The new variant of Creutzfeldt-Jakob disease

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Summary
New variant Creutzfeldt-Jakob disease (nvCJD) is a novel human transmissible spongiform encephalopathy which was first identified in 1996 in the United Kingdom (UK). Subsequent scientific studies have revealed that the strain of the transmissible agent responsible for nvCJD is identical to that of the bovine spongiform encephalopathy (BSE) agent, and the disease has been considered as 'human BSE'. By 31 December 1999, 52 cases of nvCJD had been reported (49 cases in the UK, two cases in France and one case in the Republic of Ireland). All these individuals were under 53 years of age and all those tested were methionine homozygotes at codon 129 of the prion protein gene. The number of cases of nvCJD likely to occur in the future is impossible to estimate because of multiple uncertainties, in particular the disease incubation period, the degree of exposure to the infective agent and the susceptibility of other genetic subtypes. Continued surveillance of both BSE and CJD is required in the UK and in other countries, to ensure that the scale of this potential epidemic is adequately monitored and that all possible steps are taken to prevent further human exposure to the BSE agent.

Keywords

Introduction
Creutzfeldt-Jakob disease (CJD) is a rare and fatal neurodegenerative condition that occurs throughout the world (71). The disease is classified as a transmissible spongiform encephalopathy (TSE) because of the characteristic spongy degeneration shown in the grey matter of the brain in those affected and because of the ability of the disease to be transmitted to laboratory animals (40). Alternatively known as prion diseases, TSEs also naturally affect a range of animal species including sheep, goats, cattle, deer, mink and cats (9). Historically, no evidence of a link between CJD and any of the animal TSEs has been demonstrated (91). However, in 1996, ten cases of a new variant of CJD (nvCJD) were reported in the United Kingdom (UK) (94) and epidemiological and other scientific studies now indicate that this new disease is almost certainly caused by the bovine spongiform encephalopathy (BSE) agent (17, 21, 59).

The occurrence of BSE and nvCJD has had wide-ranging implications for animal and public health. The epidemic of BSE has led to restrictions in practices in many countries and the introduction of new policies to prevent the spread of TSEs. The Office International des Epizooties in partnership with the World Health Organization (WHO) has been promoting surveillance of human and animal TSEs throughout the world. Collaborative meetings have been held to strengthen TSE surveillance activities and make recommendations relevant to the protection of animal and public health (98, 99, 100).

The concern over nvCJD is intensified by multiple uncertainties; as yet, it is not known how many people are incubating the disease, whether nvCJD will spread to the rest of Europe and beyond, and whether the disorder can be transmitted via surgical instruments or blood products. These concerns are further magnified by the current lack of a
presymptomatic screening test or effective treatment and the unknown nature of the transmissible agent.

In this review a brief synopsis of the human TSEs is presented, followed by a detailed overview of nvCJD.

**Historical background**

In 1920, Hans Gerhard Creutzfeldt, a German neuroscientist, reported the case of a twenty-two-year-old woman who died following a history of progressive cerebral dysfunction (25). A year later, another German neuroscientist, Alfons Maria Jakob, described five further cases (60, 61, 62), and in 1922, the term ‘Creutzfeldt-Jakob disease’ was coined (86). Nomenclature was problematic over the ensuing decades, and more than fifty synonyms have been used to describe the disease now known as CJD (41). To confuse matters further, CJD was often used as a convenient diagnosis for any unexplained rapidly progressive dementia, and many cases (including the original patient of Creutzfeldt) would not have met modern criteria for CJD (72).

A major breakthrough in understanding CJD came through the study of kuru. This fatal progressive cerebellar ataxia was epidemic in the 1950s and 1960s among tribespeople of the eastern highlands of Papua New Guinea (35). The search for the aetiology of kuru was initially unfruitful, but a clue came from the neuropathology of the disease, which was noted to be reminiscent of scrapie, a fatal neurodegenerative disease of sheep and goats (47). Scrapie had been known for over two centuries and, in 1936, had been shown to be experimentally transmissible between sheep following inoculation of central nervous system (CNS) tissue (26). Kuru was successfully transmitted to laboratory animals via intracerebral inoculation of neural tissue in 1965 (36). This finding, combined with the results of anthropological studies led to the conclusion that kuru arose through cannibalism (42). Animal transmission experiments were subsequently initiated with CNS tissue from a wide range of neurodegenerative conditions, but only those using brain tissue from cases of CJD (a disorder with many neuropathological similarities to kuru) were successful (40).

Transmissibility established a ‘gold standard’ for TSE diagnosis, but also raised the possibility that these diseases could be transmitted to humans, either from humans or from animals. The former concern was realised in 1974 when the first report of iatrogenic CJD was published (29); approximately two hundred such cases have now been documented. However, despite all subtypes of CJD being experimentally transmissible, the majority of cases occur sporadically without evidence of an acquired infection (Table I). Furthermore, a minority of cases are inherited. The multiple modes of acquisition of the human TSEs sets them apart from other diseases and explanation of the nature of the transmissible agent has been a great scientific challenge.

### Table I

The human transmissible spongiform encephalopathies

<table>
<thead>
<tr>
<th>Human transmissible spongiform encephalopathy</th>
<th>First reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creutzfeldt-Jakob disease*</td>
<td>1921</td>
</tr>
<tr>
<td>Sporadic (85% of cases)</td>
<td>1921</td>
</tr>
<tr>
<td>Familial (10%-15% of cases)</td>
<td>1924</td>
</tr>
<tr>
<td>Iatrogenic (&lt;5% of cases)</td>
<td>1974</td>
</tr>
<tr>
<td>New variant</td>
<td>1996</td>
</tr>
<tr>
<td>Gerstmann-Sträussler-Scheinker disease</td>
<td>1938</td>
</tr>
<tr>
<td>Kuru</td>
<td>1957</td>
</tr>
<tr>
<td>Fatal familial insomnia</td>
<td>1986</td>
</tr>
</tbody>
</table>

* While generally percentages are as stated, these may vary from country to country

The precise nature of the TSE agent remains unknown despite intensive investigation. Although initially considered a ‘slow-virus’ (34), exhaustive efforts to detect such an agent have been unrewarding (64). Furthermore, a conspicuous lack of inflammatory response and the remarkable resistance of the infective pathogen to virucidal treatments, such as ultraviolet and ionising radiation, argue against a viral aetiology. Increasing evidence now suggests that a host-encoded protein, prion protein (PrP), plays a crucial role and constitutes a major component of the transmissible agent (79). The hypothetical infectious pathogen, termed the ‘prion’ (78), is thought to consist largely, if not wholly, of a ‘twisted’ form of normal PrP. The normal (cellular) form of PrP is termed PrP<sup>c</sup> and the twisted isoform found in the disease state is termed PrP<sup>Sc</sup> (the scrapie isoform). These two forms of the same protein have different chemical properties which allow the two to be distinguished, in particular, PrP<sup>Sc</sup> shows a greater resistance to proteinase K digestion. Normal PrP is a ubiquitous, membrane bound protein whose function is not well understood, but may be important for cellular resistance to oxidative stress by influencing the activity of Cu/Zn superoxide dismutase (11). The prion hypothesis states that once produced, PrP<sup>Sc</sup> promotes the conversion of more PrP<sup>c</sup> to PrP<sup>Sc</sup>. Thus, a chain reaction is set in motion with increasing amounts of PrP<sup>Sc</sup> being transformed into the pathological PrP<sup>Sc</sup> isoform. The mechanism by which PrP<sup>Sc</sup> causes tissue damage is still unclear. The prion theory may help to explain the central paradox of CJD, namely: how the disease can develop as an inherited, sporadic and infective disorder. It is suggested that the mutations associated with familial disease render the mutant PrP inherently unstable, with an increased tendency to twist into the PrP<sup>Sc</sup> isoform. In sporadic disease, the initial PrP<sup>Sc</sup> needed to seed the conversion of PrP<sup>c</sup> to PrP<sup>Sc</sup> could arise as a rare spontaneous event, perhaps due to a somatic mutation of the PrP gene in one or more cells. Finally, in the infective forms of disease, the inoculated PrP<sup>Sc</sup> would initiate the chain reaction of conversion of host PrP<sup>c</sup> to PrP<sup>Sc</sup>.
Although the prion hypothesis elegantly explains many of the observed phenomena of the TSE agent and diseases, it has not been conclusively proved, and the presence of multiple agent strains (15, 16), as observed in sheep scrapie, is arguably more compatible with a viral-like pathogen. A further theory, the 'virino hypothesis', combines elements of the prion and viral hypotheses and postulates that the infectious particle consists of an informational molecule containing nucleic acid that is closely associated with PrP (53).

Epidemiology and clinical features of sporadic, familial and iatrogenic Creutzfeldt-Jakob disease

Four aetiological groups of CJD have been described, namely: sporadic, familial, iatrogenic and nvCJD.

Sporadic Creutzfeldt-Jakob disease

Epidemiology

Sporadic CJD has an incidence of approximately 0.5-1.5 cases per million persons per year, a rate that is fairly consistent wherever in the world surveillance has occurred (93). The onset of disease typically occurs between the ages of 55 and 80 and is rare at the extremes of age (Fig. 1) (14); males and females are affected equally (14). Risk factors for the development of sporadic CJD have been investigated in multiple studies, but no dietary, occupational or past medical factors have been consistently implicated (90, 91).

Clinical features

The classical clinical triad of sporadic CJD is a rapidly progressive dementia, myoclonus and a periodic electroencephalogram (EEG). However, the disease phenotype is quite variable, and in practice, only approximately half of pathologically confirmed cases show all these features (13). Patients usually present with cognitive decline, ataxia, behavioural disturbance or visual symptoms, either alone or in combination (14). Dementia is invariably present during the course of the illness, and myoclonus, although a rare presenting feature, is observed at some stage in approximately 80% of cases. As the disease progresses, multi-focal CNS failure occurs with increasing global cognitive dysfunction, urinary incontinence, ataxia and dependency, culminating in the patient becoming bedbound, mute and unresponsive. Terminally, patients are usually rigid, often cortically blind and develop dysphagia, predisposing to aspiration and pneumonia, the most common cause of death. The median and mean duration of illness are 4.5 and 8 months, respectively, only 4% of cases survive longer than two years (14).

Familial prion disease

Genetic forms of human prion disease are associated with one of the twenty-three mutations identified to date of the PrP gene (located on the short arm of chromosome 20 and termed PRNP). Inheritance is autosomal dominant, with penetrance dependent upon the specific mutation, but usually almost complete in carriers who reach very old age. Hereditary disease strikes, on average, a decade earlier than sporadic CJD and can range in duration from a few months to over two decades (14, 77). Although the clinicopathological phenotype of familial CJD is often indistinguishable from sporadic CJD, many atypical patterns of illness have been described, such as
protracted psychiatric disturbance without dementia (83), and presentation with a spastic paraparesis (65).

Gerstmann-Sträussler-Scheinker disease (GSS) (80) and fatal familial insomnia (FFI) (18, 80) can be considered as variants of familial CJD, as both are transmissible neurodegenerative conditions associated with PRNP mutations. The clinical picture of GSS is characterised by progressive cerebellar ataxia and the pathological signs are multicentric amyloid plaques in the cerebellum. Patients with FFI typically present with severe insomnia and autonomic failure. More typical features of CJD appear as the disease progresses. The pathological hallmarks of FFI are marked thalamic gliosis with little or no cerebral spongiform change (74).

**Codon 129 influence on Creutzfeldt-Jakob disease**

At codon 129 of PRNP, a polymorphism exists, coding for either methionine (M) or valine (V). Of sporadic CJD cases, 71% are homozygous for methionine (MM), compared to 39% of controls (1). Cases of sporadic CJD with valine homozygosity tend to have a longer duration of illness, are more likely to present at a young age and only rarely have a periodic EEG (1). The importance of codon 129 genotype in new variant and iatrogenic CJD is discussed later.

**Iatrogenic Creutzfeldt-Jakob disease**

Approximately two hundred cases of iatrogenic CJD have been documented to date (Table II). The majority arose through the use of cadaver-derived human growth hormone (HGH), with the other causes being contaminated human gonadotropin, dura mater grafts, corneal transplants and neurosurgical instruments (10).

**Kuru**

Kuru is a TSE that was identified in the Fore-speaking people of Papua New Guinea. The disease causes a progressive cerebellar syndrome, progressing to total incapacitation and death, usually within three to nine months. The disease resulted from the practice of ritualistic cannibalism, which allowed conjunctival, nasal, skin, mucosal and gastrointestinal contamination with infectious brain tissue. Kuru has gradually been disappearing since cannibalistic rituals ceased, towards the end of the 1950s and, with the passage of time, progressively older age groups have become free of kuru.

**Diagnostic tests**

Diagnostic tests for nvCJD are discussed later in the paper.

**Routine blood tests**

Routine haematological and biochemical investigations, including inflammatory markers, are usually normal in CJD and other TSEs. In just over one-third of CJD cases, the liver function tests are mildly deranged (92), often in the form of transiently elevated transaminases. The reason for this is not known.

**Electroencephalography**

Approximately 60% of sporadic cases are reported to develop the characteristic appearance of 0.5-2 Hz generalised bi/triphasic periodic complexes, the remaining cases usually show only non-specific slow wave abnormalities (14) (Fig. 2).

**Cerebrospinal fluid**

The cerebrospinal fluid (CSF) of patients affected with CJD typically contains no inflammatory cells (14). An elevated protein concentration (0.5 g/l or greater) occurs in approximately one-third of cases (92). Although CSF has been shown to transmit disease (14), the pathological isoform of PrP has not been detected in CSF (or blood or serum) by currently available methods.

Recent reports indicate that the detection of the 14-3-3 protein (a ‘marker of neuronal death’), in the CSF, using Western blotting, is a technique which is both highly sensitive and specific for the diagnosis of sporadic CJD (55, 107). Revised clinical diagnostic criteria for sporadic CJD, incorporating a positive 14-3-3 CSF result have recently been published by the WHO (101).

**Table II**

**Summary of all proven or highly probable cases of iatrogenic Creutzfeldt-Jakob disease**

<table>
<thead>
<tr>
<th>Mode of infection</th>
<th>Number of patients</th>
<th>Entry of agent into brain</th>
<th>Mean incubation period (range)</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stereotactic electrodes</td>
<td>2</td>
<td>Intracerebral</td>
<td>18 months (16-20)</td>
<td>Dementia/cerebellar</td>
</tr>
<tr>
<td>Neurosurgery</td>
<td>4</td>
<td>Intracerebral</td>
<td>20 months (15-28)</td>
<td>Visual/dementia/cerebellar</td>
</tr>
<tr>
<td>Corneal transplant</td>
<td>3</td>
<td>Optic nerve</td>
<td>13 months (16 months-30 years)</td>
<td>Dementia/cerebellar</td>
</tr>
<tr>
<td>Dura matter graft</td>
<td>83</td>
<td>Cerebral surface</td>
<td>5.5 years (1.5-12)</td>
<td>Cerebellar (visual/dementia) (c)</td>
</tr>
<tr>
<td>Gonadotropin</td>
<td>4</td>
<td>Haematogenous</td>
<td>13 years (12-15)</td>
<td>Cerebellar</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>&gt;100</td>
<td>Haematogenous</td>
<td>12 years (5-30)</td>
<td>Cerebellar (b)</td>
</tr>
</tbody>
</table>

a) Median
b) Clinical information not available for all cases
c) Calculated from the midpoint of hormone therapy to the onset of symptoms of Creutzfeldt-Jakob disease

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Fig. 2
Typical period electroencephalogram as seen in sporadic Creutzfeldt-Jakob disease (top)
Electroencephalogram in new variant Creutzfeldt-Jakob disease does not show periodic complexes (bottom)
 Neuroimaging

The main role of neuroimaging in patients suspected of being affected with CJD has traditionally been to exclude other conditions. The results of computerised tomography (CT) are usually normal in sporadic CJD (37), but sometimes atrophy is found, especially in patients with a protracted illness (38). Magnetic resonance imaging (MRI) scans show increased signal in the basal ganglia on T2- and proton-density-weighted images in approximately 80% of sporadic cases (31). Fast fluid-attenuated inversion-recovery (FLAIR) and diffusion-weighted imaging, both relatively new MRI processes, can make these basal ganglia abnormalities more prominent.

 Neuropathology

Neuropathology has been the mainstay for diagnosis of human TSE for many decades. The characteristic neuropathological features include spongiform change, neuronal loss, reactive proliferation of astrocytes and microglia and the accumulation of PrPSc in the brain (4). Spongiform change is the term given to the characteristic vacuolation of the grey matter occurring in the TSE, usually in nerve cell processes, but occasionally in neuronal cell bodies (70). These vacuoles measure from 2 mm to 20 mm and can coalesce to form larger cyst-like cavities (Fig. 3a). The distribution of the pathological changes in TSE is highly variable both from one region of the brain to another, and between cases (6). Pathological phenotypic variation is a well-recognised phenomenon in sporadic, inherited and acquired forms of human TSE. In sporadic CJD, evidence suggests that the codon 129 PrP genotype and the PrP biochemical subtypes are major influences on the pattern of pathology in the brain (75).

The development of techniques to detect the accumulation of PrP in the brain represents a major advance in the diagnosis and study of TSE. The abnormal form of PrP accumulates in the CNS in all forms of TSE, and in human diseases a wide range of patterns of accumulation have been described, including perivascular, synaptic, neuronal and plaques (6). In some forms of human TSE, fibrillary amyloid plaques are visible in routinely stained sections of the brain. These amyloid plaques are particularly striking in kuru, and different forms of PrP amyloid deposits occur in familial TSE, particularly GSS, and in some forms of acquired human TSE, including CJD in growth hormone recipients. In sporadic CJD, amyloid plaques characteristically occur in individuals who are heterozygotes at PRNP codon 129 (75) (Fig. 3b). Immunocytochemistry shows strong labelling of these plaques and can demonstrate smaller plaque-like PrP deposits which are not detectable on routine stains. The marked variability in the pathology of human TSE necessitates comprehensive neuropathological study following autopsy. Consensus guidelines for autopsy protocols, tissue handling and storage in cases of CJD have been published, along with recommendations for diagnosis of human TSE by neuropathological techniques, including PrP immunocytochemistry (7, 19, 57).

The accumulation of PrP in the brain can also be demonstrated in unfixed tissue by biochemical techniques, usually by Western blotting (75). Using these techniques, the presence of disease-associated PrP can be demonstrated after enzymatic treatment of brain homogenate to remove any normal PrP, leaving the partially digested disease-associated isoform. Western blotting not only allows the confirmation of the presence of disease-associated PrP, but can also be used to study PrP subtypes in terms of differential protein glycosylation, and in terms of the molecular weight of the unglycosylated fragment after proteinase K digestion. This allows subclassification of PrP subtypes which appear to have an important influence in determining disease phenotype in sporadic CJD (76). Differential PrP subtypes may be related to different strain types of the transmissible agent, although this matter is still under investigation.

The differential pathological diagnosis of CJD is greatly facilitated by the use of PrP immunocytochemistry and Western blotting, since the accumulation of proteinase K-resistant PrP is specific for human TSE. Using conventional techniques, a wide range of differential diagnoses exist for human TSE, since spongiform change can occur in many other conditions, including Alzheimer's disease, Lewy body diseases, cerebral oedema and various metabolic disorders affecting the brain (6).

History of the identification of new variant Creutzfeldt-Jakob disease

In 1990, surveillance of CJD was re instituted in the UK in response to concern that BSE might be transmitted to humans and cause CJD. It was postulated that if this were to occur, detection may arise due to one of the following scenarios:

- a general increase in the incidence of CJD in the UK
- an increased occurrence of CJD in individuals at risk of occupational exposure to the BSE agent
- the emergence of a new clinicopathological phenotype of CJD.

The incidence of CJD in the UK has shown a general increase with time since surveillance was first instigated in the 1980s. However, similar trends have occurred in other countries in which BSE has not been reported, supporting the hypothesis that the increasing CJD incidence in the UK from 1980 to the mid-1990s was due to improved case ascertainment (23).
a) The cerebral cortex in sporadic CJD shows characteristic spongiform change in the neuropil of the grey matter.

Haematoxylin and eosin stain, x150

b) A rounded 'kuru-type' amyloid plaque (centre) is present in the cerebellar cortex in a case of sporadic CJD.

Haematoxylin and eosin stain, x200

c) A large florid plaque (centre) is present in the occipital cortex in a case of nvCJD, surrounded by spongiform change.

Haematoxylin and eosin stain, x200

d) The pulvinar in the thalamus in nvCJD shows widespread astrocytosis (astrocytes are stained brown).

Immunocytochemistry for glial fibrillary acidic protein, x200

e) Numerous clusters of PrP plaques (brown) are present in the occipital cortex in nvCJD.

Immunocytochemistry for PrP, x200

f) The tonsil in nvCJD shows PrP accumulation (brown) within germinal centres.

Immunocytochemistry for PrP, x40

Fig. 3
Pathological features of Creutzfeldt-Jakob disease
In the early to mid-1990s, an increased incidence of CJD was reported among dairy farmers, most of whom had worked on farms with cases of BSE. This led to much concern that BSE was the cause of occupationally acquired CJD in these farmers (44). However, none of these cases exhibited an unusual clinicopathological phenotype, and a similarly high incidence of CJD in dairy farmers was seen in other countries of Europe where BSE had never been reported or had a very low incidence (23). This suggested that there was an increased incidence of CJD in dairy farmers for reasons unrelated directly to BSE, such as ascertainment bias arising though the more vigorous investigation of farmers because of the concern of a connection between human and animal TSEs. Subsequent strain typing studies have failed to identify the BSE strain type as the cause of any of the cases of CJD in farm workers in the UK (17, 49).

In the latter half of 1995, staff at the UK National CJD Surveillance Unit (CJDSU) had become increasingly concerned about the number of referrals of young suspect cases of CJD. The identification of two young cases led to intensive review of the clinical and pathological features of previous young patients identified by the CJDSU and published cases from elsewhere (5, 10). No cases similar to the recent young patients were discovered. One patient who died aged sixteen in 1980 of possible CJD was of particular interest and neuropathology specimens were requested for review. Bearing in mind the possibility that previous young cases of CJD might have been mis-diagnosed, in December 1995, details of suspect but unconfirmed cases of subacute sclerosing panencephalitis (SSPE — a cause of young onset dementia) were reviewed through the co-operation of the SSPE register, although no suspect CJD cases were identified.

By January 1996, five cases of CJD in young people had been confirmed as exhibiting unusual neuropathological appearances, including plaque deposition. Review of the clinical features of the confirmed and suspect cases suggested a common clinical picture with early psychiatric symptoms, progressive ataxia, and a relatively prolonged duration of illness in comparison with previous experience of CJD. One possible explanation for the unusual clinicopathological phenotype was that the cases were hereditary forms of CJD; analysis of the PrP genotype in these cases therefore became a high priority.

By February 1996, ten cases of CJD in young people had been identified, eight of which had been neuropathologically confirmed. A crucial issue was whether similar cases were being identified in other countries and in late February, countries collaborating in the concerted study of CJD in Europe (France, Germany, Italy, the Netherlands and Slovakia) were asked to send details of all cases of CJD in people aged less than forty-five years. Seven such cases were identified but only one case, in France, suggested a possibility of a similar phenotype, and in this case neuropathological information was not yet available. On 7 March 1996, details of full PRNP sequencing became available in three of the cases which had occurred in the UK, revealing no evidence of a mutation. It had already been established that no mutation had occurred in the two initial teenage cases and one further young case. Detailed information on putative risk factors for CJD was available in eight cases and none had a history of potential iatrogenic exposure. No explanation for the occurrence of this ‘cluster’ of young cases was apparent, raising the possibility of a causal link with BSE. Details of the cases were presented at the UK Spongiform Encephalopathy Advisory Committee on 8 March 1996 and one recommendation was that the clinical and pathological features of these patients should be discussed with independent experts.

In the following week, discussions with senior neurologists and neuropathologists provided support for the hypothetical novelty of these cases and two further cases were confirmed neuropathologically. Review of the neuropathology of a case of CJD in a sixteen-year-old from the UK, who died in 1980, revealed an appearance typical of sporadic CJD and review of the clinical and pathological features of previous cases in patients aged less than forty-five, in the UK (and one case from Poland) showed distinct differences from the recently identified cases in young people. In summary, between March 1995 and 20 March 1996, ten unusual cases of CJD were identified in the UK and investigations suggested the following:

- the cases shared a common and unusual clinical phenotype
- the neuropathological appearances were similar in all cases and probably novel
- no known risk factor, including PRNP mutations, existed in any of the cases
- no linking factor, such as occupational exposure, existed between the cases
- the cases were occurring only in the UK.

The identification of these cases by the CJDSU was announced in the UK Parliament on 20 March, and details of the cases were published in the Lancet on 6 April 1996 in an article entitled 'A new variant of Creutzfeldt-Jakob disease in the United Kingdom' (94).

Up to the end of December 1999, a total of forty-nine deaths fromnvCJD have been recorded in the UK, forty-seven of these were confirmed neuropathologically, and two were clinically probable cases who died but did not undergo pathological examination.
Clinical and investigative features of new variant Creutzfeldt-Jakob disease

Detailed clinical and investigative features have been published regarding the first fourteen cases of nvCJD identified in the UK (102, 104) and the first case in France (20). More recent articles describe the psychiatric features of thirty-three cases (95) and the pathology of the disease (56). Further papers reporting clinical diagnostic criteria (96) and MRI imaging (106) are in press at the time of writing.

Early features

The two striking early features of nvCJD are psychiatric and sensory disturbance, both of which are relatively unusual in sporadic CJD.

In a review of the psychiatric features of thirty-three cases of nvCJD (Table III), all but one exhibited psychiatric symptoms in the early stages of the illness, and in most, these were the first manifestation of the illness (95). The single case without definite psychiatric symptoms was emotionally labile for some months prior to the onset of neurological symptoms and signs. The psychiatric symptoms initially occurred in isolation in the majority of cases, but in four cases, forgetfulness was reported at the onset, in addition to the psychiatric symptoms.

Table III
Psychiatric features of new variant Creutzfeldt-Jakob disease
(n = 33)

<table>
<thead>
<tr>
<th>Psychiatric symptom</th>
<th>Number of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression</td>
<td>16</td>
</tr>
<tr>
<td>Anxiety</td>
<td>17</td>
</tr>
<tr>
<td>Withdrawal</td>
<td>18</td>
</tr>
<tr>
<td>Aggression/irritability</td>
<td>18</td>
</tr>
<tr>
<td>First rank symptoms</td>
<td>5</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>17</td>
</tr>
<tr>
<td>Delusions</td>
<td>18</td>
</tr>
<tr>
<td>Forgetfulness at onset</td>
<td>4</td>
</tr>
<tr>
<td>Suicidal ideation</td>
<td>3</td>
</tr>
</tbody>
</table>

In seventeen of the thirty-three cases, pain in the limbs or joints, or painful or unpleasant paraesthesia or dysesthesia was present. The sensory symptoms usually occurred in combination with psychiatric disturbance, prior to the development of other neurological symptoms and signs some months later. In six of the thirty-three cases, the painful limb or sensory symptoms were present from the onset, and in all of the seventeen cases, the pain or sensory symptoms persisted for weeks or months. Five of the first fourteen patients underwent either electromyography or nerve conduction studies, or both. These were normal in four cases and abnormal in one, which showed minor changes of uncertain significance. The sensory symptoms are therefore presumed to be of central origin, perhaps thalamic. Other early neurological symptoms during the 'psychiatric' phase included gait imbalance and, in a small minority of cases, blurred or double vision, dysarthria, dysgeusia, involuntary movements, or dysgraphia. A few patients stopped driving, some after road traffic accidents.

Seventeen of the thirty-three patients suffered visual or auditory hallucinations and five exhibited first rank schizophrenic symptoms. Aggression and irritability were prominent features in eighteen cases, and in a minority, this was associated with tantrums or aggressive outbursts, including a small minority with violent behaviour. Two cases exhibited transient suicidal ideation and one patient wrote notes indicating suicidal intent.

Fleeting delusions were noted as an unusual feature in the original description of the psychiatric features of the first fourteen patients (102). Of the subsequent nineteen cases, eight described fleeting delusions, including beliefs that a child was missing, that they were pregnant and that they were possessed by the devil. In two cases, the delusions became persistent. The psychiatric diagnosis in the majority of cases was depression, although in some cases the possibility of an 'organic' basis was suspected clinically, particularly in those cases with associated forgetfulness. In a small number of cases, a psychotic illness was suspected, and in two cases, the psychiatric symptoms were thought to be hysterical or functional. The psychiatric treatment given to patients reflected these diagnoses: nineteen of the thirty-three patients received anti-depressants, often initially prescribed by the general practitioner, five received antipsychotic medication and one was treated with electroconvulsive therapy. Ten patients received no specific psychiatric treatment.

Clinical course

The following paragraphs refer to the clinical course reported for the first fourteen cases of nvCJD in the UK (104). The subsequent cases have shown similar features.

Although a minority suffered from forgetfulness or mild unsteadiness of gait from an early stage, frank neurological signs were not apparent for many months after disease onset in any of the cases (median: 6.25 months; range: 4-24.5 months; Table IV). After the onset of frank neurological dysfunction, mainly in the form of ataxia, the illness rapidly progressed with global cognitive impairment, involuntary movements, incontinence of urine and progressive immobility, leading to increasing dependency, unresponsiveness and mutism. Pre-terminally, the patients were usually akinetic mute and at least three developed cortical blindness. Dysphagia usually developed, predisposing to aspiration and pneumonia, the most frequent terminal
Table IV
New variant Creutzfeldt-Jakob disease: months (median) to clinical milestones (n = 14)

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>Time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forgetfulness</td>
<td>5.0</td>
</tr>
<tr>
<td>Ataxia</td>
<td>5.5</td>
</tr>
<tr>
<td>Psychiatrist consulted</td>
<td>5.5</td>
</tr>
<tr>
<td>Neurological signs</td>
<td>6.5</td>
</tr>
<tr>
<td>Neurologist consulted</td>
<td>6.8</td>
</tr>
<tr>
<td>Myoclonus/chorea</td>
<td>8.3</td>
</tr>
<tr>
<td>Mutism</td>
<td>10.5</td>
</tr>
<tr>
<td>Bedbound</td>
<td>11.0</td>
</tr>
<tr>
<td>Death</td>
<td>14.0</td>
</tr>
</tbody>
</table>

The median delay from developing unsteadiness to becoming bedbound was five months (range: 2.5-12.5 months), and from bedbound to death, 1.5 months (range: 1 week-21 months). Most patients had minor fluctuations in the clinical course, with cognitive or neurological dysfunction varying over a few hours or days, often related to a change of medication or infective episode.

The terminal stage, after the development of progressive cognitive impairment and involuntary movements, was similar to the late stages of sporadic CJD. The median total clinical duration of illness was 14 months (range: 9.5-38 months; Table IV).

Clinical signs
The first neurological sign was cerebellar (limb or gait ataxia) in nine cases. This occurred in isolation in three cases, or in combination with either involuntary movements, pyramidal signs, primitive reflexes, or sensory signs, in the others. The remaining cases first developed either pyramidal signs, dysarthria, or involuntary movements, although unsteadiness of gait was noted within weeks even in these cases. Those with the longest delay to the development of neurological signs had a long prodrome with mild personality change or forgetfulness followed by sensory disturbance. The majority of cases developed primitive reflexes, cerebellar and pyramidal signs. All fourteen patients developed persistent involuntary movements, initially chorea (seven cases) or myoclonus (seven cases), and five of the seven patients who initially had chorea were later noted to also have myoclonus. Although seven cases were not formally noted to have chorea, four of these were described as 'fidgety'. Tremor was an early feature in four cases, and dystonic movements were noted during the clinical course in five. Seven cases were noted to have upgaze paresis, an uncommon feature of sporadic CJD, after the development of other focal neurological signs.

Investigations

Electroencephalogram
Each of the fourteen cases underwent electroencephalograph on multiple occasions (two to five times). The characteristic periodic periodic EEG pattern associated with sporadic CJD was not reported (Fig. 2), even though four patients underwent recordings in the final month of illness (104). Interestingly, in kuru and HGH-related CJD, conditions similar to nvCJD which are peripherally acquired, the EEG is also non-periodic.

Cerebrospinal fluid analysis
All of the fourteen cases initially reported underwent CSF analysis. A leukocyte response was not recorded in any of the cases. Four patients had slightly raised CSF protein concentration (0.5-0.9 g/l), but in nine patients the level was normal. The 14-3-3 protein analysis was positive in only two of the five cases reported (104). A report of an analysis of the CSF markers, 14-3-3 protein, neuron-specific enolase and tau protein is in press (46).

Neuroimaging
Ten of the first fourteen nvCJD cases in the UK underwent brain CT. Eight patients were reported to have normal scans and two showed nonspecific minor abnormalities, probably of no significance.

Three early cases were noted to have posterior thalamic (pulvinar) high signal on MRI scanning (Fig. 4) (36, 54, 104). This prompted the review of MRI scans from cases of nvCJD, revealing that twenty-eight had definite bilateral pulvinar high signal, an abnormality not seen in any of the control group (106).

Abnormal areas of cerebral perfusion were detected in both cases that were subjected to single-photon emission CT studies (27). A single patient underwent positron emission tomography, the results of which were normal (54).

Genetic analysis
Sequencing of the open reading frame of PRNP identified no mutations in the nvCJD cases screened to date. All of these cases have the MM genotype at codon 129, thereby differing significantly from controls (39%) and sporadic CJD (79%) (1). The reason for this is interesting to postulate. The ability to transmit a TSE between species appears to be determined, in part, by the homology of the central residues of the PrP structures of the two species (which includes residue 129) (87). Recent studies suggest that, unlike humans, bovines only code for methionine at the equivalent site to the human codon 129 polymorphism (84). Therefore, if nvCJD is due to infection with the BSE agent, and bovine PrP is usually, if not always, homozygous for methionine at codon 129, the MM genotype in humans may confer an increased susceptibility to bovine prions (103). Even if these assumptions are correct, it would be premature to conclude that individuals possessing the codon 129 genotypes MV or VV cannot develop nvCJD.
polymorphism of the ovine PrP gene at codon 136 has been shown to influence incubation period after experimental exposure to BSE (43), and in HGH-related CJD, codon 129 genotype influences incubation period (28). Therefore, should further cases of nvCJD occur, patients with alternative codon 129 genotypes could possibly be affected, but these cases may have relatively prolonged incubation periods.

Tonsil biopsy

The abnormal isoform of PrP has been detected in lymphoid follicles in palatine tonsil biopsies taken from sheep with a genetic susceptibility to scrapie. The animals studied normally develop scrapie aged twenty-five months, but biopsies taken from six asymptomatic sheep approximately ten months after birth were positive for the abnormal PrP isoform (85). This prompted a study of palatine tonsillar tissues in human TSEs. Positive results were first demonstrated in tissue taken at necropsy from a nvCJD case (50). A subsequent analysis of various lymphoid tissues, including palatine tonsil, obtained at necropsy, failed to identify abnormal PrP in a small number of cases of GSS and sporadic CJD (63). A more recent and larger study of sporadic and nvCJD cases has confirmed the previous findings in ante- and post-mortem tonsillar tissue (51). These results suggest that ante-mortem tonsil biopsy may be useful as a presymptomatic diagnostic test for nvCJD, but not for other forms of CJD. The use of this procedure in patients suspected to have CJD is controversial, and tonsil biopsy should be considered in the appropriate clinical context, for example, in cases with possible nvCJD without evidence of bilateral pulvinar high signal on MRI brain scanning (105).

Neuropathology

Neuropathological studies were of paramount importance in identifying nvCJD as a distinct entity. In contrast to the pathological variations that occur between cases of sporadic, acquired and familial TSE, the neuropathology of nvCJD is relatively stereotyped, although variations in the severity of the pathology occur from case to case. The diagnostic neuropathological features of this disorder are summarised in Figure 5. The most striking abnormality is the presence of large amyloid plaques in the brain which occur in the cerebral and cerebellar cortex, particularly in the occipital cortex. These plaques are large, fibrillary and surrounded by a rim or corona of spongiform change (Fig. 3c). The plaques resemble the florid plaques first described in experimental transmission of Icelandic scrapie into mice (33), and which also occur in several other unrelated TSEs, including chronic wasting disease in white-tailed deer (Odocoileus virginianus) (97). Spongiform change in nvCJD is variable in distribution and frequently occurs as a patchy abnormality in the cerebral cortex, often at the base of gyri, and in a random distribution within the cerebellar cortex. However, spongiform change is most conspicuous in the basal ganglia, particularly the caudate nucleus and putamen. In the thalamus, the principal neuropathological abnormality is severe neuronal loss and astrocytosis in the posterior nuclei, particularly the pulvinar (Fig. 3d). This neuropathological abnormality is the likely substrate for the abnormal MRI signal identified in this region of the brain in vivo. A similar pattern of astrocytosis occurs in the midbrain in the periaqueductal grey matter, the distribution of which is consistent with the abnormalities of eye movements frequently described in patients with nvCJD.
However, the most striking abnormality in nvCJD is the massive accumulation of disease-associated PrP in a distribution unlike any other human TSE. This not only involves the large florid plaques, but occurs as multiple small plaques which are frequently clustered together in the neuropil (Fig. 3e). In addition, amorphous pericellular and perivascular deposits of PrP occur throughout the cerebellar cortex and in the cerebral cortex. In the basal thalamus and ganglia, a different pattern of PrP accumulation occurs; PrP is present in neurones and around axons in the basal ganglia and a synaptic pattern of staining is seen in the thalamus with only a few plaques identified. PrP also accumulates in the brain stem and in the grey matter of the spinal cord in a perineuronal and synaptic distribution, and has been identified outside the CNS in dorsal root and trigeminal ganglia. Disease-associated PrP also occurs in lymphoid tissues throughout the body in nvCJD, localising in follicular dendritic cells within germinal centres in the tonsil (Fig. 3f), lymph nodes, spleen and gut-associated lymphoid tissue (see below).

Another unique feature of nvCJD is the appearance of the disease-associated form of PrP in Western blotting studies, where a predominance of the diglycosylated form of the protein is detected. This is the consistent finding in all cases tested, and in all affected brain regions. This pattern of PrP glycosylation is different from other forms of human TSE, but resembles the PrP glycosylation pattern in BSE and related disorders (see below). Similar biochemical findings have been demonstrated in Western blotting studies for PrP in the tonsil and other lymphoid tissues (see below).

The quantitative and qualitative differences between the neuropathology of nvCJD and sporadic CJD have been studied in considerable detail using both subjective (observer) analysis and semi-automated image analysis techniques. These techniques confirm the subjective impressions that the neuropathology of nvCJD is clearly distinct from other human prion diseases (58). Review of CJD cases in many other countries across the world has not revealed any cases with the classical neuropathological features of nvCJD outside the UK, with the exception of two cases of nvCJD reported in France and one in the Republic of Ireland.

### Epidemiology of new variant Creutzfeldt-Jakob disease

#### Age distribution and sex

The mean age at onset of the fifty-two cases of nvCJD identified up to the end of December 1999 was twenty-nine years (range 16-52 years) compared to sixty-five years for sporadic CJD (Fig. 1). Twenty-three cases were male. The reason for the relatively young age of the nvCJD cases is unknown. One hypothesis is that young people had a greater exposure to the BSE agent due to diet. Which foods presented the highest infective risk is unknown, but it has been speculated that those containing mechanically recovered meat (MRM) are likely contenders. These would include some types of beefburgers, sausages and meat pies. British dietary surveys of age-related eating habits have shown that beefburger consumption markedly declines with age (45), as does the intake of meat pies and pastries, albeit to a much lesser extent. However, no significant age-related change has been recorded in consumption of sausages. Explanation of the age distribution of nvCJD by diet alone is problematic, as the age-related exposure to the likely relevant meat products does not appear sufficiently marked to explain the complete absence of cases in persons over the age of fifty-five.

Other hypotheses to explain the young age of the nvCJD cases include a failure of case ascertainment in the elderly, infection acquired through childhood vaccination and a shorter incubation period of the BSE agent in young people. However, none of these theories are supported by the available data. A genuine age-related susceptibility to the BSE agent therefore appears to exist. The reason for this can only be speculated upon. One suggestion is that the gastrointestinal lymphoreticular system plays an important role in facilitating entry of infection, and that with increasing age, changes in these tissues hinder invasion of the transmissible agent.

#### Geographical distribution of new variant Creutzfeldt-Jakob disease in Great Britain

An analysis performed on the thirty-four cases of nvCJD identified in Great Britain up to 31 December 1998 showed no evidence of geographical clustering (Fig. 6). An earlier study of the twenty-six cases of nvCJD identified in Great Britain up to 31 August 1998 failed to identify any clustering of the residences of patients in proximity to rendering plants during the 1980s (24).

The analyses below are based on data from thirty-five cases of nvCJD and twenty-five age- and sex-matched hospital controls (73).

#### Medical risk factors

Two cases had a definite history of blood transfusion. One had received blood following a road traffic accident some eighteen years before disease onset, while the second had received an exchange transfusion at birth, twenty-four years before disease onset. A further case might have received a transfusion during a hysterectomy approximately one year prior to disease onset. Three of the controls had also received blood transfusions.

Fourteen of the cases had no history of any operation or surgical procedure (other than dental procedures) compared with five of the controls. Therefore no evidence exists to suggest that cases were more likely than controls to have undergone operations in the past. However, this finding should be interpreted with some caution, because of the
potential bias associated with the use of a control group chosen from hospital patients.

Dietary risk factors for new variant Creutzfeldt-Jakob disease

The infective dose required to transmit BSE orally to humans is unknown, but it is noteworthy that lemurs showed evidence of infection after being fed as little as 0.5 g of BSE-infected bovine brain (8).

The reported consumption of various types of meat by cases and controls after 1985 is shown in Table V. One case was excluded from the analysis because consumption of meat and meat products after 1985 was unknown.

Table V
Results of a comparison between cases of new variant Creutzfeldt-Jakob disease and controls with regard to consumption of different types of meat after 1985

<table>
<thead>
<tr>
<th>Type of meat</th>
<th>Percentage of cases (n=34)</th>
<th>Percentage of controls (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamb</td>
<td>91</td>
<td>88</td>
</tr>
<tr>
<td>Pork</td>
<td>94</td>
<td>92</td>
</tr>
<tr>
<td>Beef</td>
<td>97</td>
<td>96</td>
</tr>
<tr>
<td>Venison</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Veal</td>
<td>18</td>
<td>28</td>
</tr>
<tr>
<td>Poultry</td>
<td>97</td>
<td>96</td>
</tr>
</tbody>
</table>

Almost all cases and controls had eaten lamb, pork, beef or poultry since 1985. Eight cases and six controls were reported to have eaten venison since 1985, while six cases and seven controls had eaten veal. Of the thirty-two cases and twenty-four controls who had eaten beef since 1985, cases were reported to have consumed beef more frequently than the controls (test for trend: p = 0.02) (Table VI). This finding may be due to recall bias, as previously reported for results from a UK case-control dietary study of sporadic CJD. No evidence was found to suggest that cases consumed lamb, pork, venison or poultry more frequently than controls (p > 0.10).

Table VI
Frequency of consumption of beef, since 1985, by cases of new variant Creutzfeldt-Jakob disease and controls

<table>
<thead>
<tr>
<th>Frequency of beef consumption</th>
<th>Percentage of cases (n=32)</th>
<th>Percentage of controls (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once a month</td>
<td>9</td>
<td>38</td>
</tr>
<tr>
<td>Once a month or more</td>
<td>19</td>
<td>29</td>
</tr>
<tr>
<td>Once a week or more</td>
<td>72</td>
<td>33</td>
</tr>
</tbody>
</table>

Most cases and controls had eaten sausages, liver, puddings, burgers and meat pies since 1985 (Table VII). Approximately one-third to a half of cases and controls were reported to have eaten kidneys, haggis and faggots. No cases or controls were reported to have ever eaten eyes after 1985, while only one control was reported to have tried 'a spoonful' of brains on one occasion, in Italy, in the mid-1980s. Only one case (post 1985) and one control (prior to 1985) were recorded as having eaten sweetbreads. No striking differences were detected between the proportions of cases and controls eating any of these items.

A potential route through which bovine brain and spinal cord may have entered the human food supply is through MRM. Data on the frequency of consumption of three of the food...
Table VII
Results of a comparison between cases of new variant Creutzfeldt-Jakob disease and controls with regard to consumption of various animal products, since 1985

<table>
<thead>
<tr>
<th>Types of animal products consumed</th>
<th>Percentage of cases (n=34)</th>
<th>Percentage of controls (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sausages</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>Liver</td>
<td>59</td>
<td>84</td>
</tr>
<tr>
<td>Kidney</td>
<td>32</td>
<td>42</td>
</tr>
<tr>
<td>Meat puddings</td>
<td>59</td>
<td>48</td>
</tr>
<tr>
<td>Haggis</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>Burgers</td>
<td>94</td>
<td>83</td>
</tr>
<tr>
<td>Meat pies</td>
<td>92</td>
<td>83</td>
</tr>
<tr>
<td>Faggots</td>
<td>38</td>
<td>29</td>
</tr>
</tbody>
</table>

items listed above which might contain MRM (sausages, burgers and meat pies) were combined to provide an estimate of the frequency with which cases might have been exposed to MRM in the period since 1985 (Table VIII). Where the frequency of consumption of one or more of these food items was unknown, the modal consumption of that food item amongst the other cases (or controls for the control group) was taken. This analysis does not indicate any major differences in consumption of these food items between cases and controls. However, a case-control study based on such a small number of cases, and reliant on dietary histories obtained from the relatives of the patients is rather a weak tool to demonstrate a dietary cause of nvCJD. Dietary exposure to the BSE agent during the 1980s is still considered the most likely reason for the occurrence of nvCJD, in the absence of another more plausible hypothesis.

Table VIII
Comparison of new variant Creutzfeldt-Jakob disease cases and controls with respect to the frequency of consumption of sausages, burgers or meat pies, since 1985

<table>
<thead>
<tr>
<th>Frequency of consumption of sausages, burgers or meat pies</th>
<th>Percentage of cases (n=34)</th>
<th>Percentage of controls (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once a month</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Several times a month</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>More than once a week</td>
<td>79</td>
<td>83</td>
</tr>
</tbody>
</table>

Occupation and new variant Creutzfeldt-Jakob disease
Table IX presents a list of occupations for cases and controls. Both cases and controls had experience of a wide range of occupations, without any occupation being particularly common among cases. Therefore, no obvious evidence exists of an occupational risk for nvCJD.

Table IX
List of lifetime occupations for thirty-five cases of new variant Creutzfeldt-Jakob disease and twenty-five controls

<table>
<thead>
<tr>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clerical worker (x 9)</td>
<td>Clerical worker (x 8)</td>
</tr>
<tr>
<td>Catering worker (x 9)</td>
<td>Catering worker (x 5)</td>
</tr>
<tr>
<td>Shop assistant/retail worker (x 8)</td>
<td>Shop assistant/retail worker (x 5)</td>
</tr>
<tr>
<td>Factory worker (x 5)</td>
<td>Factory worker (x 2)</td>
</tr>
<tr>
<td>Engineer (x 3)</td>
<td>Engineer (x 3)</td>
</tr>
<tr>
<td>Nurse (x 3)</td>
<td>Hairdresser (x 2)</td>
</tr>
<tr>
<td>Bar worker (x 3)</td>
<td>Dental nurse</td>
</tr>
<tr>
<td>Cleaner/domestic (x 2)</td>
<td>Cleaner/domestic</td>
</tr>
<tr>
<td>Computing (x 2)</td>
<td>Chicken factory worker</td>
</tr>
<tr>
<td>Horticulture (x 2)</td>
<td>Management worker</td>
</tr>
<tr>
<td>Cable layer (x 2)</td>
<td>Bar worker</td>
</tr>
<tr>
<td>Kennels (x 2)</td>
<td>Miner</td>
</tr>
<tr>
<td>Driver (x 2)</td>
<td>Fireman</td>
</tr>
<tr>
<td>Hairdresser</td>
<td>Fairground worker</td>
</tr>
<tr>
<td>Stable hand</td>
<td>Performer</td>
</tr>
<tr>
<td>Solicitor</td>
<td>Road layer</td>
</tr>
<tr>
<td>Debt collector</td>
<td>Labouwer</td>
</tr>
<tr>
<td>Launderette worker</td>
<td>Bottling worker</td>
</tr>
<tr>
<td>Forestry worker</td>
<td>Drivers/porter</td>
</tr>
<tr>
<td>Royal Air Force policeman</td>
<td>Painter/Decorator</td>
</tr>
<tr>
<td>Energy industry</td>
<td>Sewing machinist</td>
</tr>
<tr>
<td>Newspaper delivery worker</td>
<td>Care attendant</td>
</tr>
<tr>
<td>Coastguard</td>
<td>Toolmaker</td>
</tr>
<tr>
<td>Plumber</td>
<td>Printing worker</td>
</tr>
<tr>
<td>Milkman</td>
<td>Roofer</td>
</tr>
<tr>
<td>Dental assistant</td>
<td>Technical writer</td>
</tr>
<tr>
<td>Receptionist</td>
<td>Translator</td>
</tr>
<tr>
<td>Painting models</td>
<td>Interviewer</td>
</tr>
<tr>
<td>Pet shop worker</td>
<td>French polisher</td>
</tr>
<tr>
<td>Dustman</td>
<td>Livestock farmer</td>
</tr>
<tr>
<td>Naval radio operator</td>
<td></td>
</tr>
<tr>
<td>Labourer</td>
<td></td>
</tr>
<tr>
<td>Butcher</td>
<td></td>
</tr>
<tr>
<td>Metal cutter</td>
<td></td>
</tr>
<tr>
<td>Paper recycling worker</td>
<td></td>
</tr>
<tr>
<td>Draught exclusion worker</td>
<td></td>
</tr>
</tbody>
</table>

Evidence of a link between new variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy

Temporo-spatial association
To date, all but two of the cases of nvCJD reported had been UK residents. Over 99% of cases of BSE have occurred in the UK, therefore providing a strong spatial link with nvCJD. Two patients with nvCJD had never visited the UK and were
residents in France, a country with an extremely small number of cases of BSE relative to the UK. However, the occurrence of this case is not incompatible with the hypothesis that the BSE agent is the source of nvCJD, as France was a major importer of bovine products from the UK.

Figure 7 shows the temporal association between the occurrence of BSE and nvCJD. Exposure of the human population to the BSE agent in the UK is likely to have been greatest in the 1980s, especially towards the end of that decade, before the introduction of a ban on the use of specified bovine offal. This would be consistent with an incubation period of between six and twelve years for the first cases of nvCJD, a range entirely compatible with the known incubation period of other TSEs.

**Biological strain type: transmission characteristics in mice**

The most persuasive experimental evidence to suggest that nvCJD is due to the BSE agent is supplied by the well established technique of biological strain typing. The 'strain' of a TSE can be defined by characteristic features when transmitted to mice. For example, when inoculated into genetically similar mice, a particular scrapie strain leads to a consistent incubation period and pattern of neuropathology. Using a range of genetically distinct mice allows a 'strain profile' based on incubation period and neuropathological distribution of lesions to be produced. Such experiments have shown that scrapie exists as over twenty distinct strains, whereas BSE is due to a single major strain of agent which is distinct from those of scrapie. Furthermore, the transmission characteristics of feline spongiform encephalopathy, the novel spongiform encephalopathies of greater kudu (Tragelaphus strepsiceros) and nyala (T. angasii), and experimental transmissions of the BSE agent to sheep, goats and pigs, resemble those of the BSE agent, and thus provide supportive evidence for a causative association between BSE and the new spongiform encephalopathies affecting captive wild ruminants and domestic and wild cats (16). Similar strain typing experiments performed subsequently for sporadic CJD and nvCJD have shown that the nvCJD agent has the strain characteristics of the BSE agent and that these characteristics differ from those of the limited number of sporadic CJD cases tested (17).

**Molecular strain type**

Proponents of the protein-only theory have hypothesised that the phenotypic variability observed in TSEs is determined by the three-dimensional structure and glycosylation of disease-associated PrP. These structural variations are reflected in different Western blotting patterns, which are used as markers of the 'molecular strain'. New variant CJD has a characteristic molecular strain, not seen in any other form of CJD, but observed in cattle with BSE and in other animal species, including monkeys, either experimentally or naturally infected by BSE, thus providing further evidence that nvCJD is due to the BSE agent (21).

**Transmission of bovine spongiform encephalopathy to primates**

Three cynomolgus macaques (Macaca fascicularis), inoculated by the intracerebral route with BSE brain homogenate, developed a spongiform encephalopathy with neuropathological features similar to nvCJD, in particular the distribution of spongiform change and the typical morphology of the plaques (68). Although this supports the
hypothesis that nvCJD is caused by BSE, the significance of the study is tempered by the results of transmission studies of BSE in common marmosets (Callithrix jacchus), which did not show typical florid plaques (3).

Factors determining the future number of cases of new variant Creutzfeldt-Jakob disease

Estimation of the future number of cases of nvCJD is hindered by a number of unknown variables including the following:
- the size of the 'species barrier' between humans and bovines
- the length of the incubation period of the BSE agent in humans infected via the oral route
- the level of dose required to cause infection by the oral route
- the influence of genetic factors, both of PRNP and other genes
- the proportion of the population exposed to the BSE agent through diet
- whether secondary iatrogenic cases will occur
- whether maternal transmission will occur.

The species barrier

The existence of a 'barrier' to transmission of a TSE between species is well documented. This results in a smaller infective dose being required and disease occurring more quickly when a TSE is transmitted between animals of species A compared to transmission from species B to species A. The size of the species barrier between bovines and humans has implications for the susceptibility of humans to the BSE agent. Although the bovine/bovine species barrier cannot be measured directly, techniques are available to aid assessment of this possibility. A major determinant of the species barrier is thought to be the ease with which donor and host PrP molecules interact. This is in turn related to the similarity of the PrP amino acid structure in the donor and the host. Different species have different PRNP sequences and hence different PrP structure. The efficiency of in vitro PrP interaction between different species has been investigated by Raymond et al., who found that the conversion of normal human PrP by the BSE agent was much less efficient than the conversion of normal bovine PrP by the BSE agent (81).

In vivo transmission studies have shown that sheep with the A136 R171 PrP genotype and hamsters seemed to be resistant to BSE. The conversion of normal ovine A136 R171 PrP and hamster PrP by the BSE agent was found to be less efficient than the conversion of normal human PrP by the BSE agent. These results imply that the species barrier between bovines and humans is considerable, but is smaller than that between bovines and certain animals that appear to be resistant to BSE.

Another model to assess the susceptibility of humans to the BSE agent used 'humanised' transgenic mice. The PrP gene of these mice is replaced by human PRNP (48). Since PRNP is thought to be an important determinant of the species barrier, this allows scientists to use mice as surrogates for testing human susceptibility to the BSE agent. When inoculated with the BSE agent, the humanised transgenic mice were shown to be clinically affected with a neurodegenerative disease, but after a much longer incubation period and with a lower incidence of successful transmission than when inoculated with the sporadic CJD agent (48). This suggests human susceptibility to the BSE agent but with a considerable species barrier. However, this reassurance should be interpreted with caution, as the humanised transgenic mice were homozygous for valine at codon 129, in contrast to all the cases of nvCJD tested to date which are methionine homozygotes. If the experiment had used transgenic mice homozygous for methionine at codon 129, the mice may have shown greater susceptibility to BSE.

Incubation period of the bovine spongiform encephalopathy agent in humans

Although the incubation period of the BSE agent in humans is unknown, estimates have been made, based on information from studies of human and animal TSEs. Perhaps the most comparable disease to nvCJD is kuru, as the oral route is assumed to be an important route of infection in both conditions. The average incubation period of kuru has not been established, but the youngest patient was aged four years at onset of symptoms and cases have been described over thirty-five years after the cessation of cannibalism (69). It is also helpful to consider HGH-related iatrogenic CJD when assessing the possible incubation period for the BSE agent in humans, as this is also a peripherally acquired TSE. The average incubation period for HGH-related CJD is estimated to be thirteen years (P. Brown, personal communication), although this may be an underestimate, as cases with longer incubation periods may yet appear. The average incubation period of the BSE agent in humans may be reasonably assumed to exceed this figure because of the species barrier effect. However, other unknown factors will also affect the incubation period, in particular the dose of the agent, route of exposure and possible, as yet unidentified, genetic factors. These factors make it difficult to draw any firm conclusions as to the average incubation period of the BSE agent in humans.

Estimation of the size of the potential new variant Creutzfeldt-Jakob disease epidemic

Three studies have attempted to predict the size of the nvCJD epidemic, taking into account various assumptions relating to key unknown variables. Two of the studies concluded that the size of the epidemic was not possible to predict accurately, with estimates ranging from as low as less than ten to as high as ten million cases (22, 39). However, the third study
predicted that the number of cases was not likely to exceed a few hundred, and most likely would be a hundred or less (88). The authors concur with the conclusions of the former two studies, that the size of the nvCJD epidemic is not possible to predict accurately and that sufficient data to draw any firmer conclusions is not likely to be available for several years.

Treatment

No treatment has been proven to halt the course of any form of CJD, although a number of specific therapies have been tested including magnesium, amantadine, interferon and other antiviral agents. Steroids, acyclovir or thiamine are frequently administered in the hope that the patients may have an occult, treatable condition such as a cerebral vasculitis, viral infection or Wernicke’s encephalopathy. None of these therapies have an appreciable effect on CJD. Amphotericin B (an antifungal drug), and doxorubicin (an anti-cancer agent), have been found to delay death in hamsters or mice experimentally infected with scrapie. However, these drugs are potentially toxic and need to be injected around the time of infection, or shortly afterwards, to be most effective. Treatment with amphotericin B has been attempted in human CJD without effect. Prophylactic administration of Congo red (a sulphonated amyloid-binding dye commonly used as a histological stain for amyloids) before or shortly after experimental scrapie infection, can significantly delay the onset of clinical disease in hamsters. This compound has also been shown to inhibit the replication of scrapie infectivity in cell culture, but has not been used as a therapy in humans. A number of other therapeutic strategies have been suggested, including treatment with polyanions, such as dextran sulphate 500 and pentosan polysulphate, which are known to prolong the lifespan of mice infected with scrapie (30), or the use of compounds that inhibit PrPse replication by interfering with amyloid formation.

Possible risk of new variant Creutzfeldt-Jakob disease from blood products and surgery

Available data from epidemiological studies have not indicated that surgical instruments used outside the CNS or blood transfusion are an iatrogenic cause of CJD, although such studies do not have the power to exclude the rare occurrence (82). The appearance of nvCJD has raised new concerns about such modes of iatrogenic transmission (89). Given the possible oral route of infection and the novel strain of infectious agent, the distribution of tissue infectivity in nvCJD may differ from that of other forms of CJD. This is supported by the demonstration of the abnormal PrP isoform in multiple lymphoid tissues in nvCJD, including in the vermiform appendix eight months prior to the onset of symptoms of nvCJD in one case (52). Furthermore, B lymphocytes have recently been shown to be required for neuroinvasion following peripheral inoculation of TSEs (66). The concern related to the possible transmission of nvCJD led to the UK authorities adopting leucodepletion of donated blood and importing plasma for use in plasma derivatives from outside the UK (32). However, the data on the involvement of the lymphoid system in nvCJD should not be over-interpreted. The lymphoid cell type that stains for the disease-associated form of PrP in nvCJD is the non-circulating follicular dendritic cell, and although B-cells appear to be required for neuroinvasion, this may be via a maturation role on follicular dendritic cells rather than by actually ‘carrying’ infection themselves (66, 67). The weight of evidence suggests that the risk of transmission of any TSE in the health care setting by blood or surgical instruments used outside the CNS is remote. Transmission studies using blood from cases of nvCJD are currently ongoing.

Conclusions

New variant CJD is a novel human TSE which almost certainly arises from exposure to the BSE agent. Although current evidence suggests that oral exposure to the BSE agent through meat products consumed in the 1980s in the UK is the most likely route of exposure, other possibilities, such as occupational exposure, cannot be excluded. The clinical and neuropathological features of this disease are distinctive, and criteria for diagnosis have been established. Disease-associated PrP in this disorder is present outside the CNS in lymphoid tissues, raising concern of iatrogenic transmission via blood or surgical instruments. At present, estimation of the likely number of future cases of nvCJD in the UK and elsewhere, is difficult because of the unknown incubation period of this disorder, the uncertain routes of exposure and the lack of knowledge regarding the infectious dose for the BSE agent in humans. Furthermore, although all cases so far have occurred in individuals who are methionine homozygotes at PRNP codon 129, other genetic subgroups in the population could also be susceptible to this disorder, perhaps with a longer incubation period. Disease modelling in transgenic mice may help to provide some of the answers to these important questions. Continued surveillance of both BSE and CJD is required in the UK and in other countries, to ensure that the scale of this potential epidemic is adequately monitored and that all possible steps are taken to prevent further human exposure to the BSE agent.

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**Nouveau variant de la maladie de Creutzfeldt-Jakob**

M. Zeidler & J.W. Ironside

**Résumé**

Le nouveau variant de la maladie de Creutzfeldt-Jakob (nvMCJ) est une nouvelle encéphalopathie spongiforme transmissible affectant l’homme, identifiée pour la première fois en 1996 au Royaume-Uni. Des études scientifiques ultérieures ont montré que la souche de l’agent pathogène responsable du nvMCJ était identique à celle de l’agent de l’encéphalopathie spongiforme bovine (ESB). La maladie a donc été considérée comme une « ESB humaine ». Au 31 décembre 1999, 52 cas de nvMCJ avaient été signalés (dont 49 cas au Royaume-Uni, un en république d’Irlande et deux en France). Les sujets atteints étaient tous âgés de moins de 53 ans et ceux qui ont été testés étaient homozygotes pour la méthionine au codon 129 du gène codant la protéine du prion. Il est impossible de prédire combien de nouveaux cas de nvMCJ sont susceptibles d’apparaître à l’avenir, du fait de nombreuses incertitudes qui subsistent encore, notamment sur la période d’incubation de la maladie, le degré d’exposition à l’agent infectieux et la sensibilité des autres sous-types génétiques. Une surveillance permanente de l’ESB et de la maladie de Creutzfeldt-Jacob est nécessaire au Royaume-Uni et dans d’autres pays, afin de s’assurer que l’ampleur de cette épidémie potentielle est bien surveillée et que toutes les mesures possibles sont prises pour éviter l’exposition d’autres personnes à l’agent de l’ESB.

**Mots-clés**


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**Enfermedad de Creutzfeldt-Jakob, nuevo variante**

M. Zeidler & J.W. Ironside

**Resumen**

La enfermedad de Creutzfeldt-Jakob, nuevo variante (ECJnv) es una nueva encefalopatía espongiforme humana transmisible, identificada por primera vez en el Reino Unido en 1996. Estudios científicos ulteriores han revelado que la cepa del agente transmisible que provoca la ECJnv es idéntica a la del agente de la encefalopatía espongiforme bovina (EEB); de ahí la consideración de “EEB humana” que se ha dado a esa enfermedad. A fecha de 31 de diciembre de 1999 se habían comunicado 52 casos de ECJnv (49 casos en el Reino Unido, uno en la
República de Irlanda y dos en Francia. Todos los individuos afectados tenían menos de 53 años de edad y los que fueron sometidos a pruebas presentaban homocigosis (para la metionina) en el codón 129 del gen que codifica la proteína del prión. Dadas las numerosas incertidumbres que subsisten, especialmente en cuanto al período de incubación de la enfermedad, el grado de exposición al agente infeccioso y la susceptibilidad de otros subtipos génicos, resulta imposible estimar el número de casos de ECJnv que pueden aparecer en el futuro. Es preciso vigilar estrechamente la evolución de la EEB y la enfermedad de Creutzfeldt-Jakob tanto en el Reino Unido como en otros países. Sólo así se podrá seguir y calibrar adecuadamente la amplitud de esta epidemia potencial, y conseguir tomar todas las medidas posibles para impedir que más personas se vean expuestas al agente de la EEB.

Palabras clave

References


