the preparation of plasma derivatives, i.e., clotting factors; the use of disposal instruments for certain surgical and dental procedures; and universal leucoreduction of all components used for transfusion.[62] Following a further risk assessment initiated by the Department of Health, selected groups of patients were informed that they could be considered to have a "small increased risk of carrying the vCJD agent" following receipt of certain batches of plasma products. These groups included hemophiliacs and those affected with other bleeding disorders and those with primary or secondary immunodeficiencies.[62,63]

It was not until 2004 that blood from vCJD-infected humans was shown to pose a significant risk of acquiring prion infection. This followed the identification of 2 potential cases of blood-transfusion acquired vCJD.[19,64] A few years later saw the identification of another case of apparent transfusion-acquired vCJD.[65] These data were collated and cases presented in detail in 2006.[41,66] A fourth occurrence of transfusion-acquired vCJD was subsequently identified and all cases have been summarized in a recent review.[67] The 4 affected individuals were from the UK and all received non-leucoreduced red cell concentrates from UK donors, who were asymptomatic of infection at the time of donation but later died from vCJD. The transfusions took place between 1996 and 1999. Of the 4 transfusion recipients, 3 developed a clinical infection consistent with previously identified cases of vCJD (i.e., diseased-associated prion protein was evident in brain and peripheral lymphoid tissues examined post-mortem).[17] These individuals were identified as being methionine homozygous at codon 129 in the *PRNP* gene. The remaining transfusion recipient showed no clinical signs associated with vCJD or other neurological-type conditions and there was no evidence of disease-associated prion protein in the individual's brain and indeed the patient died of causes unrelated to vCJD.[19] Disease-associated prion protein was identified in selected lymphoid tissues such as the spleen and a cervical lymph node. Unlike the 3 clinical cases previously reported, this recipient was identified as having a different *PRNP* genotype being heterozygous (MV) at codon 129.

A surveillance program (established by the National CJD Research and Surveillance Unit and the UK Hemophilia Centre Doctors Organisation) identified the first case of vCJD infection in a hemophiliac patient.[18,68,69] The study examined biopsy and autopsy samples of lymphoid or brain tissue from a small numbers of samples submitted for investigation. The individual was an elderly male who resided in the UK. In conjunction with surgical procedures, the patient received numerous units of non-leucoreduced red cells and thousands of units of Factor VIII. The factor VIII was prepared from UK plasma pools and it was found that some of the pooled-plasma could be traced back to a donor who died from vCJD. The individual showed no signs of vCJD or other neurological conditions and was MV at codon 129 in the *PRNP* gene. Upon repeated examination, a specific area of spleen was positive for the abnormal form of the prion protein. A subsequent risk assessment found that of all possible sources of vCJD infection, including dietary exposure, the mostly likely was determined as treatment with UK-sourced clotting factors.[70] To date, there have been no further cases of vCJD acquired following the transfusion of blood, blood components or clotting factors. While there has been no documented evidence, to date, of the transmission of sCJD infection following blood transfusion in humans,[41] a recent, though limited study, has reported the presence of disease-associated infectivity in plasma obtained from 2 patients affected with sCJD.[71]

Although estimates of the infectious titer of blood from patients with vCJD are low[71,72] it has been demonstrated that blood and components from asymptomatic individuals appear capable of transmitting vCJD-infection following blood transfusion. Major efforts have been made toward the development of screening assays and diagnostic tests for vCJD in blood[72-75]; the development and implementation of prion reduction filters[76-80]; understanding the numbers of individuals who may be sub-clinically affected with vCJD[39,81-84] and what this really means in terms of further spread of vCJD. There are significant challenges to be faced in each of these areas, which are further confounded by the absence of an available treatment for vCJD.

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Prevalence of Asymptomatic vCJD Infection

The UK population had a wide exposure to the BSE agent through contaminated meat products in the food chain in the 1980s and early 1990s, resulting in 177 definite cases of vCJD to date. Of the cases genotyped, all were methionine homozygotes at codon 129 in the *PRNP* gene. However there are ongoing concerns over vCJD infection in other codon 129 genotypes with potentially longer incubation periods. This has prompted a series of tissue–based studies on the prevalence of vCJD infection in lymphoid tissues (appendices and tonsils) removed surgically as part of treatment for appendicitis, tonsillitis, and related disorders in otherwise healthy individuals with no neurological symptoms. Variant CJD differs from other human prion diseases in the widespread involvement of lymphoid tissues by the causative agent which is detectable in follicular dendritic cells and is associated with infectivity.[16,85]

Review of paraffin-embedded appendices that had been resected from a small number of individuals before the onset of vCJD revealed that prion protein was detectable in the lymphoid follicles in the wall of the appendix for at least 2 y prior to the onset of vCJD symptoms.[86] This observation allowed the possibility of a large-scale retrospective survey of appendix and tonsil tissues from histopathology departments across the UK to determine the extent of asymptomatic vCJD infection as revealed by immunohistochemistry on paraffin-embedded tissues. These studies have proven challenging in terms of logistics and ethics and have proceeded on the basis of using anonymised specimens that are not directly linkable to any individual. The first of these studies reported in 2004 an estimated prevalence of asymptomatic vCJD infection in 237 per million in 4000 individuals in the UK (3 in 12 674 positive specimens tested), but with very wide 95% confidence intervals (49–692 per million).[83] Two subsequent prospective studies on tonsil tissues collected frozen tissue samples as well as paraffin-embedded tissues, which allowed the use of enzyme immunoassays and western blotting in addition to immunohistochemistry for the detection of the abnormal prion protein.[81,82,87] No positives were detected in the frozen tissue samples from either study (2000 in Frosh et al.[87]; 32 661 in Clewley et al,[81]). Immunohistochemistry was subsequently performed on 10 075 samples from the de Marco et al.[82] study, with 1 apparent positive detected.

In order to resolve the findings from these studies, a larger unlinked and anonymised immunohistochemical survey was performed on archived paraffin-embedded appendix samples from 41 histopathology departments in the UK.[39] Of the 32 441 samples assessed, 16 were positive for abnormal prion protein, giving an overall prevalence of 493 per million (95% confidence intervals 282–801 per million), which is broadly in keeping with the results of the earlier study by Hilton et al.[83] *PRNP* codon 129 genotype analysis of the positive cases showed that all possible genotypes were involved, with a predominance of the valine homozygous genotype,[39] as for the Hilton et al. study,[40] and in contrast with the definite cases of vCJD identified to date. These findings have a wide range of implications, including the need for continuing surveillance of human prion diseases in the UK and the risks of secondary vCJD transmission from asymptomatic infected individuals via surgical instruments or blood transfusion; the latter is now the subject of a UK Parliamentary Inquiry (Parliamentary Select Committee on Science and Technology, 2013[88]).

Diagnostics and Treatment

The diagnosis of vCJD rests on recognizing the typical phenotype and applying appropriate specialist investigations, in particular MRI brain scan (**Fig. 4**). The clinical features are remarkably stereotyped. There is an initial phase of around 6 mo dominated by psychiatric symptoms, including depression, delusions, and anxiety[89] followed by the rapid development of neurological features,[90] typically confusion, ataxia, and involuntary movements, which may be choreiform, dystonic, or myoclonic. The duration of illness from onset to death averages 14 mo[91] in contrast to sCJD in which the mean survival is 4 mo.

The electroencephalogram does not show the periodic sharp wave complexes that are seen in sCJD, except rarely in the terminal stages of the illness[92] and the CSF 14–3-3 immunoassay is only positive in about half the cases.[93] The CSF RT-QuIC has been negative in vCJD in all assays to date. The most helpful investigation is MRI brain scan, which shows high signal in the pulvinar region of the thalamus, the so-called hockey stick sign, on FLAIR (**Fig. 4**) and DWI sequences in over 90% of cases.[94]

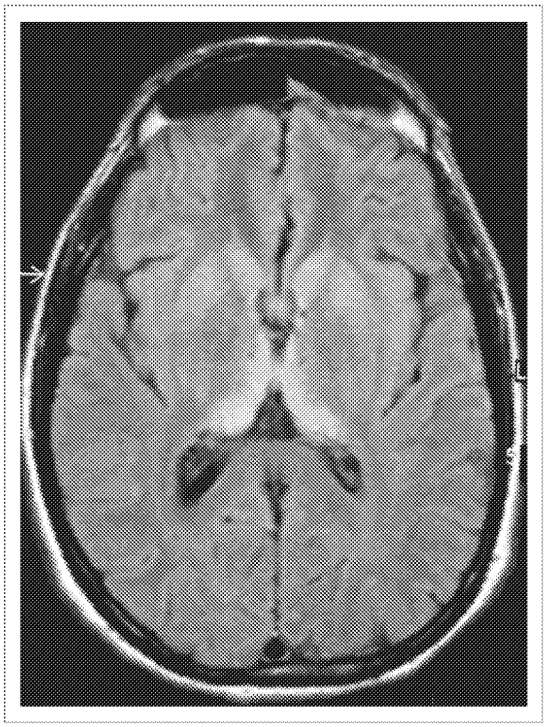

Figure 4. MRI brain scan in variant CJD. FLAIR axial section at the level of the basal ganglia showing bilateral symmetrical dorsomedial and pulvinar thalamic hyperintensity. Courtesy of Dr David Summers.

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Tonsil biopsy shows immunostaining and deposition of type 2B or type 4 PrP^{res} in the majority of cases,[95,96] but this test is invasive, and definitive diagnosis rests on neuropathological examination of brain tissue, usually at post-mortem.

Highly sensitive and specific diagnostic criteria, including a combination of core clinical features and the results of MRI brain scan and pathology, have been formulated[97] and validated.[98] Cases classified as definite or probable are reported by international surveillance systems as the likelihood of accurate diagnosis in possible cases is uncertain.

The phenotype in cases of vCJD in an MV or VV genetic background cannot be predicted and continued vigilance is necessary in order to identify such cases.

Treatment of vCJD has been attempted using a range of medications, but none have been proven to be effective. Initial reports of improvement following treatment with quinacrine have not been confirmed in observational trials[99,100] and this drug is no longer used in vCJD treatment. Studies in animal models raised the possibility that pentosan polysulphate (PPS) might be a candidate treatment for vCJD[101] and extended survival has been reported in a small number of treated cases.[102,103] However, this medication has to be given by intraventricular infusion, requiring a neurosurgical procedure, and treated patients continued to decline with no reversal of severe neurological deficits.[104] Post-mortem examination of one case of vCJD treated with PPS showed extensive and severe pathology.[105] Some cases of vCJD received doxycycline with no obvious benefit and a controlled trial in sCJD has not demonstrated efficacy.[99]

Emergence of Novel Strains

Identification of novel strains involves veterinary and medical vigilance, but it also requires a proper and full characterization of known prion agents. While the deployment of wild-type mouse panels, transgenic mice, and non-human primates all rapidly concluded that vCJD was a novel human prion strain related to BSE (see above), determining how many distinct human prion strains there are has proved surprisingly difficult, especially for sCJD. Transmission studies in humanised transgenic mice and non-human primates point to 4 major groups within sporadic, iatrogenic CJD, Kuru, and some genetic CJD cases, termed M1, V1, M2, and V2.[22,106] Sporadic fatal insomnia and fatal familial insomnia (FFI) together may represent a sixth strain[107] and 2 further transmissible phenotypes can be derived from GSS disease: one involving a transmissible amyloid phenotype, the other a fully transmissible spongiform encephalopathy.[108,109] The transmission properties of PrP cerebral amyloid angiopathy and Variably ProteaseSensitive Prionopathy (VPSPr) remain to be reported. The relationship between human disease phenotypes, agent strain, and prion biochemistry is further complicated by the now widely recognized phenomenon of distinct PrP^{res} type co-occurrence in the sCJD, vCJD and VPSPr brain.[15,24,110]

Surveillance for BSE in cattle, sheep, and goats has identified new (or newly discovered) animal prion diseases including atypical scrapie in sheep and so called H- and L-type BSE in cattle. These along with chronic wasting disease (CWD) in deer and elk represent a potential zoonotic risk to human health that is hard to quantify. While Wilson et al.[111] have shown no transmission of CWD, BASE, H-type BSE, and atypical scrapie to mice expressing wild-type levels of human PrP, Kong et al.[112] demonstrated transmission of BASE to an alternative line of mice expressing wild-type levels of human PrP. In contrast, Beringue et al.[113] showed transmission of BASE to mice overexpressing human PrP but no evidence of H-type BSE transmission, furthermore no evidence of CWD transmission to overexpressing mice has been identified.[114,115] This difference in transmission results may be due to different genetic backgrounds or differences in PrP expression levels between the different mouse lines. An alternative to modeling the species barrier is the cell-free conversion assay which points to CWD as the animal prion disease with the greatest zoonotic potential, after (and very much less than) BSE.[116]

Surveillance

Continued surveillance for long-term effects of BSE exposure in the UK human population appears necessary for the foreseeable future in order to discount possible second wave epidemics that might depend on genetic susceptibility, subclinical infection, and secondary transmission or disease in defined "at risk" patient groups such as hemophiliacs or patient groups in which full ascertainment is difficult, such as the elderly.

However, an additional concern is associated with idiopathic human prion disease. Sporadic CJD is not a uniform condition and the phenotype is clearly influenced by the codon 129 genotype of the patient and the prion protein type that accumulates in their brain. The etiological basis of the condition might be presumed to be spontaneously occurring, but this is not known with certainty in general, or in individual specific cases. Neither are the molecular mechanisms of spontaneous conversion of the prion protein to its pathogenic form well understood or easily investigated. Additionally, surveillance identifies apparently sporadic cases of human prion disease that do not fit well into currently accepted classification systems. This is exemplified by the recent identification of a new human prion disease (VPSPr by Gambetti et al.[117]) and its prospective and retrospective identification in other countries subsequently.[118,110] The true prevalence, the relationship to sCJD and the risk to public health of VPSPr are yet to be determined.

Conclusions

Since the identification of vCJD we have made progress in identifying routes of infection, controlling further infection, producing models of disease, developing decontamination procedures, and understanding susceptibility to disease. The vCJD epidemic in the UK now appears to be in decline and it appears that the control measures in food production and blood supplies have prevented further vCJD cases arising through dietary/infected blood exposure.

Despite this, there are still ongoing concerns over cases of vCJD arising in countries where little or no exposure to UK meat products have occurred, the presence of subclinical vCJD in the

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UK population with the possibility of further human-to-human transmission and the identification of new strains of human prion disease. These scenarios necessitate ongoing studies in understanding transmission properties, disease diagnosis, and therapeutics. The identification of novel human prion diseases and the current estimates of subclinical vCJD infections show the importance of continued CJD surveillance and maintaining control measures already in place to protect human health.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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