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Variant Creutzfel

INTRODUCTION

Creutzfeldt–Jakob Disease (CJD) has been the focus of much attention in recent years due to the identification of a new variant now known as variant CJD (vCJD). Experimental evidence has shown that vCJD is due to human infection with the causative agent of Bovine Spongiform Encephalopathy (BSE), which was epidemic in UK cattle in the 1980s and early 1990s. Although vCJD remains rare, its existence has many implications. Clinicians have to face many questions from patients, relatives and other health care professionals. This review aims to present some of the scientific background, help health care professionals recognize the typical clinical picture and thus aid diagnosis.

CJD is a human transmissible spongiform encephalopathy (TSE). Uniquely, it is a disease that exists in sporadic, inherited and infectious forms. It is a rare, invariably fatal neurodegenerative disease that can be difficult to diagnose during life, and neuropathological examination of the brain is still the only means of definitive diagnosis. TSEs are only transmissible in specific circumstances and are not conventionally infectious, they affect humans and many different animal species.

The names of two physicians are linked to CJD. However, most modern commentators do not accept that Creutzfeldt described the illness that now bears his

dt-Jakob Disease

name, Jakob should be credited alone with the first published description in 1921 (Katscher 1988). Following the original clinicopathological description, many 'variants' of the disease were described, causing considerable nosological confusion. Matters have been clarified by the discovery of transmissibility, the determination of the role of the prion protein gene and its protein product (the prion protein, PrP), and the ability to detect PrP in tissue using immunocytochemistry. However, there is still unfortunate confusion about the recognized subtypes, especially by those unaware of the history of CJD prior to the emergence of BSE and vCJD. It is difficult to think of another disease, of a comparable rarity, which has been the subject of such media interest and public health protective measures. The accumulating understanding of CJD is the product of clinical and epidemiological research on the one hand, and pathological and molecular biological research on the other. These disciplines are sometimes presented as being separate worlds, but they act in complementary ways, as the story of CJD so clearly shows.

TYPES OF CJD

Sporadic CJD

This form makes up 85% of all CJD, with a worldwide incidence of around one case per million population per year. It primarily affects the middle-aged and elderly, the mean age of onset is 65 years with a range from 15 to 94 years. The classical presentation is with a rapidly progressive dementia and features of widespread neurological involvement, particularly including ataxia and myoclonus. It is a surprisingly rapidly progressive illness, given its neurodegenerative pathological characteristics. The median duration of illness is 4.5 months (range 1–74 months). Around three-quarters of patients are dead within six months of their initial symptoms. Most routine investigations are normal, including brain CT. The EEG is potentially helpful with characteristic generalized periodic (0.5–2 Hertz) triphasic complexes in two thirds of cases. The detection of a brain-specific protein (14-3-3) in the CSF of more than 90% of cases is particularly helpful. The brain MRI may show changes in the cortex, and particularly in the caudate and putamen.

Clinical criteria allow categorization into 'Probable' or 'Possible' cases (Table 1). A diagnosis of 'Probable' sporadic CJD, using these criteria has an accuracy of over 95% but a definite diagnosis requires neuropathological examination. The aetiology remains unclear despite extensive research; however, it is thought that it is due either to a somatic mutation of the prion protein gene or to a spontaneous protein structural change, in one or more cells in the brain.

Table 1 Diagnostic Criteria for Sporadic CJD, Rotterdam, 1998

I	RAPIDLY PROGRESSIVE DEMENTIA	
II	A	MYOCLONUS
	B	VISUAL OR CEREBELLAR SYMPTOMS
	C	PYRAMIDAL OR EXTRAPYRAMIDAL FEATURES
	D	AKINETIC MUTISM
III	TYPICAL EEG	
	DEFINITE	NEUROPATHOLOGICAL/IMMUNOCYTOCHEMICALLY CONFIRMED
	PROBABLE	I, PLUS AT LEAST TWO OF CRITERION II, PLUS CRITERION III, OR, POSSIBLE AND +VE 14-3-3 IN CSF
	POSSIBLE	CRITERION I, PLUS AT LEAST TWO OF CRITERION II, AND DURATION LESS THAN TWO YEARS

Iatrogenic CJD

Contamination with CJD infectivity from other individuals was first reported in 1974. It has occurred in people who had received contaminated human pituitary derived growth hormone, human gonadotrophins, human dura mater grafts and corneal grafts. There are also reported instances of transmission by neurosurgical instruments and intracerebral EEG depth-electrodes.

Genetic CJD

This is a very rare autosomal dominant condition, with more than 20 known disease-causing mutations of the prion protein gene located on the short arm of chromosome 20. It is possible to test a patient's blood sample for these mutations.

Variant CJD

vCJD was first reported in March 1996 (Will *et al* 1996a). As a result of active CJD surveillance in the UK, carried out by the National CJD Surveillance Unit since 1990, a number

of young patients were noticed who had a new and distinct clinico-pathological phenotype. The European Collaborative surveillance system made it easy to ascertain that identical cases were not being identified at that time in other European countries. Subsequent investigation linked this form to the epidemic of BSE in UK cattle.

CJD: AETIOLOGY and PATHOPHYSIOLOGY

PrP: The Prion Protein

Transmisable Spongiform Encephalopathies (TSEs) – or prion diseases – in humans and animals are all associated with the accumulation of an abnormal isoform of a normal host protein (prion protein) in the central nervous system (Prusiner *et al*. 1998). The normal protein in man is encoded by a gene on chromosome 20 and the entire open reading frame is contained within a single exon. The normal isoform of the prion protein (PrPc) is expressed in many tissues, but levels of expression are highest in central nervous system neurones. The function of the normal protein is uncertain. A potential role in synaptic function is suggested by studies in transgenic animals which do not express this normal protein, but more recent biochemical studies have indicated that PrPc can act as a copper binding protein and may have a role in protecting cells against oxidative stress. There is also some evidence to suggest that PrPc may have a role in signal transduction.

In prion diseases, PrPc becomes altered to the disease-associated isoform, PrPSc. PrPSc has no sequence differences from PrPc, being encoded by the same normal host gene, but there is a major difference in the conformation of the two forms, with PrPSc containing less -helix structure and relatively more -sheet structure (Prusiner *et al*. 1998). The predominance of -sheet structure confers a remarkable resistance to degradation and allows the protein to accumulate within the central nervous system as amyloid fibrils. The prion hypothesis states that this abnormal isoform of the protein is either the infectious agent, or the most significant component of it (Prusiner 1982). However, the protein-only hypothesis has still to explain adequately the existence of multiple different strains of TSE agents, all with different biological behaviours. Recent structural analysis and studies on the conformation of PrPSc indicates that the relatively unstructured N-terminus of the protein may be the region that confers strain specificity.

Genetics

The prion protein gene contains a polymorphic locus at codon 129 that can encode either methionine or valine. This polymorphism is of major importance in determining disease susceptibility in both the sporadic and acquired forms of CJD, and in potentially influencing the disease phenotype, particularly in sporadic CJD (Table 2). In addition, cases of familial prion diseases in humans are associated with point mutations in the prion protein gene, for example the codon 102 proline to leucine mutation, which is associated with the Gerstmann–Sträussler–Scheinker syndrome. In addition to these point mutations, familial cases of CJD have been associated with insertions into the octapeptide repeat region of the gene (Parchi *et al*. 1998). These abnormalities may produce a wide variety of clinical diseases, not all of which have been recognized as CJD before autopsy confirmation. It is therefore important in the investigation of any case of suspected CJD to undertake analysis of the prion protein gene to determine the codon 129 polymorphism status and to screen for the presence of any potentially pathogenic mutations or insertions. Around 10% of all cases of CJD have been found to occur as familial disorders, associated with prion protein gene mutations or insertions (Parchi *et al*. 1998).

BOVINE SPONGIFORM ENCEPHALOPATHY

General Background

Bovine spongiform encephalopathy (BSE) was first reported in the UK in 1987 (Wells *et al*. 1987). This epidemic disease of cattle was

Table 2 Prion protein gene codon 129 polymorphisms in normal and CJD patients: percentage distribution

	NORMAL POPULATION	SPORADIC CJD	VARIANT CJD
MM	37	74	100
MV	51	15	0
VV	12	11	0

probably transmitted by animal feed contaminated with the BSE agent. The precise origins of BSE are uncertain. Many workers believe that the BSE agent arose from a mutated form of scrapie (the endemic prion disease in sheep within the UK); others suggest there was a previously unrecognized rare sporadic cattle disease that was then transmitted to many cattle. The transmission of BSE was probably a result of the practice of rendering the carcasses of dead animals to produce a high-protein material, meat and bone meal (MBM), which was then used as animal feed particularly in the dairy industry. Changes that occurred within the rendering industry (including a reduction of the rendering temperatures) may have contributed to the persistence of the BSE agent in animal feed. The BSE epidemic reached its peak in 1992–93 when up to 1200 cases per week were being identified. Since then, the disease has declined sharply, but is not yet completely eliminated. It has been estimated that at least 700 000 cattle in the UK were infected with BSE and that a large number of these animals may have entered the human food chain (Anderson *et al*. 1996). Not all infected animals necessarily had clinical signs of disease prior to slaughter, as the incubation period in cattle is around 4–5 years.

How was human health protected?

- The MBM ban for animal feed in 1988–89 was important in preventing further transmission of BSE and in protecting animal health.
- Human health was protected from BSE by the specified bovine offal (SBO) ban in 1989–90 when tissues that may have contained the highest levels of prion infectivity (brain, spinal cord, spleen and other lymphoid organs) were banned from human consumption.
- However, vertebral columns could be used in the production of Mechanically Recovered Meat (MRM) until late 1995, and this may have allowed continuing infectivity to enter food, via the presence of residual spinal cord.
- Human health was further protected in 1996 by the 'over 30 months' scheme in which only animals under the age of 30 months were used for human consumption. By this stage most cases of BSE were occurring in animals much older than 30 months of age.

Although the BSE epidemic has declined in the UK, there is evidence of increasing numbers of BSE cases in other European countries, particularly in France, Germany, and Portugal. But BSE case ascertainment does depend on the type of surveillance, i.e. active or passive. Switzerland has recently instituted active BSE surveillance, with a resulting rise in recognized cases. Most countries with BSE acquired the disease as a result of importing infected animals from the UK, or by the importation of contaminated meat and bone meal from the UK. Regulations are in place to try to prevent human food contamination with potentially infectious tissues. It is a matter of concern that a recent analysis of German sausages revealed CNS tissue in some retail brands (Lucker *et al*. 2000).

Human exposure to BSE could arise via a number of routes (Table 3). Of these, the most likely is oral exposure to the BSE agent through the food chain, particularly prior to the Specified Bovine Offal (SBO) ban, and the ban on the use of vertebral columns in the production of Mechanically Recovered Meat (MRM).

BSE has infected a range of other species in the UK including a number of antelope species in zoos (as a result of exposure to meat and bone meal animal feed) and to domestic cats (over 85 cats in the UK have died from the novel prion disorder feline spongiform encephalopathy, FSE) and a smaller number of large wild cats in zoos, also as a result of dietary exposure. Experimental strain typing in mice using brain material from BSE, FSE and the novel TSE in antelopes indicated these are all due to a single strain of agent, which exhibits markedly different properties in terms

Table 3 Potential routes of human exposure to BSE

I	DIETARY ROUTE, THROUGH MEAT PRODUCTS THAT HAVE BEEN CONTAMINATED WITH TISSUE CONTAINING BSE INFECTIVITY, E.G. MECHANICALLY RECOVERED MEAT CONTAINING NEURAL TISSUE.
II	OCCUPATIONAL ROUTE, IN INDIVIDUALS WHO HANDLE ANIMALS OR THE CARCASSES OF ANIMALS INFECTED WITH BSE, E.G. ABATTOIR WORKERS, BUTCHERS, VETERINARIANS AND FARMERS.
III	IATROGENIC EXPOSURE, THROUGH VACCINES AND OTHER MEDICINAL PRODUCTS MADE USING BOVINE MATERIALS.

of the disease incubation period and pattern of neuropathology in the mouse brain when compared with scrapie. The BSE agent, unlike scrapie, appears to be more successful in causing disease in unrelated species following oral exposure. Hence the possibility that humans might develop a BSE-related disorder following oral exposure to the agent through the food chain.

Evidence that BSE is the cause of vCJD

There are three lines of evidence supporting a causal relationship between BSE and vCJD.

BSE and vCJD are caused by the same strain of agent

There is accumulating laboratory evidence that the agents are identical. Biochemical analysis of PrPSc by Western blotting from cases of BSE and other BSE-related diseases in animals and vCJD shows a similar glycoform ratio, suggesting a common agent strain. This suggestion was subsequently confirmed in terms of biological behaviour. In mice experiments, vCJD transmitted with a similar incubation period and pattern of neuropathology in the brain to BSE, which was in turn markedly different from transmissions of sporadic CJD and scrapie (Ferguson *et al*. 1996). Similar findings where identified in transmissions into transgenic mice (with the human PrP gene inserted), and a recent study of transmission of BSE and vCJD to the mice with the bovine PrP gene inserted on a null background confirmed this result (Scott *et al*. 1999). This experimental model may well be the most sensitive for the detection of BSE-related infections. In addition, studies of experimental BSE inoculated intracerebrally into Macaque monkeys unexpectedly showed neuropathological findings very similar to vCJD.

The disease passed from cattle to man

Epidemiological studies showed that the country with the highest number of BSE cases (UK) also has the highest number of vCJD cases. There is no evidence for a third, separate source of both BSE and vCJD. Furthermore, the time interval between the first identification of vCJD and the earlier identification of BSE was appropriate for transmission of a prion disease across a 'species barrier'. This concept came from earlier experimental studies of prion diseases where transmission of these agents from one species to another tended to prolong the incubation period. Transmission from cattle to man remains an overwhelmingly likely hypothesis with no reasonable alternative suggestion.

The means of transmission was via food contamination

This remains the most likely means of transmission, but there is as yet no formal proof of the dietary theory (Knight *et al*. 1999).

VARIANT CJD: THE GENERAL PICTURE

Variant CJD has a clinical and neuropathological picture that is distinct from other forms of CJD (Zeidler *et al*. 1997a). It presents as a progressive neuropsychiatric disorder, usually in a young person, and can have characteristic MRI brain findings in up to 70% of cases (Will *et al*. 2000). The typical presentation is with psychiatric or behavioural disturbance (Will *et al*. 1999; Zeidler *et al*. 1997b), often accompanied by sensory symptoms (MacLeod *et al*. 2000), with the subsequent development of ataxia, involuntary movements and dementia. Variant CJD has a relatively long duration of illness compared with sporadic CJD: the median duration being 13 months (range 6–39 months) compared with 4.5 months in sporadic disease. At present, the only definitive diagnostic test is neuropathology. All cases to date have had a similar overall clinico-pathological phenotype including the one elderly patient (Lorains *et al*. 2001). There are currently 104 definite and probable cases in the UK (Table 4). It is still therefore a very rare disease but currently there is an increasing incidence of about 35% per year (approximating to a doubling of cases every 3.3 years; 95% CI 2–9.6 years) (National CJD Surveillance Unit 2000; Andrews NJ, pers. comm. 2001). As we do not know the exact route of infection with the BSE prion protein, or the incubation period of vCJD, it is difficult to calculate potential exposure and the size of any future epidemic. Some groups have tried to estimate this but with a wide range in the predicted

upper limit of total cases from a few hundred to hundreds of thousands (Cousens *et al* 1997; Ghani *et al*. 2000).

Making the diagnosis

Variant CJD usually presents in a young person, in their twenties or thirties, although there have been both younger and much older cases (Table 4). In the majority of cases, the first symptoms are psychiatric and many have sensory symptoms at onset. Common psychiatric symptoms include:

- depression (most common)
- withdrawal
- aggression and irritability
- anxiety or fear
- hallucinations and delusions
- first rank symptoms and suicidal ideation

have also been seen.

The psychiatric symptoms characteristically do not respond to antidepressant or psychotropic medication (Will *et al*. 1999).

Sensory symptoms are a prominent feature and those commonly reported include:

- limb pain
- paraesthesia and dysaesthesia
- numbness
- cold feet and cold or burning sensations

(MacLeod *et al*. 2000).

These symptoms predominantly affect the limbs but have also been reported in the cheek and trunk. They may be asymmetrical or even unilateral in distribution. It has been postulated that they are of thalamic origin.

Both sensory and psychiatric symptoms usually precede the development of other symptoms and any neurological signs by some months, and tend to be persistent. Therefore, these patients can first present to specialities other than neurology, in particular to psychiatry. Diagnosis in the early stages may be very difficult. There are more common and mundane causes of, for example, a syndrome of depression and limb pains in a young individual.

Definite neurological signs occur at a mean of approximately six months after the initial symptoms, although some patients may have mild cognitive impairment from the onset. Some have such severe sensory symptoms that investigations are warranted prior to the development of other neurological symptoms or signs (MacLeod *et al*. 2000; Will *et al*. 2000). Subsequently, ataxia, involuntary movements and cognitive problems develop.

An unsteady gait is often the first physical complaint, which rapidly becomes obvious ataxia, affecting the limbs and/or trunk.

The development of a movement disorder can also impair mobility and is characteristic of this disease. Patients often initially appear restless and fidgety, with progression most commonly to choreiform movements of the limbs and/or dystonia or myoclonus. Myoclonus often appears later in the disease course and can evolve from choreiform movements or dystonia; it can be spontaneous and/or stimulus sensitive (Will *et al*. 2000).

Cognitive impairment is usually subtle at first but quickly progresses to frank dementia.

Variant CJD is characterized by its relentless and rapid progression. Mobility deteriorates and in the terminal stages patients are usually bed-bound. Many lose the ability to communicate due to a combination of dysarthria, dysphonia and cognitive impairment, and eventually become mute. Later in the disease course, cortical blindness can occur but is less common than in sporadic CJD. As the disease progresses, primitive reflexes (grasp, rooting, pout and palmomental) are often evident. Myoclonus becomes more prominent and may become generalized, and a startle reflex may develop. Limb rigidity becomes

TABLE 4 CURRENT FIGURES FOR VARIANT CJD

TOTAL CASES OF VCJD, 1ST MARCH 1995–12TH JULY 2001	104
PATIENTS WHO HAVE DIED	
DEFINITE	81
PROBABLE (NO POST MORTEM) CASES	11
PROBABLE (AWAITING POST MORTEM RESULT)	5
7 PROBABLE PATIENTS ARE STILL ALIVE	
MEAN AGE AT ONSET	27 YEARS (RANGE 12–74)*
MEDIAN AGE AT ONSET	26 YEARS
MEAN AGE AT DEATH (FOR THOSE WHO HAVE DIED)	29 YEARS (RANGE 14–74)*
MEDIAN AGE AT DEATH	28 YEARS
MEAN DURATION OF ILLNESS	13 MONTHS (RANGE 6–39)
55 MALES: 49 FEMALES	
91 CASES TESTED ARE MM AT CODON 129 OF THE PRP GENE	
DEATHS FROM DEFINITE OR PROBABLE VCJD SPLIT BY REGIONS OF THE UK	
ENGLAND	80
SCOTLAND	17
WALES	4
N IRELAND	1
DIED ABROAD	2

*ALL BUT ONE OF THESE PATIENTS IS AGED 12–53 YEARS.

more pronounced. Some patients also develop pyramidal signs with spasticity, hyperreflexia and extensor planters. Dysphagia and subsequent aspiration often develop. The terminal stages of vCJD are very similar to sporadic CJD with progression to a state of helplessness and akinetic mutism.

Investigations

Patients who may potentially have a diagnosis of vCJD are usually referred to a neurologist and should be extensively investigated to ensure that treatable conditions are excluded.

Routine investigations such as blood tests are normal (this should include copper and caeruloplasmin assays), although some patients have a mild transient disturbance of liver function. To completely exclude Wilson's disease, urinary copper should also be measured and the patient undergo slit lamp examination looking for Kayser-Fleischer rings.

Cerebrospinal fluid (CSF) is usually examined. In all forms of CJD, including variant, the CSF is essentially normal. The white cell count should be normal. CSF protein is usually normal but in 10–15% of sporadic and variant cases the total protein is mildly to moderately elevated. Brain specific protein (BSP) analysis can be useful in aiding diagnosis Green *et al*. 2000; Will *et al*. 1996b). The brain specific protein 14-3-3 may be elevated in approximately 50% of cases of vCJD but this is a less sensitive test than for sporadic CJD. It is important to note that BSP analysis is not an absolute diagnostic nor a screening test and is only valuable if the patient's clinical picture is generally consistent with CJD. A negative test does not exclude the diagnosis and false positive results can occur if the CSF is bloodstained, has a high white cell count, if the patient has had recent seizures, traumatic brain injury, a stroke, encephalitis or a paraneoplastic syndrome. The National CJD Surveillance Unit is currently the only laboratory that performs brain specific protein analysis in the UK as a clinical service.

Magnetic resonance imaging (MRI) of the brain is an important investigation as specific abnormalities can be seen in vCJD that are of diagnostic value, i.e. high signal in the posterior thalamic nuclei (pulvinar region), which is bilateral and symmetrical (Fig. 1) (Zeidler *et al*. 2000). In the correct clinical context, the 'pulvinar sign' is highly supportive of a diagnosis of vCJD. It has been seen in other disorders (e.g. benign intracranial hypertension, Alper's disease) but these have very different clinical presentations. The pulvinar sign on MR corresponds to areas of gliosis found when the brain is subsequently examined at post mortem, but it is important to recognize that this examination is often performed many months later and it is not clear if there is a direct radiological-pathological correlation. Currently, the characteristic MRI appearance is not considered definitively diagnostic but allows a previously possible case to be categorized as probable. The MR scan is usually otherwise normal, but may show atrophy in the later stages of the illness.

Most patient have an electroencephalogram (EEG), which can be normal despite significant neuropsychiatric illness. More often, it is nonspecifically abnormal with deterioration in the normal background rhythms, excessive slow wave activity or occasionally excessive fast

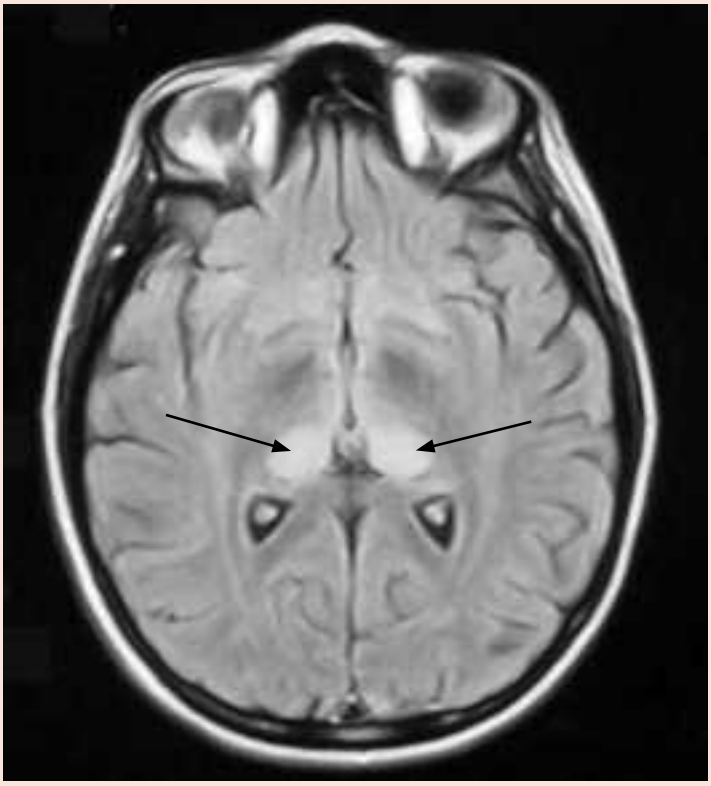


Fig. 1 MRI brain of a patient with variant CJD showing bilateral pulvinar high signal (arrows) – FLAIR image.

wave activity. The triphasic complexes seen in sporadic CJD have never been seen in vCJD.

Genetic analysis, looking for mutations and for determining the codon 129 status, can be performed on whole blood.

Tonsil biopsy is not a routine investigation, but may be considered in specific individuals. For example, in those patients who fulfil the diagnostic criteria for 'Possible vCJD' (with no 'pulvinar sign' on MRI), and in whom there is a particular need for a premortem diagnosis with a greater degree of certainty. The tonsil biopsy must to be performed by someone who understands the potential risks of infectivity. The biopsy tissue needs to be examined by a laboratory with specific experience in PrP immunocytochemistry, and preferably one with experience of the examination of lymphoreticular tissue in prion disease. A 'positive' tonsil biopsy cannot result in a diagnosis of 'Definite vCJD'.

Neuropathology remains the gold standard for diagnosis and this is commented on in greater detail later.

Cerebral biopsy, in life, may be considered in exceptional circumstances, for example if there is any possibility of an alternative, potentially treatable disease, that cannot be diagnosed without neuropathological confirmation.

Differential diagnosis

Clearly, iatrogenic and genetic CJD must be excluded by history and investigation. In iatrogenic CJD, there is a history of relevant iatrogenic exposure. Many patients with genetic CJD have a family history of a similar disorder, but not all. If genetic CJD is a consideration, the patient and their family should be referred to the local Clinical Genetics Service for appropriate counselling. Currently, in the UK, sporadic CJD is the most important differential diagnosis. Sporadic CJD can present atypically and sometimes occurs in younger patients. In some instances, these may be difficult to distinguish from vCJD but they do not have the typical MRI changes of bilateral pulvinar high signal. Sporadic CJD has characteristically different neuropathological appearances to vCJD and the distinction can be made definitively at post mortem.

Wilson's disease should be excluded as this can present as a neuropsychiatric disorder in a young person and is potentially treatable. Other diagnoses made in patients classified as at least possible vCJD include Alzheimer's disease, cerebrovascular disease, limbic encephalitis, cerebral vasculitis and infective encephalitis. Diagnoses in cases initially classified as clinically unlikely include peripheral neuropathy, Alzheimer's disease, vitamin B12 deficiency, cerebrovascular disease, Wilson's disease, possible encephalitis lethargica, corticostriatalnigral degeneration and cerebral vasculitis (Will *et al*. 2000).

Pathological diagnosis

Current World Health Organization criteria state that neuropathological examination of the brain is required for the definite diagnosis of a human prion disease. Because brain biopsy is not recommended as an investigative procedure (unless to exclude an alternative treatable disorder), the diagnosis of vCJD is usually confirmed by post mortem examination (Ironside *et al*. 2000a). This requires informed consent from the relatives, who often generously give additional permission for retention of the brain and other tissue samples for research purposes, allowing a full study of the distribution of the abnormal form of PrP throughout the body.

The pathology of human prion diseases (Ironside *et al*. 2000a) is characterized by:

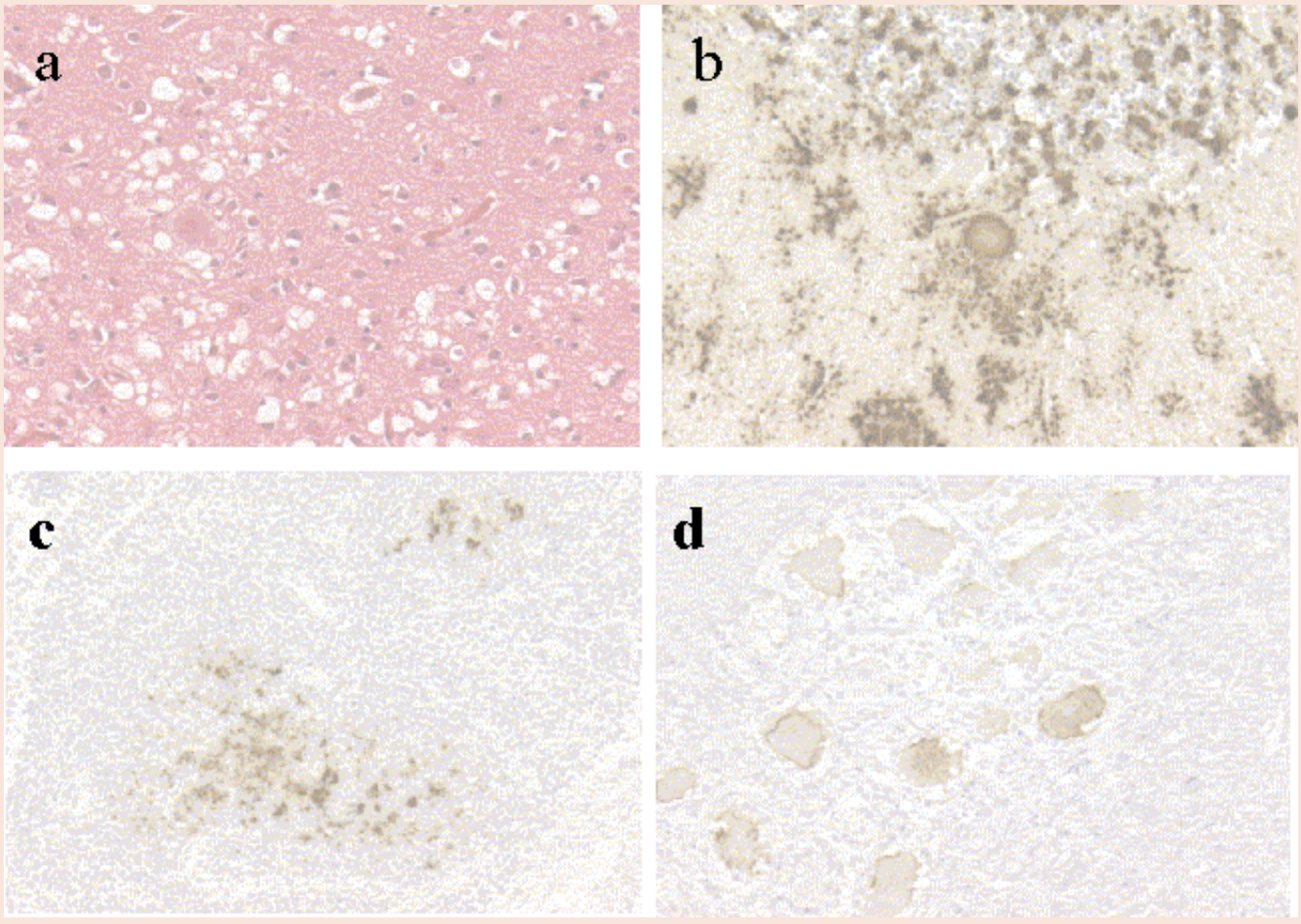
- spongiform change
- neuronal loss
- reactive astrocytic and microglial proliferation
- accumulation of PrP^{Sc} within the grey matter of the central nervous system.

There are no characteristic pathological abnormalities in other organs, and there is no evidence of an immunological response against the infectious agent. Spongiform change occurs as vacuolation within the grey matter due to swelling and distension of nerve cell processes that contain membrane-filled spaces, possibly as a consequence of conversion of PrP^{c} to PrP^{Sc}. The distribution of spongiform change, neuronal loss and PrP^{Sc} is markedly

Fig. 2 A florid plaque, the hallmark of variant CJD in the cerebral cortex, is composed of abnormal prion protein amyloid, with a dense centre, fibrillary periphery and a rim of spongiform change (haematoxylin and eosin). (a) The cerebral cortex in variant CJD contains a florid plaque (left) with spongiform change in the surrounding grey matter (Haematoxylin and eosin); (b) Immunocytochemistry for PrP in the cerebellum in variant CJD shows strong staining (brown) of a large plaque (centre), with widespread amorphous PrP deposits in the surrounding molecular and granular layer (KG9 monoclonal antibody); (c) PrP accumulation in the tonsil in variant CJD is confined to the germinal centre, with intense labelling of follicular dendritic cells and macrophages (KG9 monoclonal antibody); (d) A dorsal root ganglion in variant CJD shows variable PrP accumulation in both ganglion cells and satellite cells (KD9 monoclonal antibody).

variable within human prion diseases and, particularly in sporadic CJD, the codon 129 genotype and PrP isotype have major influences on the pattern of brain pathology.

In vCJD, there is a relatively consistent neuropathological profile, which is characterized by the presence of numerous fibrillary amyloid plaques composed of PrPSc surrounded by a rim or halo of spongiform change (Ironside *et al*. 2000a). These florid plaques have a widespread distribution in the cerebral cortex and cerebellum and, although not exclusively found in vCJD, are characteristic of this disorder (Fig. 2a). The other main neuropathological abnormalities in vCJD are severe spongiform change in the caudate nucleus and putamen, marked neuronal loss and astrocytosis in the thalamus, particularly in the posterior thalamic nuclei, corresponding to the distribution of the areas of hyperintensity in the posterior thalamus on MR scans. There is widespread accumulation of



PrPSc within the brain, particularly in the cerebral and cerebellar cortex where immunocytochemistry reveals multiple smaller plaques and amorphous deposits of PrPSc that are not detected on routinely stained histological sections (Fig. 2b). Immunocytochemical analysis is therefore essential for the diagnosis.

Variant CJD also differs from other forms of human prion disease in that there is widespread accumulation of PrPSc in lymphoid tissues throughout the body (Hill *et al*. 1999). This is most readily identified in the tonsil (Fig. 2c) and spleen, and tonsillar biopsy for the detection of PrPSc by biochemistry and Western blotting has been used on a small number of patients for clinical diagnosis (see above). The widespread involvement of lymphoid tissues in vCJD is thought to relate to the peripheral pathogenesis of this disorder, when oral exposure to the BSE agent is followed by distribution of the agent in the body, perhaps by lymphocytes. Such involvement of the lymphoreticular system probably occurs at a relatively early stage in the incubation period. Similar patterns of lymphoid tissue involvement have been found in sheep with natural scrapie, and with some experimental animal models of prion disease. The lymphoid tissue involvement in vCJD has encouraged attempts to screen large numbers of tonsillectomy and appendectomy specimens on a retrospective and anonymous basis to see if any containing PrPSc are identified. The preliminary results have been negative, but the study is continuing and will help provide data that might enable more accurate planning of future disease scenarios in relation to vCJD (Ironside *et al*. 2000b). Peripheral nervous system involvement also occurs in vCJD, especially in dorsal root ganglia (Fig. 2d)

PrPSc can also be detected by Western blot analysis of the central nervous system or lymphoid tissues, when the abnormal prion protein is demonstrated by antibody staining following digestion of the supernatant with proteinase K (Ironside *et al*. 2000a). Because the currently available antibodies to PrP detect both PrPc and PrPSc, it is necessary to completely denature PrPc by limited proteolysis of the specimen. The abnormal prion protein that remains can readily be detected on the gel, and occurs as a three-banded profile corresponding to the diglycosylated, monoglycosylated and unglycosylated forms of the protein. In vCJD, there is a characteristic PrP isoform present that has a predominant diglycosylated band. This is detectable both in the central nervous system and in lymphoid tissues and serves as a molecular 'marker' for vCJD when both the glycoform ratio and the molecular size of the unglycosylated fragment are studied (Ironside *et al*. 2000a). The presence of PrPSc in the lymphoreticular tissues is associated with infectivity, and a recent study has demonstrated transmission of infectivity from tonsil and spleen taken from patients with vCJD to experimental mice. The levels of infectivity in the lymphoreticular tissues (although around 1–2 logs lower than in the central nervous system) reinforce concerns that infectivity from these tissues might be inadvertently transmitted via surgical instruments from person to person.

TREATMENT AND CARE

There is currently no proven effective treatment for any type of CJD. Some drugs have been used in experimental animal models (Knight *et al*. 2000) but there are no current clinical trials. However, it is still important to make the diagnosis of vCJD and so avoid unnecessary investigation of the patient, and to give the patient and their family the chance to be fully informed about the diagnosis and its implications.

Once the diagnosis of vCJD has been made, treatment is supportive and palliative. Some specific treatments can be beneficial for troublesome symptoms, e.g. clonazepam or other benzodiazepines for myoclonus and agitation, and some patients have required antipsychotic medication. As bulbar dysfunction occurs in all patients as the disease progresses, artificial feeding may be considered by either NG or PEG tube.

An increasing number of patients with vCJD are now being managed at home for the duration of their illness. It is important that families and health care professionals realise that although vCJD is a transmissible disease, this does not occur via ordinary contact with the patient or body fluids, etc., and barrier nursing is unnecessary. However, invasive pro-

cedures may require specific precautions, especially if operative intervention is required.

THE FUTURE

Despite the fact that the clinical diagnosis of vCJD with a high degree of certainty is currently possible in the majority of cases, it requires expert neurological assessment and investigation and there remain difficult cases (for example, those without the MRI pulvinar change). Therefore, a definitive, minimally invasive and easily performed diagnostic test would be clinically useful and there are a number of research groups working to this end, particularly in relation to the detection of PrPSc in blood (Saborio *et al*. (YEAR)). We cannot at present predict the number of people who will develop vCJD in the UK. It also remains to be seen how many cases will occur in other countries. The present cases are assumed to be related to BSE dietary contamination and there are concerns about the possibility of secondary iatrogenic spread via surgery, blood and blood products. This has led to certain precautionary measures such as the use of disposable surgical instruments for tonsillectomy and a variety of measures in relation to blood transfusion practice. An important issue, especially for surveillance systems, is the fact that vCJD has not yet been seen in prion protein codon 129 MV or VV genotypes. It is conceivable that such individuals could have a clinical and pathological phenotype that differs from what we have come to recognize as vCJD which is, after all, BSE in MM humans. For all these reasons, it is crucial to maintain surveillance, both in the UK and in collaborative European projects.

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