UNIVERSITY OF SOUTHAMPTON

# A NEW VARIANT OF CREUTZFELDT-JAKOB DISEASE IN THE UNITED KINGDOM

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ABSTRACT

FACULTY OF MEDICINE

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By Martin Dieter Zeidler

This thesis details the first 14 cases of variant Creutzfeldt-Jakob disease (CJD) identified in the UK and reports the findings of a study to assess magnetic resonance imaging (MRI) as a diagnostic tool for variant CJD (vCJD).

Since the mid 1980s an epidemic of bovine spongiform encephalopathy (BSE) has occurred in the UK. It was postulated that if BSE was to transmit to humans it would most likely cause a spongiform encephalopathy similar to CJD. Therefore in 1990 surveillance of CJD was instituted in the UK. Case ascertainment is mostly by direct referral from neurologists and neuropathologists to the CJD Surveillance Unit in Edinburgh. Clinical details are obtained for all referred cases and patients considered likely to have CJD are assessed by a research registrar. Information on potential risk factors is collected from patients' relatives using a standard questionnaire. Similar data is obtained for an age- and sex-matched control patient in the referring hospital.

Between September 1995 and 31 December 1996 14 cases of CJD were identified with an apparently novel clinicopathological phenotype. When compared to sporadic CJD these patients were relatively young (mean age at onset 29 vs. approximately 65 years) and had atypical early features: all developed behavioural disturbance and eight experienced persistent sensory symptoms. Other unusual characteristics noted during the illness were upgaze paresis (50%) and chorea (50%). None developed the periodic electroencephalographic pattern characteristic of sporadic CJD. All shared identical and unusual neuropathological features, in particular the presence of numerous prion protein aggregates encircled by spongiform change ('florid' plaques) throughout the brain. Risk factors identified for vCJD were young age, residence in the UK and methionine homozygosity at the polymorphic codon 129 of the prion protein gene. The latter genotype occurs in about two-fifths of normal Caucasians but was observed in all cases. The case-control study failed to identify any clear dietary, past medical or occupational risk factors. This has to be interpreted with caution given the small number of patients involved. The study concluded that that vCJD is a novel disease most likely caused by BSE. This is supported by a number of studies that show that the vCJD agent and the BSE agent have the same properties.

MRI shows striatal high signal on dual echo sequences in the majority of sporadic CJD cases. In contrast two early cases of vCJD were reported to have bilateral pulvinar high signal. This prompted a study of MRI in vCJD. MRI from patients 36 patients with vCJD and 57 controls (patients with suspected CJD) were analysed. Scans were reviewed blind on two separate occasions by two neuroradiologists and scored for the distribution of changes and likely final diagnosis. Images showing bilateral pulvinar high signal were classified as diagnostic for vCJD and the degree of such changes were graded on a three point scale: minimal/equivocal, moderate and marked. Scans were later openly reassessed to reach a consensus on all abnormalities.

A radiological diagnosis of vCJD was made in 86% of case assessments and 3% of control assessments. The seven false-positive assessments in the controls were graded as minimal/equivocal in six and moderate in one (<0·5% of all control assessments). However, 80% of the assessments in the vCJD cases were graded as moderate or marked. On consensus review, 28 of 36 cases and none of 57 controls were considered to have prominent bilateral pulvinar signal - sensitivity 78% (95% CI 60-90%) and specificity 100% (95% CI 94-100%). Other common MRI features of vCJD were medial thalamic and periaqueductal grey matter high signal, and the notable absence of cerebral atrophy. Pulvinar high signal correlated with histological gliosis. The study concluded that in the appropriate clinical context the identification of bilaterally increased pulvinar signal on MRI is a useful non-invasive test for the diagnosis of vCJD.

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Table 54 is modified from Gore[27]

Table 57 is modified from Hornabrook[28]

Table 60 is Modified from the Ninth Annual Report of the UK National CJD Surveillance Unit[29]

# ABBREVIATIONS

| | |
|---|---|
| A&E | Accident and emergency |
| ACE | Angiotensin converting enzyme |
| AED | Antiepileptic drug |
| AFP | Alpha-fetoprotein |
| AIDS | Acquired immunodeficiency syndrome |
| Ala | Alanine |
| ANA | Antinuclear antibody |
| ANCA | Antineutrophil cytoplasmic antibody |
| Arg | Arginine |
| Asn | Asparagine |
| Asp | Aspartic acid |
| AST | Aspartate transaminase |
| BCG | Bacille Calmette-Guérin |
| BMJ | British Medical Journal |
| bp | Base pair |
| BSE | Bovine spongiform encephalopathy |
| C3, C4 | Complement components 3 and 4 |
| CA125 | Cancer antigen 125 |
| Ca 19-9 | Cancer antigen 19-9 |
| CD | Cluster of differentiation |
| CEA | Carcinoembryonic antigen |
| CI | Confidence interval |
| CJD | Creutzfeldt-Jakob disease |
| CMV | Cytomegalovirus |
| CNS | Central nervous system |
| CPK | Creatine phosphokinase |
| CRP | C-reactive protein |
| CSF | Cerebrospinal fluid |
| CT | Computed tomography |
| Cu | Copper |
| CVL | Central veterinary laboratory |
| CWD | Chronic wasting disease |
| Cys | Cysteine |
| D&C | Dilatation and curettage |
| Dod | Date of death |
| DNA | Deoxyribonucleic acid |
| DRPLA | Dentatorubral-pallidolusian atrophy |
| DWI | Diffusion-weighted imaging |
| ECG | Electrocardiograph |
| EDTA | Ethylenediaminetetraacetic acid |

| | |
|---|---|
| EEG | Electroencephalogram/electroencephalography |
| EM | Electron microscopy |
| EMG | Electromyography |
| ENA | Extractable nuclear antigens |
| EPIC | European prospective investigation of cancer |
| EPs | Evoked potentials |
| ESR | Erythrocyte sedimentation rate |
| EU | European Union |
| FB | Foreign body |
| FDCs | Follicular dendritic cells |
| FDG | $^{18}$F-2-fluorodeoxyglucose |
| FFI | Fatal familial insomnia |
| FH | Family history |
| FLAIR | Fluid-attenuated inversion recovery |
| FSE | Feline spongiform encephalopathy |
| FVB | Friends virus B |
| GFAP | Glial fibrillary acid protein |
| Gln | Glutamine |
| Glu | Glutamic Acid |
| Gly | Glycine |
| GP | General practitioner |
| GSS | Gerstmann-Sträussler-Scheinker disease |
| H&E | Haematoxylin and eosin |
| hGH | Human growth hormone |
| His | Histidine |
| HLA | Histocompatibility antigen |
| hPG | Human pituitary gonadotrophin |
| HRT | Hormone replacement therapy |
| HSV | Herpes simplex virus |
| HTLV1 | Human T-cell lymphotropic virus type 1 |
| HuPrP | Human prion protein |
| Hz | Hertz |
| ICD | International Classification of Diseases |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| Ile | Isoleucine |
| IP | Incubation period |
| IQ | Intelligence quotient |
| IV | Intravenous |
| LBD | Lewy body disease |
| LD | Lethal dose |

| | |
|---|---|
| Leu | Leucine |
| LFTs | Liver function tests |
| LMN | Lower motor neuron |
| LRS | Lymphoreticular system |
| Lys | Lysine |
| MAFF | Ministry of agriculture, food and fisheries |
| MBM | Meat and bone meal |
| MELAS | Mitochondrial encephalopathy, lactic acidosis and stroke-like episodes |
| Met | Methionine |
| MM | Methionine homozygous genotype |
| MMSE | Mini-mental state examination |
| Mo | Mouse |
| MRI | Magnetic resonance imaging |
| MRM | Mechanically-recovered meat |
| MRS | Magnetic resonance spectroscopy |
| MV | Methionine-valine heterozygous genotype |
| n | Number |
| N/A | Not applicable |
| NCJDSU | National Creutzfeldt-Jakob disease surveillance unit |
| NCS | Nerve conduction studies |
| NFT | Neurofibrillary tangle |
| NHS | National Health Service |
| No | Number |
| NR | Normal range |
| NS | Not stated |
| NSE | Neuron specific enolase |
| Op | Operation |
| OTC | Over the counter |
| PD | Proton density |
| PET | Positron emission tomography |
| Phe | Phenylalanine |
| PM | Post-mortem |
| PML | Progressive multifocal leucoencephalopathy |
| PRNP | Human prion protein gene |
| Pro | Proline |
| PrP | Prion protein |
| $PrP^{C}$ | The cellular form of PrP |
| $PrP^{Res}$ | The partially protease resistant form of PrP |
| $PrP^{Sc}$ | The scrapie isoform of PrP |
| Ref | Reference |
| REM | Rapid eye movement |
| SAFs | Scrapie-associated fibrils |

| | |
|---|---|
| SBO | Specified bovine offals |
| sCJD | Sporadic Creutzfeldt-Jakob disease |
| SD | Standard deviation |
| SDS-PAGE | Sodium dodecyl sulphate polyacrylamide gel electrophoresis |
| SE | Spongiform encephalopathy |
| SEAC | Spongiform Encephalopathy Advisory Committee |
| Ser | Serine |
| spCJD | Sporadic Creutzfeldt-Jakob disease |
| SPECT | Single photon emission computed tomography |
| SSPE | Subacute sclerosing panencephalitis |
| T3 | Triiodothyronine |
| T4 | Thyroxine |
| TATT | Tired all the time |
| TB | Tuberculosis |
| Tg | Transgenic |
| Thr | Threonine |
| TME | Transmissible mink encephalopathy |
| TPHA | Treponema pallidum haemagglutination assay |
| Trp | Tryptophan |
| TSE | Transmissible spongiform encephalopathy |
| TSH | Thyroid stimulating hormone |
| Tyr | Tyrosine |
| UK | United Kingdom |
| USA | United States of America |
| UV | Ultraviolet |
| Val | Valine |
| vCJD | Variant CJD (alternatively called new variant CJD) |
| vs | Versus |
| VV | Valine homozygous genotype |
| WCC | White cell count |
| WHO | World Health Organization |
| YOB | Year of birth |
| Zn | Zinc |

**Figure 1: Hans Gerhard Creutzfeldt (left) and Alfons Maria Jakob (right)**

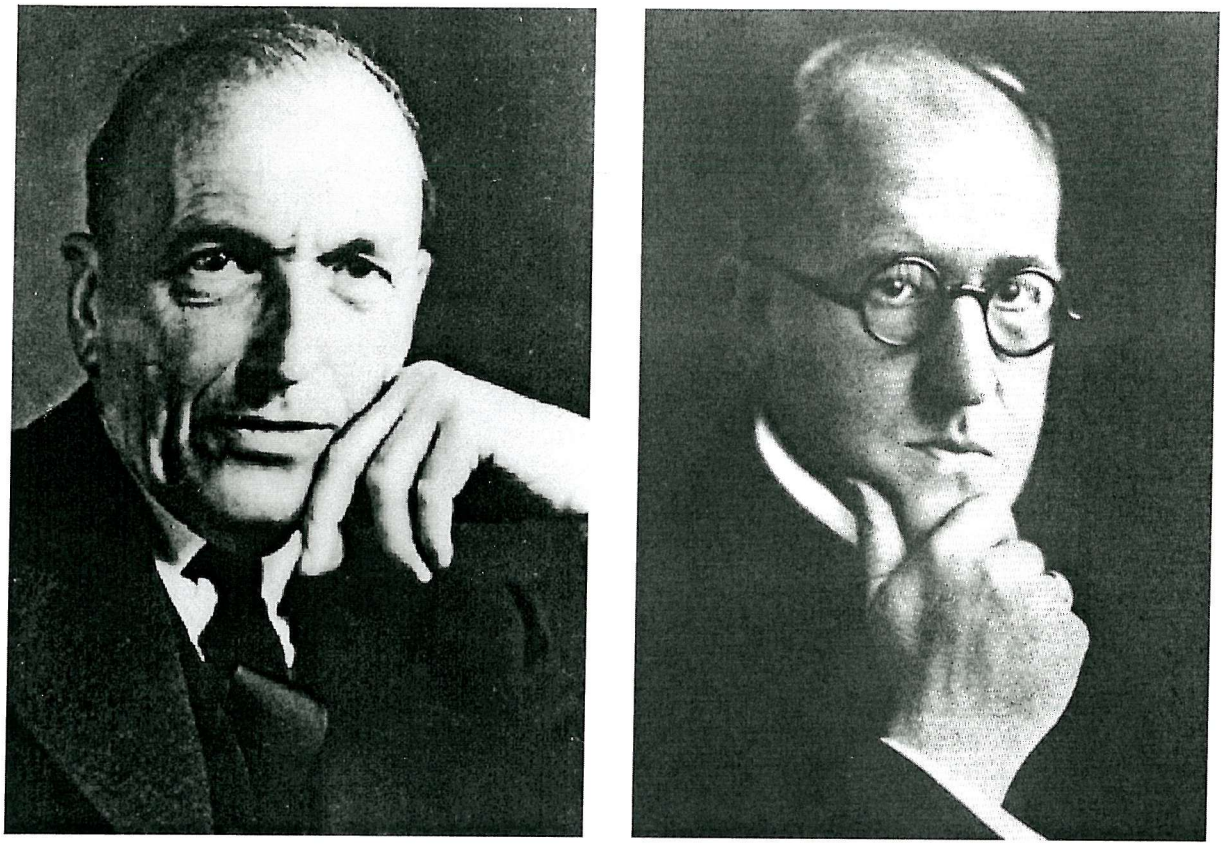
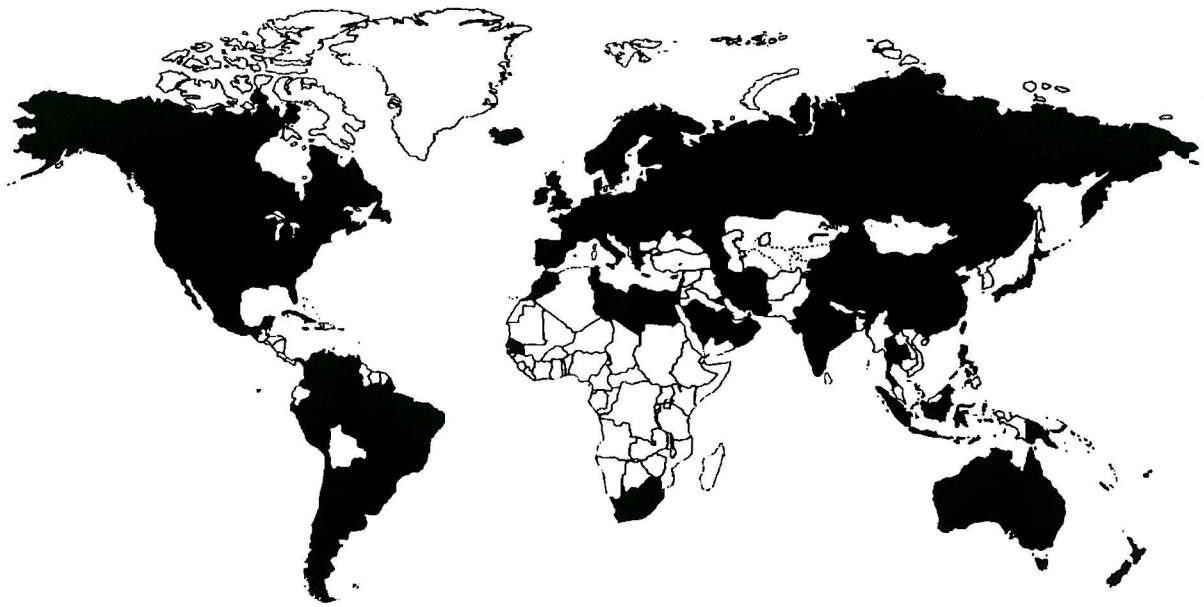


**Figure 2: Countries (in black) reporting Creutzfeldt-Jakob disease**



# INTRODUCTION

## HUMAN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES: A HISTORY

### Creutzfeldt and Jakob's original descriptions

Hans Gerhard Creutzfeldt (Figure 1) was born in Harburg, Germany on the 2nd of June 1885 and died in Kiel on the 30th of December 1964. After training in neuropathology with Ludwig Edinger he worked with Alois Alzheimer and later Walther Spielmeyer.[1] He was appointed director of the psychiatry and neurology clinic at the Christian-Albrechts University in Kiel from 1938 to 1945, and became rector of its research institute in 1948.[30]

In 1920 Creutzfeldt reported the case of a women who died aged 22 following a history of progressive cerebral dysfunction.[31] Bertha Elschker was born in 1890, the youngest of five children. Two of her sisters were 'mentally abnormal' and lived in an institution. When Bertha was nine-years-old her mother died of an unknown cause. At age 16 she was described as being childish and stubborn. Four years later she 'refused nourishment under the pretext that she wanted to become slender', her behaviour was described as indecisive, she was said to be easily influenced and had a 'remarkably awkward gait'. During the Summer of 1912 she developed 'hysterical exfoliative dermatitis' and when examined was found to have a generalised tremor and an abnormal gait with spasticity in the legs, the latter being variable and on one occasion apparently disappeared after what was considered an attack of hysterical collapse. Her gait subsequently showed a gradual improvement until May 1913 when she again became unsteady, but was also now neglecting her personal hygiene and assuming odd postures. She was admitted the following month with increasing unsteadiness and delusions that she was possessed by the devil and that both her and her sister were dead. Fever, spastic paraparesis, dysarthria, hyperaesthesia and hyperalgesia, myoclonus and a fluctuating level of consciousness were subsequently noted and she was markedly cognitively impaired. Her cerebrospinal fluid (CSF) contained seven lymphocytes and had a normal protein concentration. During her last weeks more pronounced myoclonus occurred and she became severely obtunded. In her final days she developed a herpetiform facial rash and died in apparent status epilepticus in August 1913.[32] Neuropathology revealed diffuse grey matter astrocytosis, numerous foci of tissue destruction, multiple neuronophagic nodules and many microglial rod cells, but neither spongiosis nor amyloid plaques.

Creutzfeldt had first met Bertha Elschker two months before her death when he was working as Alzheimer's assistant. They had studied her case in great detail and planned to publish a report. However the war intervened and Alzheimer died in 1915. Creutzfeldt completed the paper (in translation, 'On a particular focal disease of the central nervous system') four years later when working for Spielmeyer, who reviewed the neuropathology.[30]

Alfons Maria Jakob (Figure 1) was born in the Bavarian city of Aschaffenburg on the 2nd of July 1884.[33] On graduating from medical school in 1909 he worked with Kraeplin and Alzheimer in Munich and after the First World War he practised neuropsychiatry in Hamburg, where he became the head of the department. He held this

position up to his death age 47, which resulted from complications of a long standing osteomyelitis of his thigh.[30,34,35]

In the course of his work in Hamburg after the end First World War Jakob identified three patients with what he considered a unique syndrome. Following the study of these cases he was informed by Spielmeyer of Creutzfeldt's case and forthcoming publication,[31] and was given sections from the brain of Creutzfeldt's patient to examine. This convinced Jakob that Creutzfeldt's patient had the same disorder as his three cases.[36] Jakob's patients were a little older, but in many ways were clinically similar to Creutzfeldt's case. There were neuropathological similarities also: both Creutzfeldt and Jakob described a diffuse non-inflammatory disease process, with some foci of tissue destruction, mainly affecting the cerebral cortex, but also involving the caudate nucleus, putamen, and medial part of the thalamus, and accompanied by secondary degeneration of the corticospinal tracts down into the spinal cord.[37] In 1921 Jakob published accounts of his three patients titled in translation 'About diseases of the central nervous system with remarkable anatomical findings, spastic pseudosclerosis and disseminated encephalomyelopathy'.[37,38] A further report, 'Resembling pseudosclerosis', was published later in the same year.[39] Creutzfeldt and Jakob's cases were also considered by Spielmeyer to represent a distinct clinicopathological disorder and in 1922 he coined the term Creutzfeldt-Jakob disease (CJD).[40] The following year Jakob detailed a further case of 'spastic pseudosclerosis' in his book 'The extrapyramidal diseases'.[41]

Reviewing Creutzfeldt and Jakob's six cases suggests that only two were likely to have the entity now known as CJD. Creutzfeldt's case was extremely young, with symptoms possibly beginning as a teenager, and had a long relapsing and remitting clinical course with features that included fever and dermatitis. The hallmark pathological feature of CJD, spongiosis, was not noted and the other histological changes were not typical. Two of her siblings had a history of mental abnormality suggesting a familial disorder. Duckett argues, however, that the actual diagnosis may have been a herpes zoster encephalopathy.[30] Masters examined the original pathological sections from four of Jakob's cases (slides from case 4 were lost, but had originally been reported not to show spongiosis) and found that his first two patients did not have evidence of a spongiform encephalopathy.[42] Cases 3 and 5 did however have CJD with spongiform change and other typical features. The actual diagnosis of Jakob's three other cases remains unknown, but case 1 had a transiently positive Wassermann reaction in blood and CSF suggesting syphilis as a possible diagnosis and cases 2 and 4 gave a history of possible alcohol abuse and malnutrition and may have developed a fatal metabolic encephalopathy.

Therefore the first definite case of CJD reported was the third patient described by Jakob, Ernest Kahn, a 43-year-old soldier who died in 1919. During his nine-month illness he developed gait ataxia, ocular paralysis, dysarthria, visual hallucinations and increasing mental deterioration. Although pathologically confirmed as CJD this patient also demonstrated some atypical clinical features, in particular an initial fluctuating clinical course and the presence of lower motor neuron signs.[42,43]

A review of the literature by Nevin in 1958[44] identified only one possible case of CJD predating Creutzfeldt and Jakob's original descriptions. This patient was a 70-year-old huntsman described in a series by Fischer (case 12) in 1911 as part of a discussion on status spongiosus.[45] The patient had had a stroke 10 years previously causing a left hemiparesis and four months later developed sudden and persistent speech disturbance. The intervening history is unclear, but when examined he was noted to have receptive and nominal dysphasia, apraxia,

pyramidal signs and jerking movements of his left arm which were worse with movement. May also included this case in his list of CJD cases in which he considered there was adequate clinical and pathological data.[46]

Duckett and Stern in their detailed review of the early history of CJD have speculated why Spielmeyer proposed the term Creutzfeldt-Jakob's *disease*, when, at best, they considered that the connection between Creutzfeldt and Jakob's cases was as a neuropathological concept or syndrome, rather than a distinct disease.[30] They are particularly critical of Spielmeyer's phraseology which proposes the use of the term Creutzfeldt-Jakob disease on the basis of a hope that the characteristics would later become clear: 'The peculiar focal disorder of the cerebral cortex reported by Creutzfeldt has not remained solitary. Clinically it is above all characterised by spasms, hyperalgesias and psychiatric symptoms. In his material studied with exceptional care A. Jakob has discovered a whole series of cases of this disease. Thus we may hope that the clinical and anatomical characteristics of Creutzfeldt-Jakob disease (Jakob's spastic pseudosclerosis) *will* be well demarcated.'

The explanation for Spielmeyer's actions have been postulated to relate to the strong rivalry between his and Jakob's neuropathological departments. Spielmeyer was promoting Creutzfeldt's original contribution to a possible pathological syndrome, effected in Spielmeyer's laboratory, because Jakob and his pupils were claiming that he, Jakob, had discovered the syndrome, and that Creutzfeldt's work was anecdotal and only published after he, Jakob, had 'finished his studies'.[30] Creutzfeldt is said to have stated after the Second World War that his case did not bear any resemblance to Jakob's.[47]

Creutzfeldt and Jakob took turn in pole naming position for 70 years following their original reports. The reason why the term Creutzfeldt-Jakob disease was finally decided upon has been lost in history,[48] but perhaps most likely reflects that Creutzfeldt's paper was the first published. One other explanation is that alphabetical arrangements became in vogue, and Jarvik recalled that the change in designation was due to the tendency of mistaking Jakob for Creutzfeldt's first name, and attributing the description to a single person. Neither Creutzfeldt nor Jakob ever used or permitted their pupils to use the eponyms Creutzfeldt-Jakob disease or Jakob-Creutzfeldt disease while they were alive.[30]

**The evolution of the CJD concept**

Many of Creutzfeldt and Jakob's peers, including Jean Lhermitte, Douglas McAlpine and Kinnier Wilson, expressed considerable scepticism that CJD, as initially reported, represented a distinct nosological entity.[30] William McMenemey echoed these views and wrote that 'so-called CJD' was 'a convenient dumping ground for several instances of atypical presenile dementias which run a rapid course and have for their histology a parenchymatous degeneration of the brain with some glial hyperplasia'.[49] To confuse matters further, not only was CJD being used to describe conditions that were not CJD (by current definitions) but what we now call CJD was so ill-defined that virtually every description carried a new title.[50] CJD and its related human diseases have now amassed an impressive number of (still increasing) synonyms, totalling over 80 to date.[51,52]

The pathological hallmark of CJD is now considered to be the presence of a spongy vacuolation of the neuropil. Such changes were documented by Jakob in his 5th case and were noted in the two cases reported by Creutzfeldt's student Kirschbaum in 1924.[53] Four years later Adolf Heidenhain reported three patients of late middle age with a rapidly progressive cerebral disorder, characterised by cortical blindness in two. Pathologically, there was severe involvement of the occipital lobes - a region relatively spared in CJD as

reported up to that time. Moreover, spongy change was a prominent feature. Heidenhain thought, however, that his cases differed fundamentally from those of CJD.[54]

There matters stood until the 1950s, when several developments took place. In 1954, an English case closely resembling Heidenhain's was reported by Meyer and colleagues, who suggested that Heidenhain's syndrome was a variety of CJD.[55] In the same year, Jones and Nevin described two cases with what would now be considered the classical clinicopathological features of CJD. The patients presented in their 60s with a rapidly progressive dementia and developed widespread myoclonic jerks, pyramidal signs and generalised periodic electroencephalographic (EEG) discharges. Neuropathological examination showed diffuse neuronal loss in the cerebral cortex with striking gliosis and spongiform vacuolation.[56] A few years later Nevin and his collaborators reported eight similar cases,[44,57] but did not in any way identify this disorder as CJD. Others, however, did believe that the syndrome of rapidly progressive dementia with myoclonus and spongiform change belonged in the category of CJD because of some clinical and pathological features in common.[58-60] The early literature is well reviewed in an article by May,[46] Van Rossum's chapter in the Handbook of Clinical Neurology[61] and in Kirschbaum's monograph 'Jakob-Creutzfeldt disease',[62] all published in 1968. Kirschbaum described 150 cases in some detail and May reported that close to 200 cases had been documented since 1920, over half during the previous decade.[46] These reports described cases from all over the world, illustrating the geographical diversity of CJD (see Figure 2).

A major breakthrough in understanding CJD came through the study of kuru. This is a fatal progressive cerebellar ataxia that was epidemic in the 1950s and 1960s among tribespeople of the eastern highlands of Papua New Guinea.[63] The search for the aetiology of kuru was initially unfruitful, but a clue came from the disease's neuropathology, which was noted to be reminiscent of scrapie, a fatal neurodegenerative disease of sheep and goats.[64] 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.[65] Kuru was successfully transmitted to laboratory animals via intracerebral inoculation of brain tissue in 1965.[66] This finding combined with the results of anthropological studies lead to the conclusion that kuru arose through cannibalism.[67] Animal transmission experiments were subsequently initiated with CNS tissue from a wide range of neurodegenerative conditions, but only brain tissue from cases of CJD (a disorder with many neuropathological similarities to kuru[68]) were successful.[69] It's interesting to note that Jakob had actually suggested transmission experiments of CJD in 1923.[52]

Transmissibility placed CJD in a family of animal and human disorders referred to as the transmissible spongiform encephalopathies (TSEs). It also established a 'gold standard' for CJD diagnosis which facilitated the formulation of reliable pathological and clinical diagnostic criteria. In turn these enabled accurate case identification for a number of major studies in the 1970s and 1980s which now form the foundation of our understanding of CJD epidemiology.[70] The ability to experimentally transmit CJD also raised the possibility that the disease might be 'naturally' passed from man, or animal, to man. The former concern was realised in 1974 when the first report of iatrogenic CJD was published,[71] and over 260 such cases have now been documented.[25] However, despite all subtypes of CJD being experimentally transmissible, epidemiological studies suggest that the majority of cases occur sporadically without evidence of an acquired infection (see Table 1). Furthermore, a minority of cases are inherited. The multiple modes of acquisition of the TSEs sets them apart from other disorders and raises the question as to what kind of transmissible agent could cause such diseases.

In addressing this question, heroic efforts have been made over the past three decades to define the properties of the infective particle and the nature of the disease process of the TSEs. The transmissible agent shows remarkable resistance to viricidal and bactericidal treatments and appears to consist in part, if not entirely, of a host-encoded protein, the prion protein.[72] The one-time heretical protein-only (or 'prion') hypothesis has been championed since the early 1980s by Stanley Prusiner and has acquired sufficient scientific backing to become for many the accepted dogma. However, despite the increasing support for the prion hypothesis and the fact that, arguably, the TSEs have become the best understood of all the neurodegenerative conditions, the exact nature of the transmissible pathogen remains an unresolved and highly contentious issue.[73]

The combination of an enigmatic 'near-indestructible' infective agent and a devastating untreatable dementing disease has sparked ever-increasing interest in the TSEs among scientists and clinicians alike. This interest was intensified in the mid 1980s when a novel form of TSE was discovered in British cattle and concern was expressed that this might be transmitted to humans through eating meat.[74] Research into the TSE has generated over 7000 publications and CJD tops the 'index of interest' (the number of papers/number of cases) of medical conditions, beating its nearest rival by more than an order of magnitude.[75] Two Nobel prizes have been awarded for work related to TSEs, the first to Daniel Carleton Gajdusek for his studies on kuru[76] and the most recent to Stanley Prusiner for his contribution to the prion hypothesis.[77]

**A brief word on nomenclature**

Unfortunately much confusion surrounds the term used to describe CJD-related disorders of humans and the comparable diseases of animals, largely reflecting disagreements over the nature of the causative agent.[78-80] These conditions have commonly been referred to as transmissible spongiform encephalopathies, prion diseases, transmissible cerebral amyloidoses or slow-virus diseases. No term is perfect, but for reasons of clarity this thesis will mainly refer to these disorders as 'transmissible spongiform encephalopathies'. The terms 'slow-virus disease' and 'prion disease' are avoided as it is still not known if the transmissible agent is viral-like, containing DNA, or composed solely of abnormal prion protein (PrP). Furthermore some forms of disease have been reported in which the abnormal form of PrP can not be identified.[70,81,82] Although it has certain advantages, the term 'transmissible cerebral amyloidoses' it not used as it has traditionally been much less widely used than 'TSEs'. It is widely accepted that the group of human and animal neurodegenerative disorders in Table 1 belong to the same group of conditions, and although these will be called TSEs in this document, it is accepted that this term is also imperfect, as some of the human inherited forms of CJD may lack spongiform change neuropathologically or have yet to be transmitted.[83]

**Table 1: Human and natural animal transmissible spongiform encephalopathies**

| Transmissible spongiform encephalopathy | First report |
|---|---|
| **HUMAN** | |
| **Creutzfeldt-Jakob disease*** | |
| Sporadic (85%) | **1921**[38] |
| Familial (5-10%) | **1924**[53] |
| Iatrogenic (<5%) | **1974**[71] |
| Variant | **1996**[84] |
| **Gerstmann-Sträussler-Scheinker disease** | **1936**[85] |
| **Kuru** | **1957**[86] |
| **Fatal Familial Insomnia** | **1986**[87] |
| **ANIMAL** | |
| **Scrapie** | |
| Sheep | **1732**[88] |
| Goat | **1872**[89] |
| Moufflon | **1992**[90] |
| **Transmissible mink encephalopathy** | **1965**[91] |
| **Chronic wasting disease** | **1967**[92] |
| Mule deer | |
| Rocky Mountain elk | |
| **Bovine spongiform encephalopathy** | **1987**[93] |
| **Captive ruminants** | |
| Nyala | **1988**[94] |
| Gemsbok | **1988**[94] |
| Arabian oryx | **1990**[95] |
| Eland | **1990**[96] |
| Greater kudu | **1990**[97] |
| Scimitar-horned oryx | **1993**[98] |
| Ankole | **1996**[6] |
| Bison | **1997**[6] |
| **Feline spongiform encephalopathy** | |
| Domestic cat | **1990**[99] |
| Puma | **1992**[100] |
| Cheetah | **1992**[101] |
| Ocelot | **1994**[6] |
| Tiger | **1996**[6] |
| Lion | **1998**[6] |
| **Captive Primates** | |
| Rhesus monkey | **1996**[102] |
| Lemur | **1997**[103] |

*Percentages of CJD subtypes vary between countries

## Slow-virus concept

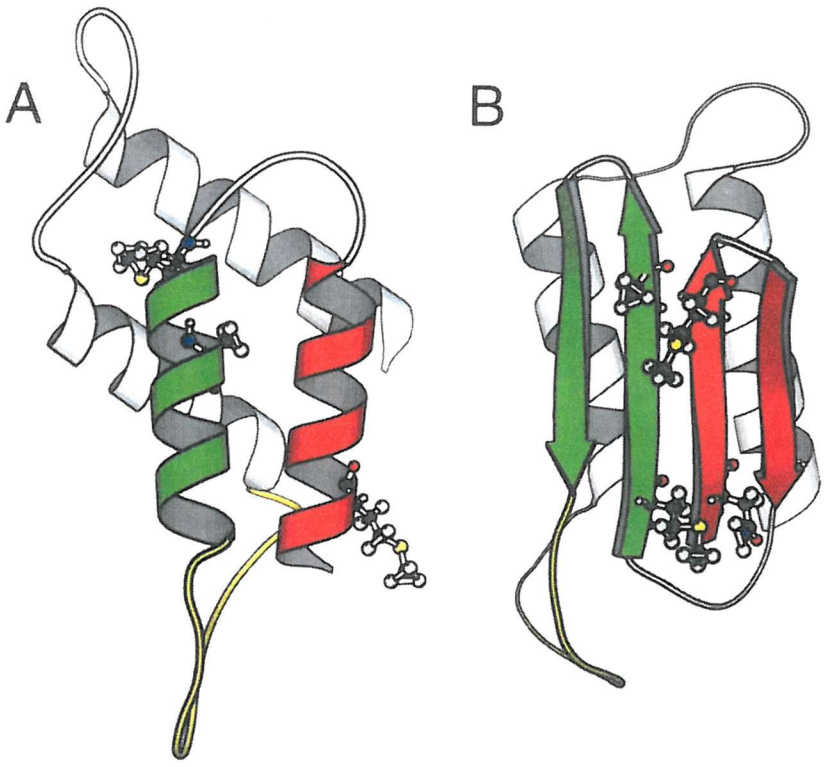
In 1954 the concept of 'slow-virus disease' was first introduced by Bjorn Sigurdsson while he was working on scrapie and visna of sheep.[104] Examples of slow-virus infections in humans include subacute sclerosing panencephalitis due to measles paramyxovirus, progressive multifocal leucoencephalopathy due to JC virus, rubella panencephalitis due to the rubella togavirus, tropical spastic paraparesis due to HTLV1 and AIDS due to the HIV virus. However, exhaustive, but unfruitful, efforts to find the 'TSE virus', and a conspicuous lack of inflammatory response, argued against a viral aetiology for TSEs. Furthermore, the infectious pathogen shows a remarkable resistance to treatments that would normally be expected to inactivate viruses (see Table 2 ).[105-108]

## Prion hypothesis

Tikvah Alper showed that scrapie infectivity survived a dose of ionising radiation that is incompatible with the biological integrity of nucleic acid, an observation that led to several theories about the agent being a membrane bound ligand, a lipid-protein-polysaccharide complex or an unadorned protein.[109] The latter hypothesis was endorsed by Griffith who published a radical theory in 1967 suggesting that the infectious agent could be a self-replicating protein.[110] Subsequent experiments showed that scrapie infectivity is associated with a partially protease-resistant protein, and in 1982 Prusiner coined the term 'prion' for the hypothetical agent.[72] He defined this as 'a proteinaceous infectious particle that lacks nucleic acid,' - the 'i' and the 'o' being switched for convenience.[111]

Advances in molecular biology thereafter contributed greatly to our knowledge of the TSE agent, often supporting Prusiner's protein-only hypothesis. Prion protein is now known to be a normal outer cellular membrane glycoprotein, expressed in many cell types,[112-114] but predominantly in neurons.[115] The normal protease-sensitive cellular form ($PrP^{C}$) is transformed into an abnormal protease-resistant isoform (termed $PrP^{Res}$ or $PrP^{Sc}$, where Sc refers to scrapie) in the disease state through a process whereby a portion of its α-helical coil structure is refolded into a β-sheet (see Figure 3).[116,117] The difference between the normal and pathological isoforms appears to be solely conformational and is achieved post-translationally. The prion hypothesis states that once produced, the abnormal isoform, $PrP^{Sc}$, acts as a template for the conversion of more $PrP^{C}$ to $PrP^{Sc}$. Thus, a chain reaction is set in motion with more and more $PrP^{C}$ being transformed into the pathological $PrP^{Sc}$ isoform (see Figure 4). The prion theory may help explain the central paradox of the TSEs: how can a disease develop as an inherited, sporadic, and infective disorder? It is suggested that the mutations associated with these hereditary disorders renders the mutant $PrP^{C}$ inherently unstable,[118-120] with a high tendency to fold into the disease-causing $PrP^{Sc}$ isoform. In sporadic disease the initial pathogenic PrP needed to seed the production of $PrP^{Sc}$ occurs as a rare spontaneous event, perhaps due to a somatic mutation of the PrP gene in one or more cells. Finally in the infective form of the disease the inoculated $PrP^{Sc}$ initiates the chain reaction of host $PrP^{C}$ conversion to $PrP^{Sc}$.

**Figure 3: Proposed three-dimensional structure (A) $PrP^C$ and (B) $PrP^{Sc}$**




In the figure of $PrP^C$ (left) helix 1 is shown in red and helix 2 in green. These helices are believed to be converted into ß-sheet structure during the formation of $PrP^{Sc}$ (right)[3]

**Figure 4: Proposed pathogenic interactions between $PrP^C$ and $PrP^{Sc}$**

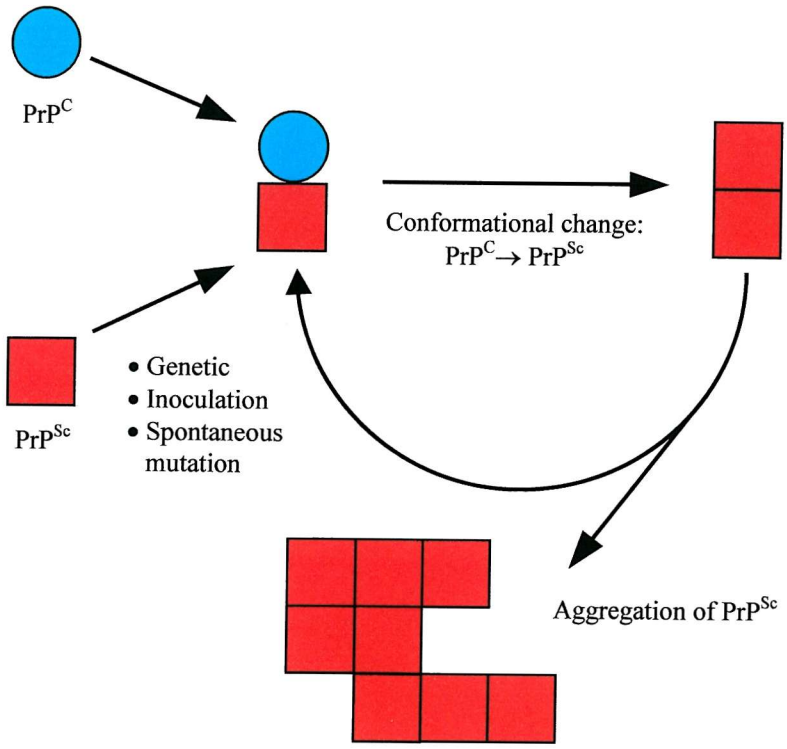


**Figure 5: Prion protein mutations (pink) and polymorphisms (blue)**

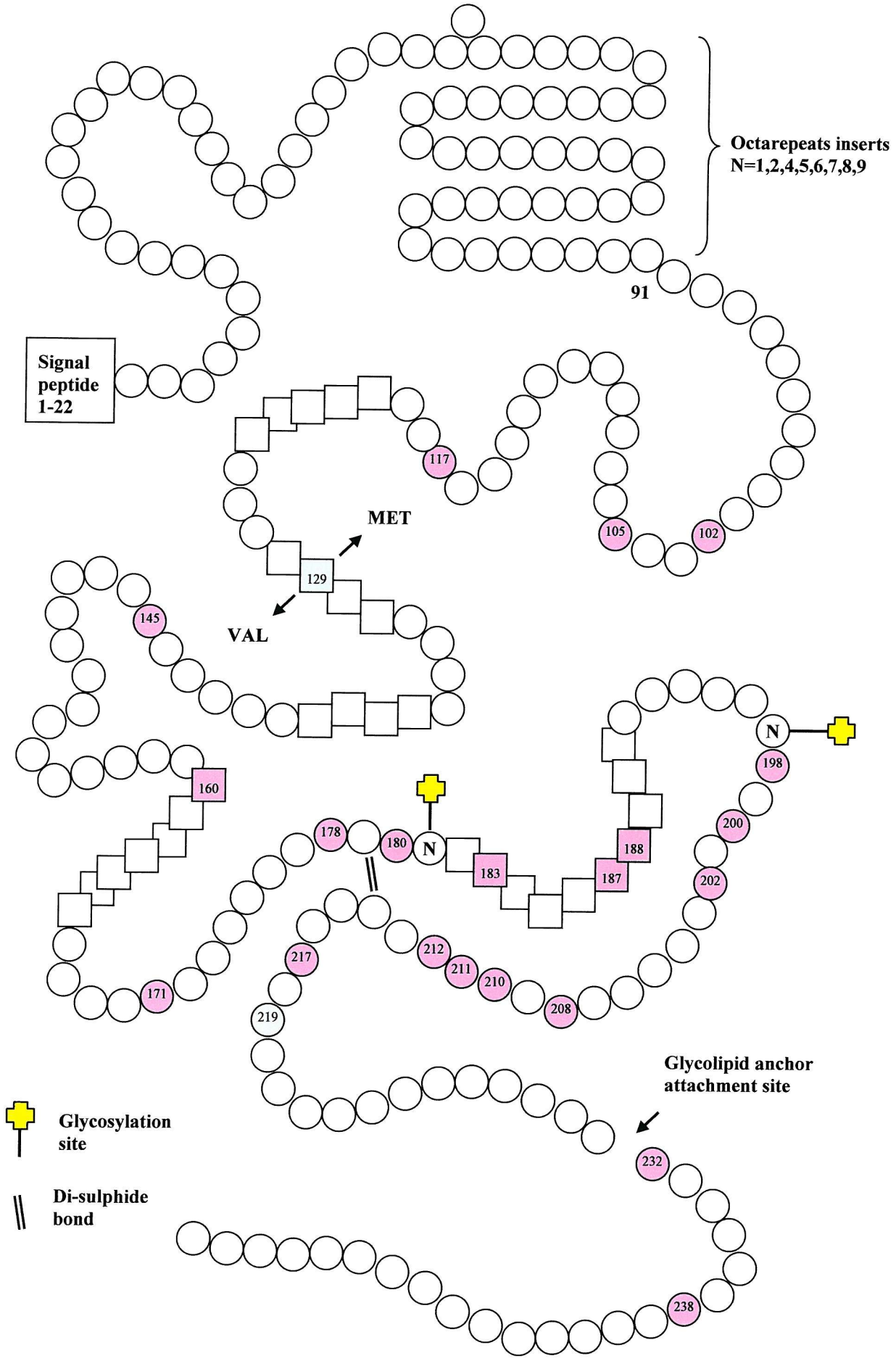


**Table 2: Chemicals and processes ineffective against TSE agents**

| Chemical disinfectants | Gaseous disinfectants | Physical processes |
|---|---|---|
| Alcohol | Ethylene oxide | Boiling |
| Ammonia | Formaldehyde | Dry heat (≤600°C)[121] |
| ß-propiolactone | | Ionising, UV or microwave radiation |
| Formalin | | |
| Hydrochloric acid | | |
| Hydrogen peroxide | | |
| Peracetic acid | | |
| Phenolics | | |
| Sodium dodecyl sulphate (5%) | | |

## Prion protein chemistry and normal function

Prion protein is the product of a gene found in organisms as diverse as the fruit fly and man.[122] Mapping of PrP genes to the short arm of human chromosome 20 and to the homologous region of the mouse chromosome 2 argues for the existence of PrP genes prior to the speciation of mammals.[123,124] The entire open reading frame of all known mammalian and avian PrP genes residues within a single exon.[125-128] In humans the PrP gene encodes a product of 253 amino acids, which includes four octapeptides contiguous with a preceding nonapeptide of similar sequence (see Figure 5). The protein is proteolytically processed in vivo to remove the 22-residue N-terminal signal peptide and the 23-residue C-terminal amino acids after addition of the glycophosphatidylinositol anchor to Ser-231.[111,129] The processed protein (size 35 kDa) contains a codon 179 to codon 214 disulphide crosslink and has glycosylation sites at Asn-181 and Asn-197.

In cells PrP moves along the usual pathways of endoplasmic reticulum and Golgi apparatus following translation, attaches to the plasma membrane during its maturity and then finally re-enters the cytoplasm. It has a half-life of 3-6 hours, cycling through the endosomal compartment from where 95% is returned to the surface with a transit time of 60 minutes.[130]

The normal function of PrP is unknown. The development and lifespan of two lines of PrP-deficient mice were indistinguishable from those of controls,[131,132] whereas two other lines reported by Sakaguchi exhibited ataxia and Purkinje cell degeneration at around 70 weeks of age.[133] In the former two lines altered sleep-wake cycles,[134] and altered synaptic behaviour have been reported,[135,136] although these findings have not been corroborated by others.[137] It has been suggested that the severe phenotype in the lines published by Sakaguchi was due to the deletion of flanking sequences that were present in the other 'normal' knockouts.[138] 'Turning off' $PrP^C$ expression by over 90% using a tetracycline transactivator in transgenic mice had no deleterious affect.[139] Therefore PrP does not appear to be vital for life. More recent studies have suggested that PrP may be important for cellular resistance to oxidative stress by influencing the activity of Cu/Zn superoxide dismutase.[140]

In the diseased host, the configurationally altered PrP is either deposited extracellularly as amyloid plaques or concentrated intracellularly in the synaptosomal region. Either way, its catabolism is impaired, showing no sign of turnover with cells,[141,142] and insoluble amyloid protein accumulates.[109]

## The mechanism of cell death

Cell death in experimental TSE has been suggested to occur due to apotosis,[143] a process that can be induced by exposure to $PrP^{Sc}$.[144,145] For cell death to occur studies indicate that the cells must express $PrP^{C}$ and microglial cells must be present.[146,147] The mechanism of $PrP^{Sc}$-induced apoptosis is unclear.

## Possible mechanism of prion replication

Two models have been postulated to explain the mechanism by which a misfolded form of PrP could catalyse the refolding of native PrP molecules into the abnormal conformation (i) the template assistance model, and (ii) the nucleation-polymerisation model.[148] In the first model, a $PrP^{Sc}$ monomer promotes the conformational conversion of $PrP^{C}$, or of a partially destabilised intermediate, to the $PrP^{Sc}$ conformation. In this model $PrP^{Sc}$ is inherently more stable than $PrP^{C}$, but kinetically inaccessible.[149] In the second model, the formation of $PrP^{Sc}$ is initiated by an aggregate of $PrP^{Sc}$ acting as a seed in a nucleation-dependant polymerisation. In contrast to the template assistance model, the $PrP^{Sc}$ monomer is less stable than $PrP^{C}$ but is stabilised upon binding to the $PrP^{Sc}$ aggregate.[150,151]

## Evidence for and against the prion hypothesis

Multiple arguments have been put forward arguing for or against the protein-only hypothesis.[152] That host PrP expression is required for disease to occur is beyond dispute, as PrP-null mice are resistant to infection. This does not, however, mean that PrP must be the agent itself as alternatively this molecule could be the receptor for the agent. Arguably the most persuasive evidence for the prion theory comes from transgenic mouse studies. The P102L mutation of Gerstmann-Sträussler-Scheinker disease (GSS) was introduced into the MoPrP transgene, and five lines of Tg(MoPrP-P101L) mice expressing high levels of mutant PrP developed spontaneous CNS degeneration consisting of widespread vacuolation of the neuropil, astrocytosis and numerous PrP amyloid plaques.[153-155] Brain extracts prepared from these mice subsequently transmitted CNS degeneration to Tg196 mice. Although some would argue that these results are difficult to explain by a mechanism other than the prion hypothesis, the results are tempered by the fact that brain tissue from the affected Tg(MoPrP-P101L) mice did not contain protease-resistant PrP[154,155] and other scientists have been unable to successfully repeat the experiment.[131,156] The definitive experiment demonstrating that normal PrP converted into the abnormal isoform in vitro can transmit disease has yet to be performed.

A number of key observations are difficult to reconcile with the prion hypothesis. The models of PrP replication described above would argue that perhaps only one, or at most a small number, of $PrP^{Sc}$ molecules should be sufficient to initiate the mass conversion of $PrP^{C}$ to $PrP^{Sc}$. However, experiments indicate that at least 100,000 molecules of $PrP^{Sc}$ are required to transmit infection by the most efficient route.[157] The decreasing incidence of sporadic CJD in the very elderly is also difficult to explain by the prion hypothesis - Wood argues if the

occurrence of sporadic CJD is due to a random event such as protein polymerisation or somatic mutation it would be expected that the incidence of disease would increase with age and that the slope or rate of such an increase might even rise with age.[158] However, probably the biggest hurdle to the protein hypothesis is the ability of the TSE agent to retain strain-specific properties independent of the primary structure of the PrP sequence (see below).

## Other hypotheses

The protein-only hypothesis is now the most popular explanation for the nature of the TSE agent, and superficially it seems difficult to conceive of a mechanism by which a conventional pathogen could explain the observed transmission characteristics of CJD, in particular the infectious nature of the hereditary form. Proponents of the 'viral' hypothesis[159-161] argue, however, that $PrP^C$ could be a receptor for a hitherto undetected virus and that upon interaction with this virus, the protein aggregates, resulting in the formation of amyloid.[162] The familial forms could be explained by the virus binding more tightly to mutant than non-mutant $PrP^C$.[163] Should such a viral-like TSE agent exist studies indicate that it would have to be very small, at most consisting of 100 nucleotides.[164] The difficulty with any viral hypothesis is that the host shows no immune response and the infective agent is remarkably resistant to processes that would be expected to destroy conventional viruses. The virino hypothesis seeks to explain these observations in the context of a DNA-containing agent. In the virino model, a host protein (presumably PrP) protects the transmissible agent's nucleic acid from degradation and prevents the host raising an immune response, since the protein/nucleic acid complex is seen as 'self'. The elegance of this model is that it reconciles the inconsistencies of the other viral and protein only hypotheses. However, it remains, like the prion theory, unproven.

A number of other suggestions have been put forward to explain the nature of the TSE agent. Bastian has proposed that this could be a spiroplasma-like pathogen,[165-167] although others have shown that Bastian's 'spiroplasma' look very similar to crystalline artefacts.[168] Neither spiroplasma nor other mycoplasmas were found in a series of CJD cases.[169] Alkaloidal glycosidase inhibitors and organophosphates have both been postulated to cause the conversion of $PrP^C$ to $PrP^{Sc}$,[170,171] although the evidence in support of these hypotheses is lacking. Ebringer and Pirt have proposed an autoimmune cause for TSEs,[172] although this model has been severely criticised.[6]

## Properties of the infectious agent and disease pathogenesis

The efficiency of TSE transmission from donor to host is dependent on several factors, including dose and route of entry. A higher dose of infectivity leads to a relatively short incubation period, with a greater probability of successful transmission. The route of infection decreases in efficiency in the following order.

| | |
|---|---|
| Intracerebral | most efficient |
| Intravenous |  |
| Intraperitoneal | |
| Subcutaneous | |
| Intragastric | least efficient |

It is of note that the intragastric route has the lowest efficiency, requiring in Syrian hamsters about $10^5$ times more infectious dose than the highly efficient intracerebral route. However, this needs to be interpreted with caution as experiments with mice using BSE brain showed that the minimal incubation period after oral and intra-cerebral challenge were comparable (435 days and 407-434 days respectively).[173]

**Pathogenesis**

Studies of experimental scrapie in rodents show that when peripheral routes of inoculation are used, including gavage and oral dosing, replication of the TSE agent occurs in the lymphoreticular system (LRS) before it can be detected in the CNS.[157,174-177] A very similar pattern with the LRS becoming infected before the CNS has been recorded in natural sheep scrapie.[178] The infective agent replicates almost immediately after oral administration in Peyer's patches[175] and can be detected in the spleen as early as four days after both intraperitoneal and, surprisingly, intracerebral infection.[179] The agent reaches the brain from the spleen probably via the visceral sympathetic fibres of the splanchnic nerves which facilitate the agent entering the mid thoracic spinal cord, from where it appears to pass caudally at a maximum rate of about 1mm/day.[157] It is interesting that splenectomy in the early stages of the disease delays neuroinvasion, illustrating the importance of the lymphoreticular system in the initial stages of infection. Once infection has passed to the brain and spinal cord it can pass centrifugally to the peripheral tissues, and this may account for the low and inconsistent infectivity at these sites.

That the LRS plays an important role in the neuroinvasion of the TSE agent is generally accepted, but which cells are involved in this process is somewhat controversial. Mice with severe combined immune deficiency, which lack B and T lymphocytes and mature follicular dendritic cells (FDCs), are relatively resistant to peripherally but not centrally inoculated TSE.[180] Experiments in PrP knockout mice which are selectively deficient in some cellular components of their immune systems[181,182] suggest that B lymphocytes and FDCs are essential for peripherally routed disease to occur. However, PrP expression on B cells is not required for prion neuroinvasion[182] and it therefore seems likely that FDCs are probably of most importance in TSE agent propagation, with B cells being required to aid FDC maturation rather than acting as sites of agent replication or transport.

**The species barrier**

An important factor determining the transmissibility of the TSE agent is the 'species barrier'. This refers to the greater difficulty that exists when trying to transfer infection across species compared to within the same species. This was discovered in the 1960s when it was found to be difficult to transmit scrapie from sheep to rodents. As a consequence of the species barrier when a TSE is transmitted from one species to another, the incubation period is usually longer than that seen on subsequent passage within the new species and there may be survivors or no transmission at all.[183] The exact cause of this effect is unclear but some argue that it results largely from the similarity between host and donor PrP sequence. This is discussed in further detail later. Prusiner argues that a third factor, in addition to donor/host PrP sequence homology and agent strain (see below), contributes to the species barrier. This factor is hypothesised to be a molecular chaperone that binds to

$PrP^{C}$ and facilitates $PrP^{Sc}$ formation. The existence of such a molecule is suggested by complex studies involving transgenic mice. Prusiner believes this chaperone to be a protein and has provisionally designated it 'protein X'.[184]

**Agent strain**

TSE agents are known to exhibit 'strain-specific' properties independent of the PrP sequence. Agent strain is reflected in transmission characteristics, in particular incubation period, and host clinical features and pathology.[185] A single source of scrapie can contain more than one strain of agent and mutation of strains has also been demonstrated.[186] These properties have been a challenge for the proponents of the protein-only hypothesis to explain, and argue that the agent contains an informational molecule such as DNA.

Prusiner has sought to reconcile the prion hypothesis with the observed strain phenomena. He postulates that $PrP^{Sc}$ could exist in various different conformations (at least one for each prion strain) with each type of prion being capable of imparting its own conformation to the $PrP^{C}$ molecule with which it interacts. Thus 'strain' is a product of the three-dimensional structure of the PrP molecule. In support of this 'molecular strain' hypothesis is the observation of distinct phenotypes termed 'hyper' and 'drowsy' in hamsters after infection with transmissible mink encephalopathy (TME). The syndromes differed with respect to clinical signs, incubation period, titre, brain lesion profile and PrP Western blotting patterns.[187,188] These banding patterns are thought to reflect different protease cleavage sites of the PrP molecule reflecting different three-dimensional conformation.

Similar studies have attempted to correlate PrP Western blotting patterns with clinicopathological subtypes of CJD. Parchi concluded that the physicochemical properties of $PrP^{Res}$ in conjunction with codon 129 genotype (see later) largely determine this phenotypic variability in CJD, and allow a molecular classification of the disease variants (see Table 3).[189] The use of this technique needs to be interpreted with caution as molecular strain has been shown to vary between brain regions.[190] In contrast, biological strain typing from different areas (cerebrum, cerebellum and olfactory bulb) has shown similar results.[191]

**Table 3: Molecular and phenotypical features of the sporadic CJD variants**

| Sporadic CJD variant | Previous classification | % of cases | DURATION (MONTHS) | Clinical features | Neuropathological features |
|---|---|---|---|---|---|
| MM1 or MV1 | Myoclonic, Heidenhain variants | 70 | 3.9 | Rapidly progressive dementia, early and prominent myoclonus, typical EEG; visual impairment or unilateral signs at onset in 40% of cases | "Classic CJD" distribution of pathology; often prominent involvement of the occipital cortex; "synaptic type" PrP staining; in addition, one-third of cases show confluent vacuoles and perivacuolar PrP staining |
| VV2 | Ataxic variant | 16 | 6.5 | Ataxia at onset, late dementia, no typical EEG in most cases | Prominent involvement of subcortical, including brainstem, nuclei; in neocortex, spongiosis is often limited to deep layers; PrP staining shows plaque-like, focal deposits, as well as prominent perineuronal staining |
| MV2 | Kuru-plaque variant | 9 | 17.1 | Ataxia in addition to progressive dementia, no typical EEG, long duration (>2years) in some cases | Similar to VV2 but with presence of amyloid-kuru plaques in the cerebellum. And more consistent plaque-like, focal PrP deposits. |
| MM2-thalamic | Thalamic variant | 2 | 15.6 | Insomnia and psychomotor hyperactivity in most cases, in addition to ataxia and cognitive impairment, no typical EEG | Prominent atrophy of the thalamus and inferior olive (no spongiosis) with little pathology in other areas; spongiosis may be absent of focal, and $PrP^{Sc}$ is detected in lower amount than in other variants. |
| MM2-cortical | Not established | 2 | 15.7 | Progressive dementia, no typical EEG | Late confluent vacuoles with perivacuolar PrP staining in all cortical layers; cerebellum is relatively spared |
| VV1 | Not established | 1 | 15.3 | Progressive dementia, no typical EEG | Severe pathology in the cerebral cortex and striatum with sparing of brain stem nuclei and cerebellum; no large confluent vacuoles, and very faint synaptic PrP staining. |

## Epidemiology

CJD occurs as a sporadic disease in about 85% of cases.[192] The incidence of CJD (sporadic, familial and iatrogenic) from published studies is approximately one case per million population per year, (see Table 4).[192,193] The disease occurs worldwide (see Figure 2) at a roughly similar rate in countries where CJD has been studied.[2] The variation of incidence around the world most likely reflects the efficiency of case detection and population demographics (i.e. developing countries with a relatively small elderly population and no surveillance system report a low incidence). Furthermore, in some countries diagnostic facilities such as EEG may not be readily available and post-mortem examination may be culturally unacceptable.[194] The possibility that other factors, perhaps genetic, contribute to the very low incidence in Africa is raised by the observation that the age adjusted mortality rate of CJD in black people in the USA was only 40% that for whites.[195] This observation may, however, have an alternative explanation such as a relatively poor access to health care facilities among black people in America compared to whites.

The sex incidence is probably equal,[46,196-198] though some studies have found a slight male preponderance[199,200] and others the reverse.[195,201-207]

For sporadic CJD, the mean age of onset is about 65 years,[2,70,193,196,197,200-203,206,208-210] but the disease has occurred in a patient of 92,[211] and one as young as 14.[212] The bell-shaped age-distribution of sporadic CJD has been seen consistently in repeated studies (see Figure 6).[206] The disease is extremely rare in young persons: the annual incidence of sporadic CJD in persons aged less than 40 and 30 is approximately 50 and five per billion respectively.[192,193,211] One explanation for the fall-off in CJD incidence in the very elderly could be that there is under-ascertainment in this group, perhaps due to misdiagnosis as Alzheimer's disease or other common cause of dementia. This hypothesis is not supported by autopsy series which show that the identification of clinically unrecognised cases of CJD is exceptional.[213] The explanation of the declining CJD incidence in persons aged over 70 remains unexplained, but one theory is that sporadic CJD is due to the acquisition of the transmissible agent in youth. If the agent infected virtually the entire population but was only pathogenic (with an incubation period of several decades) to those individuals who acquire a mutated form of agent then a bell-shaped distribution of affected individuals would occur.[214]

A number of papers report apparent geographical clusters of sporadic CJD[215-218] and some studies have shown a relatively high incidence of CJD in densely populated areas.[197,219-221] Brown reported a husband and wife who died less than five years apart from pathologically confirmed sporadic CJD when aged 53 and 55 respectively.[222] Three cases have been documented in persons marrying into families affected by CJD[197,203,218,219] and Leiderman reported the occurrence of pathologically confirmed sporadic CJD in two unrelated schoolteachers both aged 48 who shared a school wing for nine months.[223] However, all these geographical associations are most likely to be a consequence of chance occurrence or ascertainment bias.[2,203,224] Systematic national surveys of sporadic CJD suggest that cases are randomly distributed within countries.[2,206,207]

**Table 4: Annual incidence of CJD (per million) around the world**

| Country | Annual incidence | Surveillance period | Country | Annual incidence | Surveillance period |
|---|---|---|---|---|---|
| Australia[225] | 1.12 | 1993-mid 2000 | Israel[226] | 0.61 | 1997-1999 |
| Austria[225] | 1.06 | 1993-mid 2000 | Italy[225] | 0.81 | 1993-mid 2000 |
| Belgium[226] | 1.26 | 1997-1999 | Japan[2] | 0.58 | 1985-1996 |
| Canada[225] | 0.75 | 1998-mid 2000 | Netherlands[225] | 1.01 | 1993-mid 2000 |
| Chile[2] | 0.69 | 1978-1983 | New Zealand[2] | 0.88 | 1980-1989 |
| Czechoslovakia[2] | 0.66 | 1972-1986 | Norway[226] | 0.83 | 1997-1999 |
| Denmark[226] | 1.32 | 1997-1999 | Slovakia[225] | 1.18 | 1993-mid 2000 |
| Finland[226] | 1.28 | 1997-1999 | Spain[225] | 0.69 | 1993-mid 2000 |
| France[225] | 1.37 | 1993-mid 2000 | Sweden[226] | 1.42 | 1997-1999 |
| Germany[225] | 0.99 | 1993-mid 2000 | Switzerland[225] | 1.34 | 1993-1999 |
| Greece[226] | 0.54 | 1997-1999 | UK[225] | 0.97 | 1993-mid 2000 |
| Ireland[226] | 0.90 | 1997-1999 | USA[227] | 0.95 | 1979-1994 |

**Figure 6: Age-specific incidence (cases per million per year) of sporadic CJD in the UK 1990-April 2001**

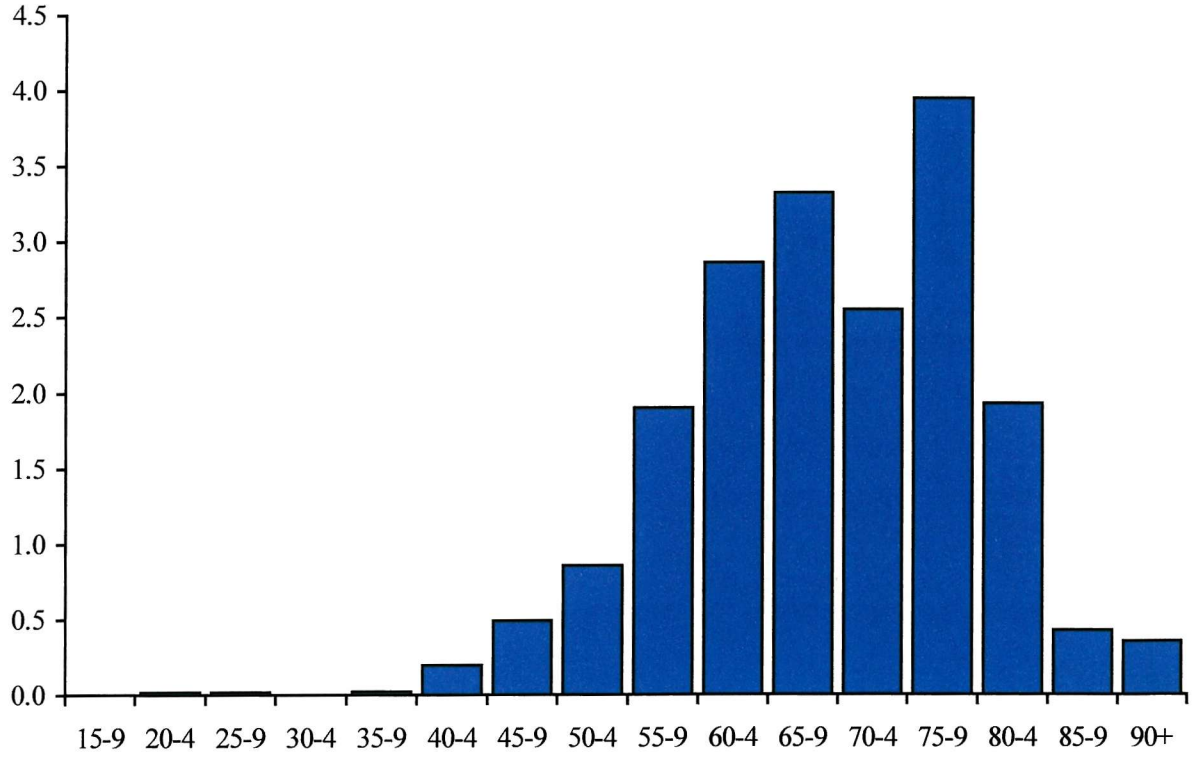


**Table 5: Significant risk factors for CJD in controlled studies**

| Study population | Method | Risk factors |
|---|---|---|
| **North America** | | |
| 1966-1970[228] | 38 'selected' cases, healthy controls | None |
| **Japan** | | |
| 1975-7[229] | 60 cases, healthy controls | Trauma in males and surgery within five years of onset |
| 1964-78[216] | 88 autopsied cases, autopsied controls | Organ resection |
| **USA** | | |
| 1970-81[230] | 26 cases, 40 controls | Trauma or surgery to head or neck, other trauma, surgery requiring sutures, tonometry |
| 1970-81[231] | As above | Roast pork, ham, rare meat, hot dogs |
| 1970-81[232] | As above | Contact with fish, rabbits, squirrels |
| **England & Wales** | | |
| 1980-1984[233] | 92 cases and 184 controls | Zoster in adult life, keeping cats, pets other than cats/dogs, dementia in family |
| **European Union** | | |
| 1993-5[234] | 405 cases and 405 controls | Consumption of raw meat and brain, exposure to leather products and fertiliser consisting of hoofs and horns |
| **Australia** | | |
| 1970-97[235] | 241 cases and 784 controls | Surgical procedures, especially eye/cataract, hysterectomy and multiple operations |

One previously popular hypothesis for the causation of sporadic CJD is that the disease results from acquisition of the scrapie agent,[236,237] for example through eating sheep's eyeballs[238] or brain.[239] In support of this theory scrapie has been shown to be experimentally transmissible via the oral route to primates.[240] Brown reports two CJD cases that raise the possibility of more exotic routes of exposure: one patient had a history of a caprine hip joint transplant and another case had previously had sexual relations with a goat.[203] In the same patient series Brown identified two shepherds with CJD, giving an annual mortality rate of 3.81 for this occupational group. However, this finding has to be interpreted with caution due the small sample size and furthermore a similar elevated mortality rate was reported for priests and nuns.[203] Perhaps the strongest argument against the hypothesis that sporadic CJD is causally related to scrapie is that there is a relatively consistent incidence of CJD around the world, including in countries with a low or no incidence of scrapie.[241] Furthermore, Chatelain

reported that in France the geographic distribution of scrapie-affected flocks was unrelated to the residential location of patients dying of CJD.[242] The occurrence of sporadic CJD in longstanding vegetarians argues against the possibility that all cases of sporadic CJD arise through dietary exposure to a scrapie-like agent of animal origin.[243,244]

Uncontrolled studies and case reports have suggested a number of other potential risk factors for sporadic CJD including contact with ferrets,[245,245] eating squirrel brains,[246,247] craniotomy,[218] medical/paramedical profession,[200] and a family history of dementia.[200] Clearly these findings have to be interpreted cautiously due to the small number of cases involved and the lack of a control group.

Case-control studies using different methodologies (see Table 5) have been conducted in Japan,[216,229] USA,[228,230-232,248,249] UK[230-233,248,249] and the European Union.[234] These studies have each identified a number of apparent risks for the development of sporadic CJD, although no factor has been consistently implicated. A meta-analysis of three of these studies consisting of 178 patients and 333 controls failed to identify any statistically significant dietary, occupational or past medical risk factors.[250] Despite the lack of clearly identifiable environmental risk factors from case-control series, due to the inherent statistical and other limitations of these studies, the possibility that sporadic CJD cases occasionally arise due to environmental exposure to an infective source can not be ruled out.[2]

## Clinical features

The presenting features of sporadic CJD are protean, presumably reflecting the disparate areas of brain first involved in the pathological process (see Annex 1).[70,196,208,210,211,251-254] A number of authors have placed an emphasis on prodromal symptoms, such as headache, fatigue, sleeping difficulties, weight loss and diarrhoea. However, the significance of these is difficult to interpret as they are non-specific and in one series many of these symptoms were just as common in a group of hospital controls.[2] Patients usually present (in order of decreasing frequency) with cognitive decline, ataxia or visual disturbance, either alone or in combination (see Annex 2).[251,255,256] Eponymous names have been given to CJD variants presenting with a pure cerebellar disorder (Brownell-Oppenheimer syndrome)[60] and cortical visual symptoms (Heidenhain syndrome),[54] these terms emanating from a time when CJD was referred to by a myriad of synonyms. Up to six percent of cases present with a stroke-like onset.[257] Other unusual presenting features includes pruritus,[258] cortical deafness[259,260] progressive aphasia[261-263] palinopsia,[264] an isolated myoclonic alien hand[265] and apparent complex partial status.[266]

Dementia is invariably present during the course of the illness and myoclonus, although a rare presenting feature, is observed at some stage in nearly all cases. Visual abnormalities are also common and include non-specific blurring, visual field defects, perceptual abnormalities and occasionally hallucinations.[267] Seizures virtually never occur at presentation and are only observed later in the clinical course in about 20% of patients. As the disease progresses multi-focal CNS failure occurs with increasing global cognitive dysfunction, ataxia, dependency and urinary incontinence, culminating in the patient becoming bedbound, mute and unresponsive. Physical pain is an uncommon feature at any stage of the illness and, due to the rapid progression of cognitive impairment, any retained insight is usually soon lost. Terminally, the patients are usually rigid, frequently

cortical blind, dysphagic (predisposing to aspiration and pneumonia, the commonest cause of death) and may develop Cheyne-Stokes respiration.

Physical signs correspond with the global CNS involvement and may include a combination of cerebellar, pyramidal, and extrapyramidal signs. Primitive reflexes, paratonic (gegenhalten) rigidity, cortical blindness and akinetic mutism are also common, whereas lower motor neuron signs are rarely observed. Myoclonus is probably the most important clinical sign. It usually shows some asymmetry; is typically arrhythmic, asynchronous and stimulus sensitive; and noted most frequently in the limbs, but also commonly affects the body and/or face. Stimulus sensitive myoclonus and/or a startle reaction[268] can occur in response to sudden noise, visual threat, touch, or muscle stretch, but usually myoclonus is also noted at rest.

The median and mean duration of illness of sporadic CJD in Western countries are approximately 4.5 and eight months respectively.[70,210] About 10% of patients survive more than a year and five percent longer than two years.[70] Exceptionally, cases with illness duration greater than five years have been described.[269] The shortest illness course is around two weeks.[211] In Japan the median and mean duration of illness is reported to be 12.8 and 16.6 months respectively.[208] Many of these Japanese cases were the panencephalopathic type, which is characterised by involvement of both grey and white matter and a relatively long clinical duration.[270] A possible explanation for the relatively prolonged survival and pathological features of the Japanese cases is intensive supportive treatment in the terminal stages of the illness. However, this seems unlikely to be the only explanation as the average duration between onset and akinetic mutism for these cases was prolonged at 10.5 months. The mean duration of illness was relatively short (six months) in a series of 30 cases of CJD in India.[209] A study of over 500 CJD cases in Europe found that the clinical features of CJD did not significantly influence illness duration except for vertigo, visual disturbances and pseudobulbar signs which were associated with a slight reduction in survival and seizures which were associated with an increased survival.[211] Female sex was also associated with a marginal, but statistically significant, prolongation of illness duration and young age at onset was more clearly linked to long duration.[211] A study of 108 German sporadic CJD cases found a statistically significant association between shortened illness duration and presence of a periodic EEG. Basal ganglia high signal on MRI scanning and positive 14-3-3 CSF protein test were not significantly associated with total length of clinical course.[271] PrP subtypes VV1, MM2 and MV2 are all associated with a statistically significant prolongation of illness duration.[189]

## Pathology

Neuropathology has been the mainstay for diagnosis of human TSEs for many decades. The characteristic features include spongiform change, neuronal loss, reactive proliferation of astrocytes and microglia and the accumulation of disease-associated PrP in the brain (see Figure 7).[272] Spongiform change is the term given to the characteristic vacuolation of the grey matter occurring in TSEs, usually in nerve cell processes but occasionally in neuronal cell bodies.[273] These vacuoles measure 2-20μm and can coalesce to form larger cyst-like cavities. The distribution of the pathological changes in TSEs is highly variable both from one region of the brain to another, and between cases.[274] Pathological phenotypic variation is a well recognised phenomenon in sporadic, inherited and acquired forms of human TSE. In sporadic CJD, there is evidence to suggest that the codon 129 PrP genotype and the PrP biochemical subtypes are major influences on the pattern of pathology in the brain.[24]

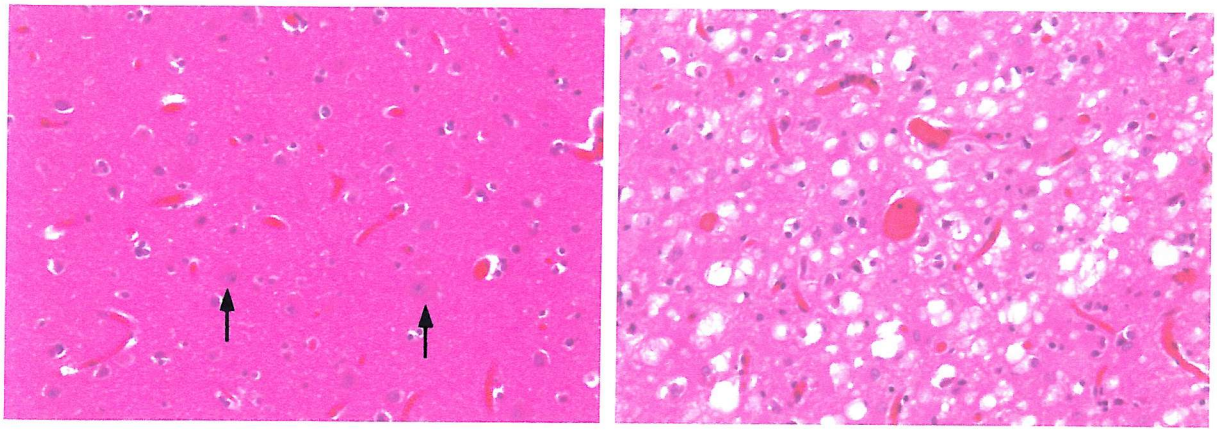
The development of techniques to detect the accumulation of PrP in the brain represent a major advance in the diagnosis and study of TSE.[275] The abnormal form of PrP accumulates in the CNS in all forms of TSE and in human diseases a wide range of patterns of accumulation have been described including perivacuolar, synaptic, neuronal and plaques.[274] In some human TSEs, 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 TSEs, particularly GSS, and in some forms of acquired human TSEs including CJD in human growth hormone (hGH) recipients. In sporadic CJD, amyloid plaques characteristically occur in individuals who are heterozygotes at codon 129.[24] 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 TSEs necessitates comprehensive neuropathological study following autopsy. Consensus guidelines for autopsy protocols, tissue handling and storage in CJD have been published, along with recommendations for diagnosis of human TSEs by neuropathological techniques, including PrP immunocytochemistry.[276-278]

The accumulation of PrP in the brain can also be demonstrated in unfixed tissue by biochemical techniques, usually by Western blotting.[24] In 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 relate to disease phenotype in sporadic CJD as described above (see Table 3).[189]

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 TSEs. Using conventional techniques, there is a wide range of differential diagnoses for human TSE, since spongiform change can occur in other conditions including Alzheimer's disease, Lewy body diseases, cerebral oedema and various metabolic disorders affecting the brain.[274] Rare cases of sporadic[279] and mutation-related (codon 181 and 188)[280] CJD and FFI[281] have been reported with negative immunocytochemistry.

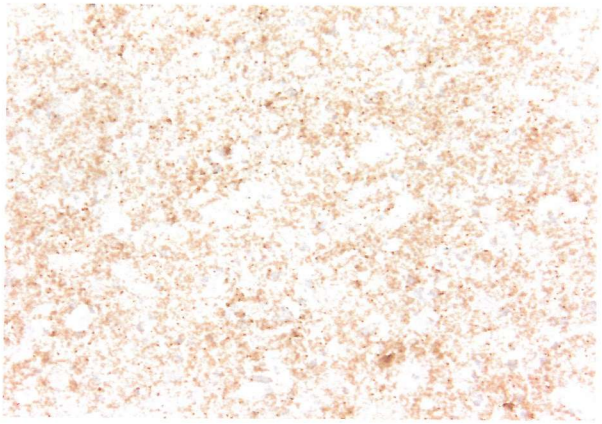
When pathological changes and infectivity first occur in the CNS in sporadic CJD is not known. Spongiform change and astrogliosis were identified in the corpus striatum of a hGH recipient who died of pneumonia but had no signs of neurological disease.[282] A study of cattle challenged orally with BSE showed that infectivity in the brain predated PrP detection by immunocytochemistry which predated vacuolar change which in turn occurred prior to the onset of symptoms. Infectivity was detected in the brain and spinal cord at 32 months, but not 28 months post inoculation, in animals that would be expected to have showed clinical signs by 37 months.[283]

**Figure 7: Pathology of CJD**



Cerebral cortex H&E x200: control (left) and in sporadic CJD (right) showing spongiform change and relative loss of neurons (arrowed in control)

Cerebral cortex GFAP x200: control (left) and in sporadic CJD (right) showing astrocytosis



Cerebral cortex KG9 x200: control (left) and in sporadic CJD (right) showing diffuse staining of PrP

## Differential diagnosis

Approximately 50% of cases referred to the UK National CJD Surveillance Unit have a final alternative diagnosis. The differential diagnosis of CJD is extensive (see Table 6). The most common condition that mimics CJD (in series of pathologically verified cases) is Alzheimer's disease followed by vascular dementia and Lewy body disease.[255,284] Treatable causes of a CJD-like syndrome are usually easily detectable by simple routine investigations. A minority of CJD suspects recover without a diagnosis being established and may be labelled as having had an 'idiopathic encephalopathy'.[285]

**Table 6: Conditions which can mimic CJD**

**Degenerative**

- Alzheimer's disease[253,255,284,286-288]
- Vascular dementia[70,253,255,284]
- Diffuse Lewy body disease[284]
- Frontotemporal dementia[253,255]
- Progressive supranuclear palsy[70]
- Huntington's disease[255]
- Familial spinocerebellar degeneration[253]
- Corticobasal degeneration[253]
- Parkinson's disease[253]

**Neoplastic**

- Brain tumours[251]
- Reticulosarcoma[251]
- Cerebral lymphoma[284]
- Angiotropic lymphoma[289]
- Metastatic carcinoma[284]

**Infective**

- Cryptococcal meningoencephalitis[290]
- Subacute sclerosing panencephalitis[70]
- Multiple cerebral abscesses[253]
- PML[251]
- Viral encephalitis[253,284]
- AIDS-dementia[291]

**Metabolic**[292]

- Hypoglycaemia[293]
- Hashimoto's encephalopathy[255,294]
- Hyperparathyroidism[295]
- Hypoxia[284]
- Hepatic encephalopathy[284]
- Mitochondrial encephalopathy[284,296]

**Drug-induced encephalopathies**

- Bismuth[297,298]
- Amitriptyline[299]
- Mianserin[300,301]
- Lithium[302-309]
- Baclofen[310]

**Other**

- Bilateral Ammon's horn atrophy[251]
- Neuroaxonal dystrophy[251]
- Extradural haematoma[70]
- Bilateral internal capsule haematomas[70]
- Diffuse subcortical gliosis[70]
- Ceroid lipofuscinosis[70]
- Multiple sclerosis[255,284]
- Sarcoidosis[70]
- Limbic encephalitis[253]
- 'Paraneoplastic syndromes'[255]

PML = progressive multifocal leucoencephalopathy

## KURU

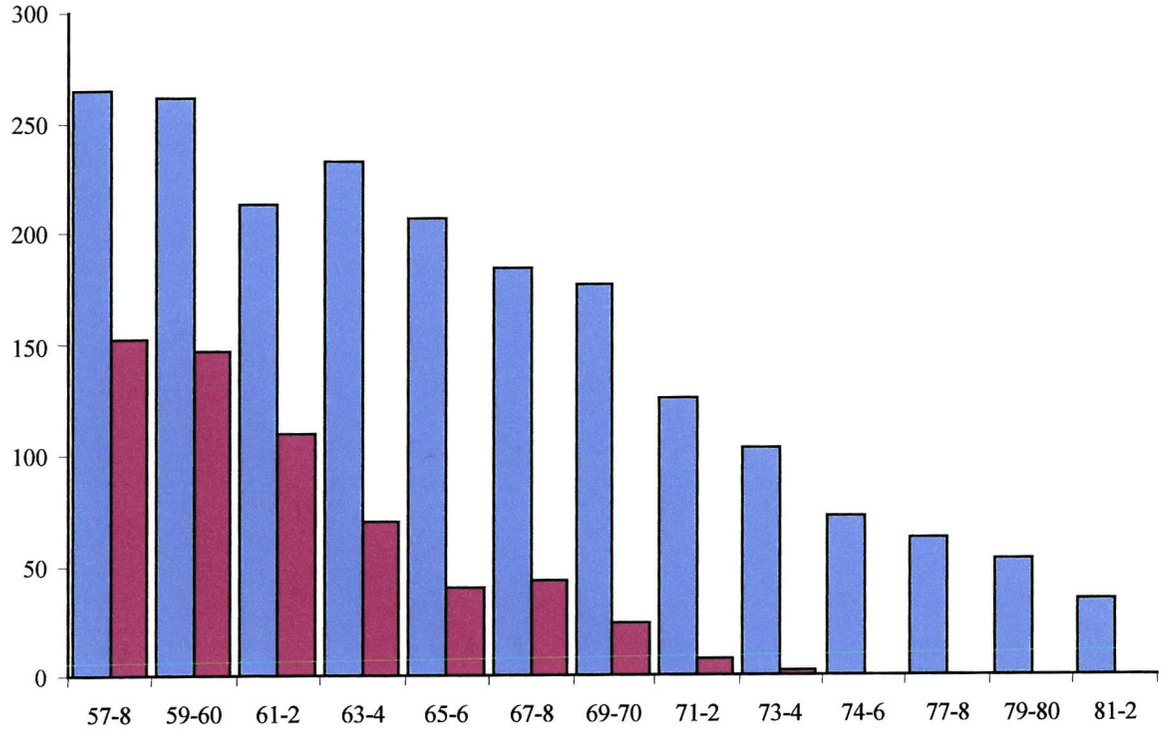
Kuru is a TSE confined to highland New Guineans living in a number of adjacent valleys in the mountainous interior of Papua New Guinea.[311] The first report was made by patrol officer JR MacArthur in 1953 and two years later Berndt described the disease in his PhD thesis.[312] However kuru had probably been occurring since the turn of the 20th century.[313] The term 'kuru' means shivering or trembling in the language of the Fore,[314] the cultural and linguistic group in which more than 80% of cases occurred.[315] The point prevalence of the disease in this population was up to 1% and the height of the epidemic occurred around 1956.[315,316] Since its discovery more than 2,600 patients have died of the disease.[316] Women and children were much more commonly affected than adult males, leading to a male/female ratio of more than 3:1 in some villages, and suggesting (incorrectly) that sex-linked genetic factors were important in disease aetiology.[317,318]

In 1959 an American veterinary pathologist, Dr William Hadlow, drew attention to the similarity between the neuropathology of kuru and scrapie.[64] It was known at this time that scrapie was transmissible and subsequently Drs Clarence Gibbs Jr. and Carleton Gajdusek demonstrated transmission of kuru to a chimpanzee in 1965.[66] Well before this result was available, the neuropathologist Klatzo had conducted an exhaustive study of the brains of 12 people with kuru,[68] commenting that they resembled only one other human disease with which he was familiar - CJD.

Although the cause of kuru was initially unclear, intensive study concluded that the disease resulted from the practice of ritualistic cannibalism, a rite of mourning and respect for dead kinsmen, with resulting conjunctival, nasal, skin, mucosal and gastrointestinal contamination with highly infectious brain tissue. For cultural reasons, men were only infrequently exposed to infectious tissues during these funeral rituals, thus explaining the relative scarcity of the disease in adult males.[67] The recognition that other tribes remained free of kuru despite cannibalistic practices similar to the Fore, led to the suggestion that kuru may have initially arisen following the ritualistic cannibalism of a sporadic or familial CJD victim in the Fore region, and it is of note that CJD has been reported in Papua New Guinea.[319,320] Kuru has gradually been disappearing since cannibalistic rituals ceased toward the end of the 1950s,[67,321] and with the passage of time progressively older age groups have become free of kuru (see Figure 8). Two or three cases still occur annually,[313] thus demonstrating that the incubation period can range from $\leq 4.5$ years (the age of the youngest victim) to $> 40$ years.

The clinical course of kuru is remarkably uniform, with cerebellar symptoms progressing to incapacitation and death, usually within nine months.[311,322] The disease has been divided into three clinical phases (see Figure 9). The first, or ambulant stage, starts with unsteadiness of stance or gait and often of the hands. This is preceded in some cases by symptoms of headache and limb pains. Dysarthria starts early, and speech progressively deteriorates as the disease advances. Convergent strabismus often appears early as well, and persists. Shivering tremors are also noted during this phase.[322] In the latter part of the first stage the patient usually takes a stick for support when walking. The second, or sedentary stage, is reached when the sufferer can no longer walk without complete support.[322] Tremors and ataxia become more severe, rigidity of the limbs often develops, associated with widespread involuntary movements, particularly myoclonus +\- choreoathetosis, and a startle reaction may be seen.[322] Emotional lability, leading to outbursts of pathological laughter, frequently occurs and although most patients show a resignation to, and a light-hearted attitude toward their illness, some patients became depressed.

**Figure 8: Decline in kuru deaths 1957-82.**




Values are number of deaths per two years (all deaths in blue and deaths under age 20 in purple).

**Figure 9: Clinical characteristics of kuru**

Left - kuru patient with stick to aid support when working in her garden. Middle - a girl with advanced kuru who requires support to stand. She has true euphoria, and a propensity to giggle and laugh. Right – kuru victim trying to execute finger-to-finger testing.

Mental slowing is apparent, but severe dementia is conspicuously absent. The third, or terminal stage, is reached when the patient is unable to sit up without support.[322] At this time ataxia, tremor, and dysarthria become progressively more severe and incapacitating. Pyramidal, extrapyramidal, and frontal release signs may be seen at this stage and in time inanition and signs of bulbar involvement develop. The patient becomes mute and unresponsive, deep decubitus ulceration and hypostatic pneumonia often occur, and the patient finally succumbs, usually, but not always, in a state of emaciation.

In keeping with the prominent cerebellar clinical features of kuru, neuropathology demonstrates macroscopic atrophy of the cerebellar vermis in most cases. Microscopically, changes are more widespread in the CNS and are characterised by marked astrocytosis throughout the brain; mild spongiform change of the grey matter; diffuse neuronal degeneration that is most severe in the cerebellum and its afferent and efferent connections; and minimal demyelination. Typical intracytoplasmic vacuolation is usually observed in the large neurons of the striatum. The most striking histological abnormality however is the presence of PrP-positive amyloid plaques, most conspicuous in the cerebellum, and occurring in about 80% of cases.

## IATROGENIC CREUTZFELDT-JAKOB DISEASE

The first documented iatrogenic transmission of any TSE was in the 1936 when over 1,200 sheep (7% of those injected) developed scrapie following inoculation with louping-ill vaccine. The vaccine contained formalin-treated sheep brain, presumed contaminated by scrapie.[323] The first report of human iatrogenic CJD was in 1974 and related to a recipient of a human cadaveric-derived corneal transplant.[71] However, probable instances of transmission through the use of neurosurgical instruments had occurred prior to 1960.[218] To date over 260 iatrogenic CJD cases have been documented (see Table 7)[25] Just over 5% of CJD cases in the UK during the period 1990-9 were iatrogenic.

**Table 7: Summary of all proven or highly probable cases of iatrogenic CJD**

| Mode of infection | No. of patients | Entry of agent into brain | Median incubation period (range) | Clinical presentation |
|---|---|---|---|---|
| Corneal transplant | 3 | Optic nerve | 16, 18, 320 months | Dementia/cerebellar |
| Stereotactic EEG | 2 | Intracerebral | 18 months (16-20) | Dementia/cerebellar |
| Neurosurgery | 4 | Intracerebral | 20 months (15-28) | Visual/dementia/ cerebellar |
| Dura mater graft | 114 | Cerebral surface* | 6 years (1.5-18)† | Cerebellar (visual/dementia)† |
| Growth hormone | 139 | Haematogenous | 12 years‡ (5-30) † | Cerebellar† |
| Gonadotrophin | 4 | Haematogenous | 13 years‡ (12-16) | Cerebellar |

*In two cases, dura was used to embolise vessels of non-CNS tissues, rather than as intracranial grafts. †Clinical information not available for all cases. ‡Calculated from the midpoint of hormone therapy to the onset of symptoms

## Transmission via neurosurgical instruments

In December 1951 a women with a rapidly progressive dementia underwent burrholes for ventriculography. She died just over a week later of pathologically-proven CJD. In January 1952 two other patients had neurosurgical procedures in the same theatre as the first case, almost certainly using common instruments - one, a 57-year-old man, had burrholes for ventriculography followed a week later by excision of a meningioma and the other, a 67-year-old man, had a craniotomy for aspiration of a temporal lobe abscess.[57] The first of these two patients was readmitted 19 months later with a four-week history of progressive dementia and died in October 1953 of pathologically proven CJD. The second patient was readmitted in November 1953 with a 12-week history of progressive dementia and died later that month of pathologically proven CJD.[218]

In February 1956 a patient who was first on the operating list underwent burrholes for ventriculography. She died the following day of pathologically-confirmed CJD. The patient third on the operating list was a 46-year-old women who had a frontal leucotomy for longstanding obsessional neurosis. This patient remained well until September 1957 when she was readmitted with a six-week history of progressive dementia. Brain biopsy confirmed CJD and the patient died a few days later.[57,218] The hospitals in which the operations took place in these cases and those in the paragraph above were both in London.

A fourth instance of probable iatrogenic transmission via neurosurgical instruments occurred in France. A 59-year-old women with symptoms and an EEG suggestive of CJD underwent a cortical biopsy in 1965 which confirmed the diagnosis. A man aged 46 was operated on three days later following a head injury. Twenty-eight months later he developed the features of CJD and died six months after this from pathologically confirmed CJD. A subsequent investigation showed that the patients were operated in the same room and probably with the same instruments, although these had been sterilised before reuse.[324]

Will[218] reports two other cases with a previous history of neurosurgical procedures and subsequent development of CJD a few years later, but in neither instance was a link with another CJD case identified. The first patient underwent burrholes for ventriculography in August 1952 as part of investigation for a suspected stroke. She was subsequently readmitted in October 1954 because of the development of a rapidly progressive dementia and died of pathologically confirmed CJD the following month. The second patient had a posterior fossa decompression in 1969, syringostomy in 1976 and became progressively demented shortly after a further syringostomy in July 1978. She developed myoclonus, became mute and her EEG was typical of CJD. She died in January 1979, but no necropsy was performed. A 15-year-old boy with clinically probable CJD and a previous history of neurosurgery has been reported in Japan. The patient had undergone an operation for arterio-venous malformation in his right occipital lobe at the age of five. Whether or not any other patient with CJD had been operated on in the same theatre is not stated.[325]

Further cases of neurosurgery-associated iatrogenic CJD were reported in 1977.[326] Two young patients (17 and 23 years), had undergone electrocorticography in Switzerland in 1974 for intractable epilepsy. During these procedures the same two silver electrodes had been inserted into their cerebral cortices for several hours. The patients developed progressive neurological disease, after a delay of 16 and 20 months, and subsequently died from histologically confirmed, and transmitted, CJD. The electrode probes used in both cases had previously been implanted for two hours into the brain of a 70-year-old women with a four-month history of mood disturbance, ataxia, mental deterioration and involuntary movements. She died three months later of

histologically confirmed CJD. The electrodes had been cleaned with benzene, disinfected with 70% ethanol and sterilised in formaldehyde between each use. Twenty-eight months after their implantation in the original CJD case, the electrodes were implanted into the frontal lobes of a chimpanzee, who, after a period of 18 months, developed an encephalopathy histologically confirmed as CJD.[327]

## Corneal transplant cases

It has been shown experimentally that it is possible to transmit TSEs by inoculation of infected material into the eye.[328-330] That the cornea is infectious has been demonstrated in various animals[331-335] including man.[334] There are, in theory, two possible routes of entry into the brain following inoculation into the eye. The first is direct, via the visual pathways.[336] The second involves peripheral spread via the blood following leakage of the inoculum from the injected eye.[337,338] Both might be relevant to man.[339]

There have been three case reports in the literature describing human to human transmission of CJD via corneal transplantation. The first of these was in 1974,[71] and this represents the only definite case of transmission by this route. The other two represent probable[340] and possible[341] cases of transmission.[342] Occasionally, other ophthalmic procedures have been reported in cases who subsequently developed CJD, such as photocoagulation,[200] cataract removal,[200] tonometry[230] or prior surgery for congenital glaucoma,[343] but these are probably incidental findings and will not be discussed further. The cases involving corneal transplantation are described below.

Duffy reported the case of a cadaveric corneal graft, the donor being a 55-year-old man living in the USA with a two-month history of ataxia, memory deficit, myoclonus and involuntary movements who was later found to have pathologically-confirmed CJD.[71] The recipient was a 55-year-old woman with Fuchs' corneal dystrophy who developed symptoms of lethargy, nausea and ataxia some 18 months after surgery, and died after a further nine months. Not only was she confirmed to have died from CJD at post mortem,[70] but a homogenate of her brain subsequently produced CJD when injected into a chimpanzee.[70] Though the transplanted cornea itself was not retained for study, this is generally accepted to be a definite case of transmission.[46,70,339,342]

Uchiyama described the case of a 63-year-old women living in Japan who developed autopsy-proven CJD 15 months following a corneal transplant.[341] Details of the donor were not given, so this is regarded as a possible case of transmission.[344,345]

Heckmann described a 45-year-old women living in Germany who developed features of CJD in 1995. She had undergone the corneal transplantation 30 years previously from a donor who had autopsy-confirmed CJD.[340] The recipient developed a rapidly progressive cerebellar syndrome with dementia, myoclonic jerks, and an (arguably) typical EEG. She died following an eight-month illness, but no histological proof of the diagnosis of CJD was ever obtained, so this case remains at best a probable case of transmission.[342]

## Dura mater cases

Human cadaveric-derived dural homografts have been used in surgical procedures since the late 1950s, particularly for neurosurgical conditions, including head trauma, cranial and spinal tumours and repair of

congenital malformations. They have also been used in general and paediatric surgery for large defects of the abdominal wall, and in maxillofacial procedures. Infectivity of dura from scrapie-infected rodents has been demonstrated with titres only two logs less than brain.[346,347]

In 1987 the first case of CJD linked with the use of a human cadaveric-derived dural homograft during a neurosurgical procedure was reported. The patient was a women aged 30 living in the USA who had had a dural patch placed in the right temporal area during surgery to resect a choleastoma in 1985, 19 months before the onset of symptoms of CJD. The diagnosis was later pathologically confirmed.[348,349]

Subsequently over 110 similar occurrences have been identified around the world (see table from Brown[25]), the greatest number occurring in Japan.[350,351] The majority of the implicated grafts were 'Lyodura', produced by a single manufacture (B. Braun Melsungen A.G.) between 1982 and 1986. During this period the manufacturing process enabled potential cross-contamination between grafts as these were often pooled. In 1987 Braun Melsungen revised their methods of Lyodura production, changing from batch to individual processing of grafts and treatment with 1N sodium hydroxide.[352] However, that latter process may not completely remove infectivity[352] and at least one case may have received a graft manufactured after the introduction of these revised procedures.[353] One of the dura mater cases was a recipient of non-commercial dura harvested from a University in Rome[354] and up to four other cases may have been recipients of a dural graft other than Lyodura.[218,351,353,355]

In Japan it is estimated that one in 3000 recipients of dural grafts during the period 1983-1987 developed CJD.[353] Hernanez-Palazon et al. reported that during 1983-4 37 patients were given a dural graft during neurosurgical operations at their hospital. At follow-up 19 had died of their original disease or unrelated cause, but four had developed CJD.[356]

The vast majority of the grafts were used during a neurosurgical procedure but transmission has been reported following use of dura as an embolisation material in the external carotid artery for a nasopharyngeal tumour[357] and in the intercostal arteries to treat aspergillus.[358]

In Lang and coauthor's comprehensive review of all published cases of dura mater-associated CJD up to 1998, they report that clinically cases are similar to sporadic CJD, but memory loss, disorders of higher cerebral functions and extrapyramidal signs were fewer, while cerebellar abnormalities were more frequent.[351] Progressive dysarthria and gait disorder/gait ataxia were prominent signs during the early stages, and myoclonus the most salient feature later (92%). The mean age at onset was 38 (youngest age at death 19, oldest about 67), mean duration of illness was 10 months and 70% had a periodic EEG. Brown reported that the site of the graft placement (supra- vs. infra-tentorial) did not affect the clinical phenotype.[25]

## Human pituitary hormone cases

Cadaveric-derived hGH has been used since 1958, mainly for the medical treatment of children with growth hormone deficiency. The hormone had been manufactured in batches, each produced from a large number of pituitary glands (up to 2000),[359] and was administered by intramuscular or subcutaneous injection. About 30,000 children had been treated with hGH by 1985. In this year the first cases of CJD in patients who had received hGH were reported, two in the USA[360,361] and a single case from the UK,[362] and subsequently nearly 140 further cases have been reported,[25] mainly in France,[363,364] the UK[360,361,365] and the USA.[109] Cases have also been

identified in Brazil[366] and New Zealand[367] in patients who received hGH manufactured in the USA,[368] and in the Netherlands[25] and Australia[369] in patients who had received locally produced hGH. The percentage of recipients of potentially contaminated hGH who developed CJD is 0.8% in the USA, 1.9% in the UK, 5.9% in France and 10.9% in New Zealand.[25] The reasons for the discrepancies are unclear, but may relate to differences in donor and recipient populations in these countries and variation in the manufacturing processes. Experimental transmission of hGH to primates has been demonstrated, but was only successful for one of 76 potentially contaminated lots produced in the USA.[370]

Four cases of CJD occurred in women treated with cadaveric-derived pituitary gonadotrophin (hPG) in Australia.[369,371,372] The total number of infertile women in Australia who received hPG was about 1500.[373]

The incubation period for hGH-related CJD has been estimated from the mid point of treatment to the onset of symptoms and was 10 years (range 6-16) in France, 16 years (8-22) in the UK, 20 years (10-30) in the USA and 26 years (14-30) in New Zealand. Combined, the average duration is about 12 years.[25] The reason for shorter incubation period in Europe is unknown but may relate to higher levels of infectivity.[2]

The largest study describing the clinical features in detail of hGH-related CJD relates to 34 French cases.[364] The average age at onset was 21 years (range 9-26).[374] Ataxic gait (94%) and visual disorders (88%), diplopia (53%) and nystagmus (47%), were the most common initial complaints. Several other symptoms were frequently detected on first examination, such as hyperphagia with weight gain, asthenia and sleep disorders. Tremor (14%) and memory disorders (12%) were less often noted at onset. All patients subsequently developed dementia and 82% myoclonus. The investigative findings are discussed later.

The main neuropathological characteristics of sporadic CJD: spongiform change, neuronal loss and astrocytosis occur in iatrogenic disease, although the distribution of lesions varies from case to case. However, the neuropathology of hGH-related cases is noteworthy as there is usually pronounced cerebellar atrophy associated with neuronal loss, widespread spongiform change and PrP amyloid plaque formation.[375] Immunocytochemistry also shows a more widespread distribution of PrP in a diffuse pattern within the cerebellar granular layer in many of these cases. Furthermore, neuropathological changes in the spinal cord, particularly the presence of PrP amyloid plaques, are more frequent in hGH-related iatrogenic cases than in sporadic CJD.[376]

## Other cases in which the possibility of acquired transmission has been raised

Case reports have described various individuals in whom the possibility of occupationally acquired CJD has been postulated. These include a 54-year-old neurosurgeon,[377] a 70-year old pathologist,[378] and a 45-year-old general surgeon who had previous worked as a pathology diener.[379] None of these individuals were known to have had worked on a CJD case. Weber reported the case of a 55-year-old orthopaedic surgeon who died of CJD in 1992. He had worked with sheep dura mater (about 150 specimens in total) and human dura mater (at least a dozen) between 1968 and 1972.[380] Two histopathology technicians have been reported to have developed CJD. The first, a 62-year-old women, had worked in a neuropathology lab for 22 years and during this period two patients with CJD had been studied, one 11 and the other 16 years before the onset of the patient's symptoms.[381] The second report of a histopathology technician related to a 75-year-old man who had been exposed to both human and animal brains before 1969 and in 1963-4 was active in dissecting sheep for

histological investigation.[382] The connection between the occurrence of CJD in these six cases and their occupation is a matter for conjecture.[383]

A number of other CJD cases have been described for whom possible iatrogenic exposure to non-CNS tissues has been reported. Tange[384] described a 54-year-old man who developed CJD four years after tympanoplasty with a homograft pericardium. Creange[385] reported a 57-year-old woman who died from CJD two years after a liver transplantation. During this procedure she also received a small amount of albumin from a large plasma pool that included a contribution from a donor who three years later died of possible sporadic CJD. Brown reported a 63-year-old man who developed CJD four years after receiving a bone graft.[25] In each of these three examples, the tissue recipients were verified by neuropathological examination to have died of CJD, and although none of the tissue donors was known to have a primary neurological illness, none had undergone autopsy. Brown states that although the occurrence of these cases should not be ignored they probably represent the chance occurrence of sporadic CJD in individuals with preceding transplantation procedures.[25]

### Conclusions

The occurrence of iatrogenic forms of CJD has been a great tragedy, that has also led to an enhanced degree of understanding of the properties of the CJD agent. The route of infection clearly influences both clinical phenotype and incubation period, factors that are important when considering the issues relevant to transmission of the BSE agent to humans. Furthermore, these cases illustrate the potential for disease transmission via medical products and surgical procedures involving both central and peripheral inoculation. Measures to prevent iatrogenic CJD via the known routes of transmission have now been widely instituted. Various authorities have recommended that the use of cadaveric dural homografts should be replaced by suitable synthetic or autologous alternatives and that recombinant growth hormone should be used instead of cadaveric growth hormone. Guidance to prevent disease spread via ocular tissues has been strengthened and it is now recommended that neurosurgical instruments used on CJD patients should be discarded. However, some authorities disagree about the need for these various cautionary measures and the risk of iatrogenic transmission may therefore arguably persist in some places. Furthermore, the risk of transmission of CJD may remain for other reasons, for example the selling of hGH to bodybuilders on the black market.[386-388]

## FAMILIAL TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

The first report of an inherited TSE relates to an affected member of the 'H' family who had a history of a neurological disorder that could be traced back through several generations. This case was described at a meeting of the Viennese Neurological and Psychiatric Association in 1912.[389] Later Gerstmann in 1928[390] and Gerstmann, Sträussler, and Scheinker in 1936[85] reported clinical presentations and neuropathological findings for several affected members of the 'H' family. These reports established the condition that is currently referred to as Gerstmann-Sträussler-Scheinker disease.

Although the case reported by Creutzfeldt in 1920 had a family history of a neurological disease this patient is not considered to have the disorder we now call CJD. The first description of familial CJD was therefore by Jakob's colleague Kirschbaum in 1924. He reported the case of Paul Backer, a 44-year-old man from northern Germany, who had died from an illness that closely resembled that which Jakob had described a few years

previously.[53] The remarkable feature of this case was that the patient's maternal grandmother, mother, and eight of her siblings had died of an unexplained neurological disease. Not long afterward, Paul Backer's sister died with a disorder that resembled her brother's, and her autopsy showed findings that were thought to be typical of CJD.[391,392] Twenty years later came a report that two of Paul Backer's children had died of CJD.[393] These observations established with certainty CJD could be transmitted from generation to generation as a dominant hereditary trait. It is now known that familial CJD in the Backer kindred is associated with a codon 178 mutation.[394,395]

Over the ensuing decades several other reports of familial TSEs were published,[396-401] but arguably these added little to the understanding of the disease process.[3] In 1973 the transmissibility of a familial TSE was first reported[402] and later in the same decade Dickinson et al, using the methods of classical genetics, identified a gene in both natural and experimental infections that determined phenotypically different strains of scrapie.[403] A major advance subsequently came through the cloning of the human PrP gene in the mid 1980s[404,405] followed within a few years by the identification of a 144-base pair insert mutation and a codon 102 mutation associated with familial CJD and GSS respectively (both in British families).[125,406] Genetic linkage was established initially for the codon 102 mutation[125] and later for the codon 200,[407,408] codon 178 (FFI),[409,410] codon 198[411] and 144 base pair insert[412] mutations.

Up to the end of 2000 point mutations associated with human TSEs have been identified at 20 loci (see Table 8) and eight different copy number insert mutations have been found (see Table 9) [413] The clinical and pathological features of these conditions show a greater degree of variation that sporadic CJD and in general occur at a younger age.[400] Penetrance is variable, but probably nearly complete for individuals who survive into very old age. Systematic surveys as part of the EUROCJD project found that familial forms of TSE represent between 1% (Netherlands) and 17% (Italy) of all cases and if countries in which mutation analysis are not available are excluded (in addition to Slovakia – see below) then 238 (9%) of 2732 cases were familial.[225] A comparable relative incidence of familial forms of TSE has been reported in Japan.[204] Some populations, (including Slovakia) have a much higher incidence due to a very high prevalence of the codon 200 mutation (discussed later). The forms of TSE associated with the most common mutations are described below.

## Familial CJD associated with the codon 200 mutation

Clusters of CJD cases have been reported in Chile,[197] the Orava population of Slovakia[414] and Libyan-born Israelis.[415] The incidence of CJD in Libyan-born Israelis is 31.3/million/year, over 30 times that reported in most other countries.[415-417] The cause of these clusters was initially unclear, but a popular hypothesis to explain the cases in Israel was that the patients had contracted scrapie through the consumption of infected sheep brain or eyeballs.[238,239,418] However it is now clear that all three of these clusters are explained by a high prevalence of persons carrying the codon 200 mutation in these populations.[345,407,419-424] Worldwide, the codon 200 mutation is commonest genetic cause of familial CJD.

The clinicopathological phenotype of this form of hereditary CJD is very similar to sporadic CJD,[413,425,426] although rarely cases with atypical features have been described such as insomnia and thalamic pathology reminiscent of fatal familial insomnia (FFI -see below)[427] or a peripheral neuropathy.[428-430] The average age at onset is 55-62 years[425,431] and the penetrance is reported as 50% by age 60,[432] 77% at age 70, 89% at age 80 and

96% if age 80 is surpassed.[433] A women carrying the codon 200 mutation, and who had two daughters die from CJD, has been reported to be in good health despite being aged 95 years.[434] Rosenmann identified five persons homozygous for the codon 200 mutation, all of whom survived to at least middle age.[435]

## Gerstmann-Sträussler-Scheinker disease

Gerstmann-Sträussler-Scheinker disease is a very rare (10-100 per billion[436]) hereditary neurodegenerative condition associated with several different mutations of the PrP gene. The disorder, which is experimentally transmissible,[401] is therefore, arguably, a variant of familial CJD. The characteristic clinicopathological profile is of a slowly progressive ataxic syndrome (average duration five years) with multicentric amyloid ('kuru') plaques in cerebellar cortex.[413] The most common genetic abnormality associated with GSS is a proline to leucine substitution at codon 102, which has been reported in over 40 kindreds,[190] including the original family described by Gerstmann, Sträussler and Scheinker.[437] This is the second most common mutation associated with familial prion disease. A difficulty with GSS is knowing whether the term should be used to refer to any patient with the classical clinicopathological features or whether its use should be restricted to individuals with a progressive neurodegenerative disease and one of the mutations characteristically associated with GSS. Or, indeed, whether the clinical, pathological and genetic characteristics are all mandatory? The problem is illustrated by the fact that a single kindred with the codon 102 mutation some cases can have a clinical picture indistinguishable from sporadic CJD.[438,439] The cause of the clinical heterogeneity remains unknown, but does not appear to be related to PrP gene polymorphisms.[440] To further complicate matters some authors refer to three forms of GSS: the typical ataxic form, a telencephalic form (dementia, parkinsonism, and pyramidal features)[334,441-444] and a variant with numerous neurofibrillary tangles pathologically.[445-447]

## Fatal familial insomnia

The first description of FFI was in 1986 and related to an Italian family.[87] Subsequently over 20 further kindreds have been described and FFI is the third most common inherited prion disease.[448,449] It appears that prior to 1986 the disease was often not recognised as a member of the CJD family of disorders and was referred to as 'selective thalamic degeneration'.[410] FFI is characterised clinically by severe insomnia and autonomic failure, and pathologically by marked thalamic gliosis and little or no spongiform change.[450] A review of 23 subjects with autopsy-proven FFI revealed a mean age at onset of 49 years (range 25-61) and duration of illness of 13 months (range 7-33).[451] Silburn reported a case aged 20.[452] The sleep disturbance in most cases was an early sign and polysomnography showed a progressive and severe decrease in total sleep time associated with reduction and/or loss of both REM and non-REM sleep. Autonomic dysfunction also occurred early and included increased lacrimation, salivation, sweating, raised body temperature, and impotence in males. Endocrine abnormalities also were noted with an increase in catecholamines and cortisol and a loss of normal circadian rhythms. More typical features of CJD appeared as the disease progressed including ataxia and myoclonus. The EEG usually showed non-specific slowing and repetitive periodic complexes were uncommon.[451]

The genetics of FFI are intriguing: in spite of the fact that a mutation at codon 178 is necessary for the development of disease, it is the presence of a polymorphism coding for methionine 'downstream' at codon 129

on the abnormal allele that appears to determine the FFI phenotype.[453] The same 178 mutation, but coding for valine at codon 129 of the affected allele, is associated with a clinicopathological phenotype clearly distinct from FFI, thus illustrating the dramatic effect on disease that can result from a subtle change in PrP structure. Riek suggests that the hydrogen bond between residues 128 and 178 may provide a structural basis for this observation.[454]

Since the original description of FFI it has become apparent that there is considerable heterogeneity, both clinical and neuropathological, between cases carrying the characteristic mutation.[281,452,455,456] Zerr reported that of eight cases from Germany none complained of severe untreatable insomnia in the early stages and one patient had neuropathological features that were more reminiscent of forms of sporadic CJD than that described in FFI.[457] A further complication emanates from reports of sporadic CJD with a clinicopathological phenotype similar to FFI (sporadic fatal insomnia).[458,459]

## Insert mutations

In 1989 a large English kindred with a history of ill-defined neurological illnesses and neuropathology, including some members with CJD was reported.[406] Affected family members had five extra copies of a repeating octapeptide coding sequence between codons 51 and 91. Records of the family show members with various neurological diagnoses dating back to the mid-19th century, including Alzheimer's disease, Huntington's disease, Parkinson's disease, myoclonic epilepsy, atypical dementia, Pick's disease, CJD and Gerstmann-Sträussler syndrome.[460] Subsequently families with two, three, six, seven and nine extra repeats have been described (see Table 9). The neurological disease in these families shows marked heterogeneity. However, several patterns have been identified. Subjects with fewer than four octapeptide inserts have a low penetrance and often a CJD phenotype with short illness duration, whereas those with more than four octarepeats have a high penetrance and often the GSS phenotype. Onset can occur as early as the third decade and as late as the ninth, with in general younger onset being associated with a greater number of repeats. Duration of illness ranges from two months to 18 years with a tendency for longer duration with a greater number of repeats. The pathological features are also variable and include CJD, GSS, mixed and non-specific phenotypes. Presence of non-amyloid elongated PrP deposits in the cerebellum molecular layer by immunostaining is said to be highly distinctive.[413]

## Codon 129 polymorphism

At codon 129 of the PrP gene there is a polymorphism coding for either methionine (Met) or valine (Val).[461] Humans are almost unique in having this polymorphism at this site. Nearly all mammalian species tested are methionine homozygous, the only exception being Wapiti deer which are leucine homozygous.[462] Palmer first demonstrated that homozygosity is more common in patients with CJD than controls.[463] This has subsequently be confirmed in multiple studies (see Table 10). The explanation for this observation is not known, but one possibility is that if, as many believe, interaction between homologous PrP molecules underlies the disease process then heterozygotes are partially protected by relatively poor interaction between their non-identical proteins.[464] However, not all populations show a significant difference in codon 129 genotypes between sporadic CJD cases and controls, e.g. Japan (see Table 10).[465]

It is now clear that codon 129 has an influence on multiple other features of human TSEs. Sporadic CJD cases 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.[465-467] The presence of at least one allele with valine at codon 129 is associated with ataxia at onset and the observation of PrP plaques on neuropathology.[189,465,467,468]

The major influence that codon 129 has on the clinicopathological phenotype of individuals with a TSE associated with the codon 178 mutation is already discussed. Several other influences in familial cases have been described,[413] for example, the age at death is significantly younger for methionine homozygotes with a 144 case pair insert[469] and the disease duration is shorter in homozygous FFI cases.[448]

Brown found that 102 (80%) of 128 iatrogenic cases were homozygous at codon 129.[25] He noted that whereas methionine homozygosity was the most important genotype in dura mater-associated CJD (74% of total), valine homozygosity made a disproportionate contribution to hGH cases (32% of total), particularly among patients in the UK (55% of total). A postulated explanation for this observation is that infected pituitaries in the UK might by chance have come mainly from valine-homozygous individuals.[25] Brown also reported on the effect of codon 129 on incubation period in iatrogenic CJD, noting that for the 43 dura mater cases with genetic information available there was no apparent association. The situation with hGH cases is somewhat more complicated as the time of infection is not precisely known because treatment with potentially contaminated hormone usually lasted for several years. Therefore an estimate has to be made for date of infection. In France heterozygous cases of CJD occurred significantly later than the homozygous cases ($p = 0.003$), using the assumption that the period of potential exposure was restricted to the years 1983 to 1985, as suggested by epidemiological data.[470] However in the UK and the USA the same restriction cannot be applied as random contamination occurred, and consequently, incubation times for each case has to be estimated using the midpoint of the total treatment period as the point of infection.[25] Using this approach, no statistically significant relationship could be demonstrated between incubation time and the codon 129 genotype in either the USA or the UK. Brown then combined the France, US and UK data, but found that a positive or negative correlation between codon 129 and incubation period could be identified dependant upon which statistical model was used. He noted that the most serious pitfall in the statistical analysis was the assumption that infection occurred at the midpoint of treatment. This could lead to a calculation artefact by hiding the possibility that the more susceptible homozygotes would be more easily infected and so contract the disease earlier in the course of treatment than would heterozygotes, thus leading to an underestimate of incubation period for the homozygotes and overestimate for the heterozygotes. In conclusion, Brown considered that it was not possible to draw any definitive judgement about the effect of genotype on incubation period in hGH cases (see Discussion section).

A study of codon 129 genotype in 80 kuru patients and 95 unaffected controls suggested that the kuru epidemic preferentially affected methionine homozygotes.[471] Cervenáková's analysis of 92 kuru cases found that homozygosity at codon 129 (particularly for methionine) was associated with an earlier age at onset and a shorter duration, but other clinical characteristics were similar for all genotypes. In nine neuropathologically examined cases, the presence of histologically recognisable plaques was limited to cases carrying at least one methionine allele.[471,472]

Various studies have tried to assess whether codon 129 has an influence on conditions other than CJD. No clear association was found in Alzheimer's disease[473] or inclusion body myositis,[474] but in a community-based sample of elderly volunteers a statistically significant association between valine homozygosity and a poor performance

on cognitive assessment was reported.[475] However, this study analysed multiple variables and an association between codon 129 valine homozygosity and cognitive impairment was not an a priori hypothesis. Therefore this interesting result should be interpreted with caution.

## Miscellaneous genetic factors

### Codon 219 polymorphism

A glutamic acid to lysine polymorphism at codon 219 was found in the general Japanese population with an allele frequency of 6%.[476] This polymorphism was not detected in Europeans.[477] Rare familial cases in which this polymorphism is carried on the same allele as a codon 102 mutation have a clinicopathological phenotype which differs from those features characteristic of cases without the polymorphism.[478] A single family with CJD associated with a codon 200 mutation and lysine at codon 219 has been reported, but it was unclear whether the phenotype of the affected patients significantly differed from other codon 200 cases.[479] A study of 85 Japanese sporadic CJD cases found that all were glutamic acid homozygous, suggesting that heterozygous individuals may be protected from developing CJD.[480]

### Prion Doppel gene

A novel human gene named Doppel that has homology to the PrP gene has been identified on chromosome 20. Analysis of the methionine to threonine codon 174 polymorphism found no significant difference in frequency between sporadic CJD cases and controls and no effect on age of onset, disease duration or PrP strain type.[481]

### Apolipoprotein E

Amouyel[482] studied 61 CJD cases and found that the epsilon 4 allele of the APOE gene was a statistically significant risk factor. However, it has been suggested that the observation may have been a consequence of an inappropriate control group,[483] and other studies have found no significant difference in the distribution of Apo E genotypes between patients with CJD and controls.[484,485]

### Histocompatibility antigens

Kuroda reported a highly significant ($p < 0.005$) increase in the frequency of the HLA-DQw3 haplotype in Japanese CJD patients compared to Japanese controls.[486] However a subsequent study of histocompatibility antigens in Finnish CJD cases did not find a link to any HLA haplotype.[487]

**Table 8: Inherited familial TSEs associated with point mutations**

| Mutation | Country/origin | Phenotype | Age | Duration | Comment |
|---|---|---|---|---|---|
| **102 Pro→Leu** 129 Met | Austria,[439] UK,[125] Canada,[488] France,[434] Germany,[489] Israel,[490] Italy,[491] Japan,[492] Mexico,[413] Poland[493] and USA[125] | GSS | 30-62 | 1-10 years | Most common GSS mutation |
| 129 Met 219 Lys | Japan[478] | GSS | 31-34 | 4 years | Less prominent cerebellar signs than above |
| **102 Pro→Leu** 129Val | USA of Italian origin[494,494] | GSS | 33 | 12 years | No dementia |
| **105 Pro→Leu** 129 Val | Japan[495-499] | GSS | 40-50 | 6-12 years | Presentation with progressive spastic paraparesis |
| **117 Ala→Val** 129 Val | France (Alsatian),[492,500] USA,[413] American family of German descent,[444] and UK.[501] | GSS | 20-64 | 1-11 years | Progressive dementia, only occasional cerebellar signs |
| **145 Tyr→Stop** 129 Met | Japan[502] | GSS | 38 | 21 years | Progressive dementia. NFT seen on pathology. |
| **160 Gln→Stop** 129 Met | Austrian family[503] | Not stated (NS) | 32, 48, 48 | >6 years | Slowly progressive course |
| **178 Asp→Asn** 129 Val | Finland,[210] Germany (Flemish origin),[395] France,[345] American (Dutch and Hungarian descent)[504] and UK | CJD | 26-56 | 9-51 months | - |
| 129 Met | America,[505] Australia,[505] Austria,[506] UK,[507] Canada,[413] Japan[505] French,[508] German[509] and Italian[508] | FFI | 20-71 | 6-33 months | - |
| **180 Val→Ile** 129 Met | Japan[496] | CJD | 66-79 | 1-2 years | Similar to sporadic CJD but slower progression |
| | Japan[510] | CJD | 85 | 1 year | 232 Met→Arg and 129 Met on other allele |
| **183 Thr→Ala** 129 Met | Brazil[511] and Germany[512] | CJD | 37-49 | 2-9 years | - |
| **187 His→Arg** 129 Val | USA[513] | GSS | 37-53 | 3-15 years All alive | - |
| **188 Thr→Arg** 129 NS | Germany[512] | NS | NS | NS | - |
| **188 Thr→Lys** | Austrian family[503] | CJD | 59 | NS | Single case |
| **188 Thr→Ala** 129 Met | Australia[280] | CJD | 82 | 4 months | Negative immunocytochemistry |
| **198 Phe→Ser** 129 Val | USA (Indiana kindred)[446] | GSS | 34-71 | 3-11 years | NFT seen on pathology |
| **200 Gln→Lys** 129 Met | Austria,[514] Slovakia,[424] Germany,[512] Chile,[424]Japan,[515] USA,[516] Sephardic Jews,[407] UK[517] and France[434] | CJD | 35-66 | 2-41 months | Most common familial CJD mutation |
| 129 Val | Austria [518] | CJD | 64 | 3 years | - |
| **202 Asp→Asn** 129 Val | UK[190] | GSS | 73 | 6 years | NFT seen on pathology |
| **208 Arg→His** 129 Met | USA[519] | CJD | 62 | 7 months | - |
| **210 Val→Ile** 129 Met | Italy,[520] France,[521] Japan[522] Chinese[523] Germany[512] | CJD | 49-70 | 3-5 months | - |
| **211 Glu →Gln** 129 Met | France[524] and Italy[525] | CJD | 42-81 | 3-32 months | - |
| **212 Gln →Pro** 129 Met | USA[526] | GSS | 60 | 8 years | No dementia. Lewy bodies. |
| **217 Gln→Arg** 129 Val | Sweden[527] | GSS | 62-66 | 5-6 years | Neurofibrillary tangles seen on pathology |
| **232 Met→Arg** 129 Met | Japan[528] | CJD | 55-70 | 4-24 months | - |
| **238 Pro→ Ser** 129 NS | Germany[512] | NS | NS | NS | - |

**Table 9: Inherited familial TSEs associated with insert mutations**

| Insert number | Country/origin | Phenotype | Age | Duration | Comment |
|---|---|---|---|---|---|
| **24 base pair** 129 Met | France[529] | CJD | 73 | 4 months | Like typical sporadic CJD |
| **48 base pair** 129 Met | USA[530] and the Netherlands[531] | CJD | 58, 61 | 3 months, 7 years | Like typical sporadic CJD |
| **96 base pair** 129 Met | UK, [532] Japan [533] and Italy[534] | CJD | 56, 65 | 2 months, 6 months | Like typical sporadic CJD |
| 129 Val | France[529] | CJD | 82 | 4 months | Like typical sporadic CJD |
| **120 base pair** 129 Met | North American of Ukraine origin[535] American[536] and Germany[512] | CJD | 26-45 | 5-15 years | CJD phenotype, but slowly progressive |
| 129 Val | America[537] and Germany[512] | CJD | NS | NS | - |
| **144 base pair** 129 Met | UK,[412,538] American, [537] Basque,[539] Japan.[540] | CJD | 22-53 | 3 months-18 years | Wide range of phenotypes |
| **168 base pair** 129 Met | Single Japanese case[541] and North American family of English origin[542]. | CJD | 23-35 | 7-13 years | Early behavioural disturbance in some cases |
| **192 base pair** 129 Met | France[543] | GSS | 21-34 | 1->12 years | Mania-like psychiatric disturbance |
| 129 Val | France[544] and the Netherlands[545]. | GSS | 21-55 | 3 months- 6 years | - |
| **216 base pair** 129 Met | UK[546] and German[547] | GSS | 32-55 | 2.5->4 years | No spongiform change |

NS = not stated

**Table 10: Codon 129 genotype (percentages) in sporadic CJD, kuru and control populations**

| | **European**[466,548-550] | | **Japanese**[465] | | **Kuru**[471,472] | |
|---|---|---|---|---|---|---|
| | **Controls n=734** | **Cases n=748** | **Controls n=179** | **Cases n=21** | **Controls n=21*** | **Cases n=92** |
| **Met-Met** | 41 | 70 | 92 | 76 | 0 | 30 |
| **Val-Val** | 10 | 16 | 0 | 5 | 38 | 25 |
| **Met-Val** | 49 | 13 | 8 | 19 | 62 | 45 |

*Unaffected individuals from the Fore region studied during 1957-59 who did not later develop kuru

## Routine blood tests

Routine haematological and biochemical investigations, including inflammatory markers, are usually normal in CJD and other TSEs.[62,253] Esmonde analysed the results from 205 CJD cases in the UK between 1985 and 1992.[551] A raised white cell count was noted in 23/189 (12%) and the ESR was elevated in a minority (median was 17mm/hr in the 30 cases with information available). He noted that in a number of cases these abnormalities may have been directly attributable to the effects of secondary infection in patients rendered immobile or with indwelling urinary catheters. Anaemia was noted in only five of 189 cases, leucopenia in three, and thrombocytopenia in one.

### Liver function abnormalities

Kirschbaum's review of the 150 CJD cases identified in the world literature up to 1967 found that 11 had pathological evidence of a fatty liver and eight others had slight degenerative parenchymal hepatic alterations with lymphocytic infiltrations.[62] No information was given on liver function tests during life. Roos reported liver abnormalities in 11 of 41 CJD cases, ranging from minimal aspartate transaminase (AST) elevation with normal liver histopathology to pathological evidence of liver damage and jaundice.[552] Other studies have found elevation of serum liver enzymes or bilirubin or both in 40-52% of cases.[253,551,553] The largest of these studies (110 cases) noted that the abnormalities were of a mild to moderate degree, and were often transient.[210,551] Reports of liver abnormalities in CJD cases in Japan have shown somewhat conflicting results. Tanaka assessed seven cases and found that all had mild hepatic enzyme dysfunction and fatty infiltration, six at autopsy and the other case on gross inspection during gastrostomy.[554,555] Iwasaki, however, did not find any abnormalities of liver enzymes or pathology in a series of nine cases.[556]

Several authors have speculated on the cause of these biochemical and pathological hepatic abnormalities. Tanaka did not consider that these were drug-induced or secondary to other recognised causes of liver disease in his cases.[555] Lanska added that he did not believe that the abnormalities could be readily explained by toxic reactions to medications, metabolic dysfunction, alcoholism, anoxia, or debility.[557] Roos suggested various possible explanations including intercurrent infection, drug toxicity, nutritional inadequacy or direct action of the infective agent.[402] However, Brown has reported successful transmission from liver in only four of 35 patients with TSE,[70] and Kitamoto did not find evidence of hepatic $PrP^{Res}$ deposition in the liver of CJD patients.[558] Will argues that most likely the hepatic abnormalities are epiphenomena, resulting from debility, drug effects or secondary infection.[553]

## Cerebrospinal fluid

### Routine analysis

The first report of CSF analysis from a series of patients with TSE was by Gajdusek in 1957. He studied 60

cases of kuru and found no pleocytosis or increase in protein or globulin.[311] Siedler reported that the CSF was normal in 89% of 62 CJD cases reported in the literature prior to 1963. In 11% there was an elevation of total protein, but this exceeded 1.0g/L in only a single case.[559] Kirschbaum's 1968 review of 150 cases noted changes in the CSF in a minority, such as an increased total protein of up to 1.2g/L, or the presence of up to 15 lymphocytes.[62]

Subsequent studies have reported an elevated protein of >0.4g/L in 37%,[252] >0.45g/L in 36%,[402] ≥0.5g/L in 55%,[210] and >0.8g/L in 8% of cases.[253] Rarely values between 1.5-2.0g/L have been documented.[210,253] An elevated protein may return to normal on repeat lumbar puncture.[402]

The percentage of CSF IgG to total protein was found to be raised >15% in three of 15 cases in one report, suggesting possible intrathecal IgG production.[253] In contrast another study found a normal IgG index in all 10 cases assessed suggesting an absence of intrathecal IgG synthesis.[210] A further study found changes in keeping with possible production of IgA within the CNS,[560] but another report of 25 cases found no evidence of intrathecally synthesised lgA or IgM.[561]

Oligoclonal IgG bands confined to the CSF are not usually found in CJD,[562] although this has been documented in four case reports.[563-566] Another patient has been described with CSF oligoclonal bands, but the paper does not state if these were also present in the serum.[567] The largest study to assess the incidence of oligoclonal bands confined to the CSF in CJD found a positive result in seven of 100 pathological proven cases.[561] However, no control data was given. The reason for a abnormal protein findings in CJD remains to be explained.

A number of studies (including a study of 300 CJD cases) have reported that CSF pleocytosis was never seen.[70,252,568] One publication described a raised cell count as 'rare'[402] and another study of 105 cases noted that a slight pleocytosis was only recorded when a repeat tap was performed in two patients.[253] Kovanen found a white cell count of 16 x $10^6$ in one case, but this was after a seizure and the count was normal a few days later.[210] Garcia Santos noted that one of a series of four dura-mater related iatrogenic cases had a raised white cell count, but neither the value nor comment were given.[569] Tsuji reported that six (11%) of 57 CJD cases showed a pleocytosis, five in the range 10-20/$mm^3$ and one a count was 303,[208] and Jacobi noted a mild pleocytosis (5-11 cells) in six of 110 (5.4 %) definite CJD cases.[561] However, in neither of these last two studies were the clinical features of the positive cases stated and no mention was made of the results of any previous or later spinal taps.

**14-3-3 protein**

Harrington found that two 30-kd proteins detected by two-dimensional electrophoresis and designated proteins 130 and 131 correlated well with a diagnosis of CJD.[570,571] However, the assay technique was complex and not practical for routine clinical use. He subsequently detected proteins 130 and 131 in normal human brain, partially sequenced their amino acids, and found that they matched the brain protein known as 14-3-3. 14-3-3 is a normal cellular protein expressed in a variety of neural and non-neural tissues and is released into the CSF as a consequence of extensive destruction of brain tissue.[572] Harrington then developed a method for detecting this protein using SDS-PAGE with immunoblotting and found that a positive result had both a sensitivity and specificity of 96% for a diagnosis of CJD (using 94 controls with other dementias), and that the specificity rose to 99% when excluding three controls with recent strokes.[573] Numerous studies have subsequently assessed the

use of the 14-3-3 protein in CJD diagnosis,[574-579] and have reported sensitivities and specificities of 88% and 100%,[574] 77% and 87%,[575] 95% and 93%,[576] 96% and 93%,[577] and 100% and 87%[568] respectively (data from pathologically confirmed CJD cases and controls with suspected CJD and a final alternative diagnosis). A study combining data from many of these and others studies found that for 222 definite CJD cases and 307 controls the sensitivity and specificity were both 93%.[579] Furthermore, the positive and negative predictive value of the 14-3-3 test in cases initially classified as clinically possible (the group in which the diagnosis is most uncertain) was 94% and 82% respectively. As a result of the high accuracy of the test it has been incorporated into WHO[194] and EU[580] criteria for a probable case of CJD. The one study that compared the sensitivity and specificity of the EEG, MRI and CSF 14-3-3 showed that the latter was the best discriminator between CJD and other rapidly progressive dementias.[255]

**Table 11: Conditions that may give a positive 14-3-3 CSF test**

| | |
|---|---|
| **Degenerative** | **Metabolic** |
| Alzheimer's disease (4/133)[578] | Hashimoto's encephalopathy[568,582,585,586] |
| Amyotrophic lateral sclerosis and dementia[581] | Hypoxic brain damage[578] |
| Cerebral amyloid angiopathy[582] | MELAS[585] |
| Corticobasal degeneration* | Barbiturate intoxication[576] |
| Dementia with Lewy bodies[577] | **Vascular** |
| Parkinson's disease (1/34)[578] | Acute stroke (4/4) [568,573,578,581] |
| | Sub-arachnoid haemorrhage[573] |
| **Neoplastic** | |
| Carcinomatous meningitis from small-cell lung cancer[583] | **Other** |
| Cerebral B-cell lymphoma* | 'Chronic lymphocytic meningitis'[577] |
| Cerebral intravascular lymphoma[568] | Delirium in Down's syndrome[568] |
| Glioblastoma/other brain tumour (3/4)[568,578] | Epilepsy (3/13)[578] |
| Paraneoplastic syndrome (10/80)[584] | Multiple sclerosis[585] |
| | Neuropathy[581] |
| **Infective** | Rett's syndrome[573] |
| Bacterial/tuberculous meningoencephalitis[585] | Tuberose sclerosis[568] |
| Herpes simplex (11/12)[573] | |
| Menigoencephalitis[568] | |
| Menigitis[585] | |
| Non-viral encephalitis* | |
| Other viral encephalitides (1/12)[573] | |

*Personal communication Alison Green. Values in brackets are cases testing positive/total tested.

The 14-3-3 protein can be detected in the CSF even in the early stages of the clinical course and, in an experimental animal TSE in a primate, in the presymptomatic phase as well.[573] Results from patients with familial TSE have been somewhat mixed, with a positive result in 28 out of 29 CJD cases related to the codon 200 mutation,[579,581] 15 of 15 codon 210 mutation cases,[579] two of five codon 102 mutation cases and none of 15

FFI cases.[579] Six of 10 iatrogenic cases tested positive.[579] Multiple other conditions may give a positive 14-3-3 CSF test result (see Table 11) and therefore it is recommended that the test is not used as a general screening test for CJD but should be reserved for use in cases where the diagnosis of CJD is considered a reasonable possibility.[194,572,578,587] A repeat CSF tap may be useful in some positive cases: Zerr reported on patients undergoing a further CSF examination at least two weeks after the first and found that 12 of 13 CJD patients were still positive, whereas all three patients with epilepsy became negative and only two out of six patients with inflammatory disorders had detectable 14-3-3 levels in the CSF in the second lumbar puncture. Both patients had still inflammatory CSF changes.[578] The 14-3-3 protein has been shown to be stable despite repeated freeze/thaw cycles or storage at up to +22$^0$C for 12 days or 4$^0$C for five weeks.[574,576,577] It is important that samples are not contaminated by excess blood cells.[577]

**CSF neuron-specific enolase, S100b and tau protein**

Neuron-specific enolase (NSE) is an enzyme synthesised almost only within the CNS and is localised in neurons and neuroendocrine cells.[588-590] Raised CSF NSE levels have been reported in various neurological disorders, including brain trauma, brain tumours, subarachnoid haemorrhage, epilepsy, CNS inflammatory diseases and acute stroke.[588,589] Wakayama and Jimi were the first authors to report elevated levels of NSE in the CSF of a small number of patients with CJD.[591-593] Although they found that the level decreased in the later stage of the illness to normal others have reported the opposite.[594] Repeated freezing-thawing or storing CSF at room temperature was not found to have a major influence on NSE level by one group[574] although Green found that the level was decreased by freezing-thawing and storage at 4$^0$C. Three studies have assessed the use of the NSE in CJD diagnosis, and have reported sensitivities and specificities of 74% and 93%,[595] 80% and 91%[574] respectively, using a cut-off at >25ng/ml, and 72% and 100%,[595] 80% and 92%[596] using a cut-off at >35ng/ml.

CSF concentrations of S100b are increased in conditions associated with neuronal loss, such as brain tumours and strokes, and in inflammatory diseases such as meningitis and Guillain-Barré syndrome.[595] The first report of raised levels in CJD was published in 1982[597] and subsequently three studies including a suitable control group have assessed the use of S-100b in CJD diagnosis. These reported sensitivities and specificities of 95% and 66% respectively, using a cut-off at >0.5ng/ml,[595] 94% and 85% respectively using a cut-off at >2.5ng/ml,[574] and 84% and 91% respectively using a cut-off at >8ng/ml.[598] Repeated freezing-thawing or storing CSF at room temperature does not seem to have a major influence on S100 level.[574,595]

Tau protein is a microtubular protein found predominantly within the axons and dendritic processes of the neurons within the CNS. Raised CSF concentrations have been found in patients with intracranial haemorrhage and brain tumours, with smaller increases reported in various slowly progressive degenerative disorders.[595] Arai reported increased CSF in a CJD patient in 1995[599] and two later studies reported that CSF tau had a sensitivity and specificity of 83% and 89% respectively using a cut-off of 1000pg/ml[595] and 100% and 95% respectively using a cut-off of 1530pg/ml.[600]

Unlike 14-3-3 protein, tests for CSF NSE, S100b and tau are not included in the current diagnostic criteria for sporadic CJD. This reflects that the latter proteins have been assessed in a relatively small number of cases and in general have shown less accuracy than 14-3-3. Furthermore, the reported sensitivities and specificities of NSE, S100b and tau given above used optimal cut-off points calculated post hoc. The observation that these cut-

offs differed between studies suggests that the underlying accuracy of these tests may be less optimistic than those reported.

### PrP detection in CSF

$PrP^{C}$ has been detected in human CSF,[601] and CSF from TSE patients has been shown to transmit disease[70] suggesting the presence of $PrP^{Sc}$. A recent study has for the first time reported a method of detection of the pathological aggregates of PrP in the CSF, but although the specificity was 100% the sensitivity was low at 21%.[602] However, this technique is still under development and shows the potential to be a potentially highly specific test for CJD.

## Electroencephalography

The EEG was first recognised as a potentially important aid to the diagnosis of CJD in 1954 when Jones described two cases with recurrent sharp wave discharges over both hemispheres.[56] A periodic EEG (see Figure 10) was later included as a key component of diagnostic criteria published by Masters in 1979.[200] The EEG appearances in CJD show considerable heterogeneity between cases, but it is possible to make some generalities regarding evolution with time and relationship to other clinical features. In the early stages of the disease the EEG may be normal,[603] and very rarely a normal EEG persists throughout the illness.[604-606] The first changes consist of a disorganisation or a decrease of the normal background activity.[70] This is followed by a gradual development of progressive slow wave abnormalities which can occur in a generalised, focal, lateralised or asymmetric fashion.[603,607-610] As the disease evolves, monophasic, biphasic, triphasic or multiphasic complexes begin to appear.[603,604] The sharp waves may occur as early as four weeks after the onset of symptoms or, more commonly, in the later stages of the clinical course.[603,611] Initially, the sharp waves occur in a sporadic fashion and may be asymmetric. After a delay of days to months, the intermittent sharp wave complexes may evolve into the characteristic pattern of continuous generalised polyphasic sharp waves having a repetition interval of 0.5-1s, a duration of 200-500ms, and an amplitude of 50-300μV.[603,612,613] The background activity between complexes consists of theta or delta waves or an attenuation of background activity.[614] During the final stage of the disease, the amplitude of EEG activity has been shown to diminish and the repetition rate of the complexes becomes more prolonged. Shortly before death, the EEG may show intermittent bursts of sharp or slow wave activity superimposed upon an almost isoelectric background.[268,609]

Focal EEG slow-wave or polyphasic sharp wave discharges may correspond to the clinical and pathological changes,[612,615-618] e.g. occipital EEG changes in CJD patients with the Heidenhain's variant,[619] but such a correlation may not occur.[603,607] Myoclonic jerks often occur in association with the periodic sharp waves, but there is not always a constant relationship and one may occur without the other.[268,603,607,620] The periodic sharp waves may be synchronous[56,608,610,614,621] or asynchronous[268,603,614,622] with myoclonus.

The periodic pattern can be suppressed transiently by various drugs including intravenous diazepam,[623,624] barbiturates,[56,625] methylphenidate,[626] and sodium amytal.[268,625] Sleep also has been reported to suppress the periodic complexes by some[609,611] but not by others.[627] Application of external tactile and auditory stimuli had variable effects on the EEG, either evoking[625] or suppressing[614,620] periodic complexes. Rhythmic stimuli such as photic stimulation or somatosensory stimuli may pace or set the frequency of the discharges when the

stimulus rate is close to the spontaneous discharge rate.[268,622] A proposed mechanism for the occurrence of periodic complexes is discussed later.

The proportion of CJD cases with periodic EEG records has been assessed in multiple studies, with values ranging from 30%[604] to over 90%[206,607,628,629] Most studies report a figure in the range 55-85%[70,196,208-210,251,253,630] However, some of these reports are likely to have overestimated the incidence of a periodic EEG because a characteristic EEG was included in the clinical case definition, and many cases were diagnosed solely on a clinical basis. A large prospective case-control analysis found that only 144 of 219 pathologically-confirmed CJD cases had a periodic EEG, as did 11 of 43 non-cases initially classified as possible or probable CJD using standard criteria. This equates to a sensitivity and specificity of 66% and 74% respectively and positive and negative predictive values of 93% and 30% respectively.[579] In the UK CJD surveillance project a typical EEG has been identified in only two (<1%) of non-cases referred as suspected CJD since 1970. The low specificity of the former study is therefore somewhat surprising and most likely reflects differing definitions of a characteristic EEG in the UK and other countries. Criteria for a typical EEG tracing have been published[194] but are not widely used.

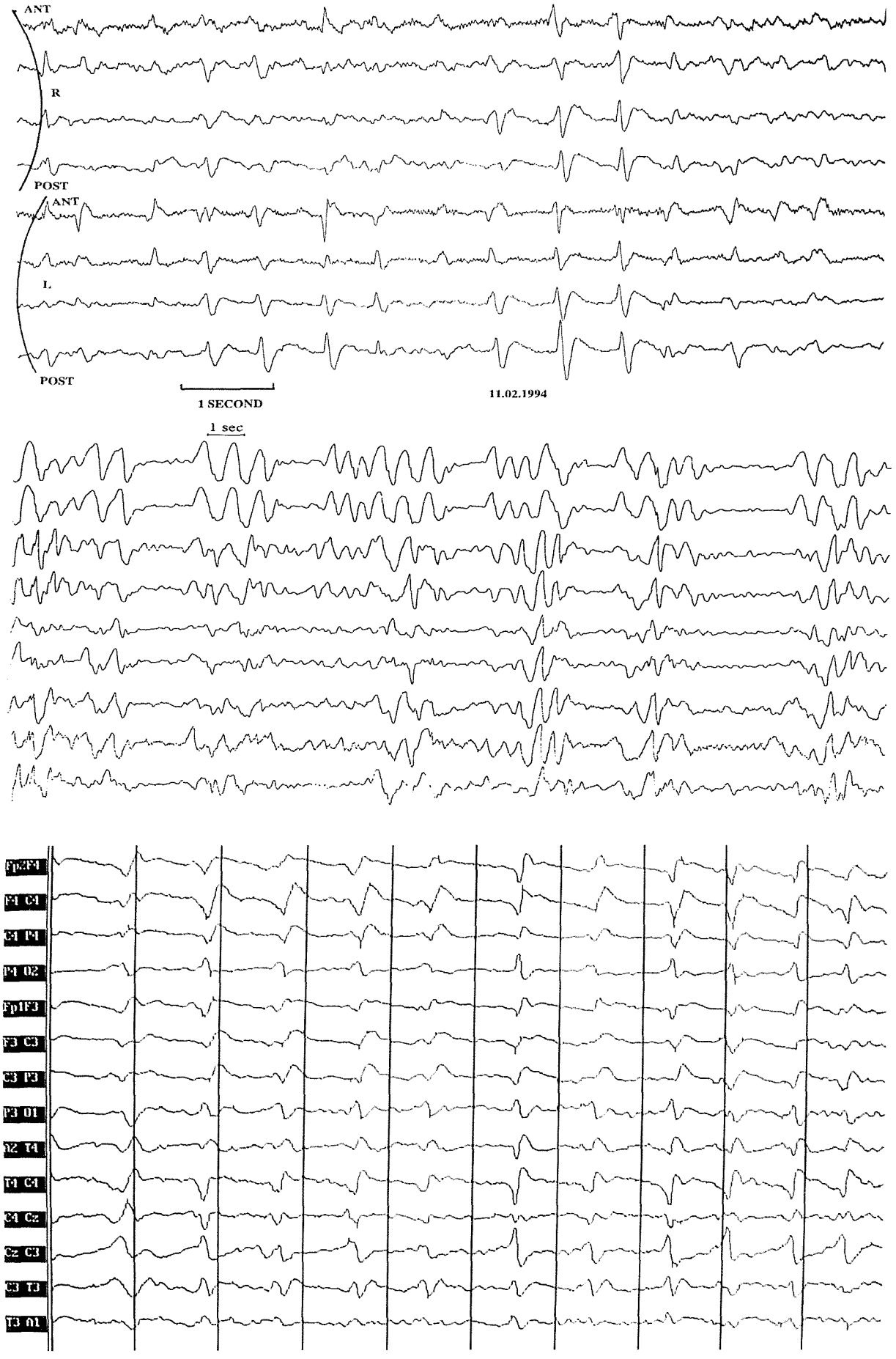
The typical EEG appearance has not been reported in kuru,[70,631] or 'classical' GSS (i.e. progressive cerebellar ataxia) and has only rarely been described in hGH-related CJD (see Figure 10).[5,364,632] The reason why patients with these forms of TSE and many with sporadic CJD fail to develop a periodic EEG is uncertain, but may relate to route of infection or strain of agent. The latter is supported by Parchi's subclassification of sporadic CJD by molecular strain type (see above) which indicates that patients with certain codon 129 genotypes and PrP electrophoretic banding patterns (VV1, MM2, MV2, VV2) only rarely or never develop period complexes.[189] Although in the correct clinical context a generalised periodic EEG is virtually diagnostic of CJD, a similar appearance has rarely been reported (but sometimes not reproduced in the paper) in other conditions, such as Alzheimer's disease (see Figure 10) or metabolic and toxic encephalopathies. (see Table 12).

**Table 12: Conditions reported to cause a CJD-like EEG**

| **EEG reproduced and typical** | **No EEG tracing reproduced** |
|---|---|
| Alzheimer's disease[255,286,579,633-635] | Lymphoma[255] |
| AIDS dementia[291] | Hyperammonaemia[637] |
| Hypoglycaemia[293] | Multiple myeloma[579] |
| Hepatic encephalopathy[636,637] | Hypo and hypernatraemia[635,637] |
| Hyperparathyroidism[295] | Multiple cerebral abscesses[637] |
| MELAS[296] | Amitriptyline toxicity[299] |
| Mianserin[300] | Metrizamide toxicity[637] |
| Lithium toxicity[305] | Lewy body disease[643] |
| Post-anoxic encephalopathy[638,639] | |
| **EEG reproduced and suggestive*** | |
| Normal pressure hydrocephalus[640] | |
| Holoprosencephaly (arhinencephaly)[641] | |
| Cerebral lipidosis.[642] | |

*Tracings reproduced in reports of the following conditions are less convincing: Binswanger's disease,[644] baclofen toxicity,[310] head injury,[645] necrotising encephalitis,[646] and progressive myoclonus epilepsy[647]

**Figure 10: Periodic EEG recordings**




Periodic EEG in Alzheimer's disease (top), hGH related CJD (middle) and sporadic CJD (bottom)

## Brain biopsy

When used to diagnose CJD, brain biopsy typically involves the removal of a small piece of non-dominant frontal cortex under general anaesthesia. Although usually diagnostic, approximately 5% of biopsies from subsequently confirmed definite cases are non-diagnostic,[70] reflecting the variable distribution of brain pathology in CJD.[648] Brain biopsy can lead to serious complications, including cerebral abscess formation or haemorrhage and, arguably,[649,650] should not be recommended as a procedure to confirm the clinical suspicion of CJD.[194]

# CEREBRAL IMAGING OF HUMAN TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

## Early imaging techniques

The earliest cerebral imaging techniques used on patients with CJD were cerebral angiography and air encephalography. Angiography does not provide any diagnostic clues[62] and air encephalography or ventriculography usually showed non-specific ventricular enlargement with or without cortical atrophy.[46]

## Computerised tomography

Computerised tomography (CT) scanning came into clinical use in 1973,[651] and the first report of CT scanning in CJD was presented at a meeting in 1976 and published the following year. Rao described rapidly progressive enlargement of the ventricles and sulci in two cases and suggested that such changes may be diagnostically useful.[652] Several subsequent case series have assessed CT scanning in CJD. Normal imaging is reported to occur in 57-80% of cases.[210,253,653,654] The most frequent abnormality is cerebral atrophy, which occurs particularly in those with a protracted illness.[655-657]A study which evaluated the distribution of atrophy found that a combination of cortical and cerebellar atrophy was most common, with only cortical or central atrophy occurring less often.[210]

Progressive atrophy on serial CT has been reported by several authors,[210,656,658-660] supporting Rao's original findings. In some cases atrophy can be extreme and a subdural hygroma as a presumed consequence of brain retraction secondary to marked atrophy has been described.[659] The atrophic process, however, varies from case to case, and may show striking discrepancies in patients with similar disease duration.[569]

Other CT features described in case reports include diffuse areas of periventricular hypointensity[661,662] loss of physiological basal ganglia hyperdensity,[663] progressive enlargement of the fourth ventricle in a case with the ataxic form of CJD,[664] low intensity in the visual cortex in a patient with visual hallucinations[665] and low intensity of the cerebral white matter in a patient with severe white matter destruction histologically.[666] The specificity of these abnormalities is unknown, but the rarity of these reports suggests they are unlikely to be sensitive indicators. No case-control study has assessed the significance of CT changes in patients with CJD and the main role of this technique is now the exclusion of alternative diagnoses.[70]

## Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a versatile imaging technique which utilises the magnetic properties of the proton nucleus of hydrogen to demonstrate the physical and chemical structures of living tissues. Radiofrequency electromagnetic energy is used to excite the hydrogen atoms in the body (particularly in water and fat), and the rate at which the excited atoms re-emit absorbed energy is used to produce a cross-sectional map of tissues, resulting in a wide range of contrast between normal and pathological tissues.

MRI images contain information from a number of different contrast mechanisms. By changing the way the radiofrequency energy is used to excite the protons (by changing the MRI sequence of energy pulses), the amount of a particular chemical or other feature of the tissues can be altered (i.e. the weighting of the image can be changed).

A number image weightings are commonly used in MRI:

- T1-weighted imaging: This provides excellent anatomical detail, but with limited contrast between normal and pathological tissue.
- T2-weighted imaging: This provides excellent depiction of many pathological processes, at the expense of some anatomical resolution.
- Proton-density (PD) imaging: This provides a rather flat image, better demonstrating blood vessels. Some pathological processes are seen more clearly on PD-imaging
- Fluid-attenuated inversion recovery (FLAIR) imaging: These images are particularly good at showing white matter disease clearly, though are also increasingly recognised to be useful in assessing grey matter disease.
- Diffusion-weighted imaging (DWI): These images are 'sensitised' to diffusion (by Brownian motion) of water molecules.

The widespread clinical use of MRI began in the early 1980s with the first published report of its use in CJD in 1985.[657] To date over 150 articles have reported on MRI in CJD or other human TSEs, including a recent series of 162 cases.[667] The three most commonly described abnormalities are cerebral atrophy, basal ganglia high signal and cortical high signal (see Figures 11-16). These changes may occur alone or in combination.

### Normal MRI

Normal appearances on MRI has been described in case reports of patients with sporadic CJD,[259,261,263-266,385,430,459,649,668-699] familial CJD,[456,520] FFI,[281,505,507,508,700] GSS,[344,488] and iatrogenic CJD linked to human cadaveric-derived dural homografts,[357,701,702] hGH,[374,703-705] and hPG.[371,372]

The proportion of CJD cases with normal MRI scans has been analysed by the UK and German CJD surveillance units. The UK study reviewed the hospital MRI reports on 96 cases and found that 41 (43%) were considered to have normal scans.[706] In the German study the MRI scans were personally reviewed by a single

author and only 44 (27%) of 162 cases were reported to have normal scans.[707] The cause of the wide discrepancy between the results of the two studies is likely to be due to methodological differences of assessment and these are discussed later.

**Atrophy**

The first report of the use of MRI in patients with CJD in 1985 described bilateral diffuse cortical atrophy.[657] Subsequent authors have described a wide variety of patterns of atrophy in sporadic CJD including generalised,[693] cerebral and cerebellar,[664] cortical/cerebral,[212,569,683,708-714] cortical and subcortical,[715] cortical with ventricular enlargement,[716] cerebral cortex and deep white matter,[717] cerebral cortex and striatum,[718] generalised more pronounced in one cerebral hemisphere,[378] cortical, central, hippocampal and caudate,[719] cerebellar,[693,720] bilateral occipital[721] (in some cases associated with visual symptoms[722,723]) and white matter degeneration in panencephalopathic CJD.[722]

In keeping with CT findings, progressive atrophy in sporadic CJD patients has been seen on serial MRI. Scans have evolved from showing no atrophy to cortical/cerebral atrophy[222,649,710,715,724,725] or atrophy involving both cortical and subcortical structures and cerebellum in a panencephalopathic case.[726] Progression from focal to generalised cerebral atrophy has been reported[722] and cortical/cerebral atrophy has been shown to become more marked on subsequent scans.[210,657,710,722,727-730]

MRI in dura mater-related CJD has been reported to show either cerebral atrophy[358,569,731] or cerebral and cerebellar atrophy.[354,732-734] One patient showed mild enlargement of the third ventricle only.[731] MRI in hGH-related cases has shown mild cerebellar vermis atrophy or[363,735] moderate cerebellar atrophy with mild dilatation of the fourth ventricle.[736] Serial MRI in hGH-related patients has been reported to evolved from normal to 'brain' atrophy[375] or mild cerebellar vermis atrophy only.[363]

The following distributions of atrophy has been reported on MRI in FFI patients, cortical,[737] cerebral,[452,738,739] frontal cortex,[740] and cerebral and cerebellar atrophy with ventricular dilatation in cases with prolonged course.[508]

In patients with GSS associated with the most common codon 102 mutation again various patterns of atrophy have been described: diffuse cerebral,[741] diffuse cerebral and cerebellar,[438] frontal cortical and cerebellar,[742] cerebellar,[490] and focal left temporal lobe.[438] Parietal atrophy was seen nine months prior to the onset of symptoms in one case who underwent repeat scanning after symptoms developed which showed progression to diffuse atrophy of all elements of the brain.[743] Although there have been no other reports of presymptomatic MRI scanning in human TSE patients five of six hamsters inoculated with scrapie showed ventricular dilatation on MRI just prior to expected onset of symptoms.[744]

Familial cases associated with insert mutations have also been reported to show various patterns of atrophy including: cerebral and cerebellar,[539,540,545] cerebellar,[543] cortical and subcortical,[543] cortical,[547,745] cerebral[460] and 'diffuse'.[535] MRI in familial CJD associated with various point mutations has shown cerebral atrophy,[522,523,746,747] cerebral and cerebellar atrophy,[748] or mild frontal atrophy.[428]

The proportion of CJD cases with atrophy on MRI brain scans has been assessed in studies from the UK and Germany mentioned above. The UK study found that 29 of 96 cases (30%) were noted to have brain atrophy.[706] The German study reported substantial brain atrophy in 47 (29%) of 162 patients. Five with serial MRI developed a massive increase in atrophy over several months. Atrophy was observed in 57% of controls indicating that this is an non-specific abnormality.[667]

**Basal ganglia and thalamic abnormalities**

The first description of basal ganglia high signal on MRI in CJD was by Gertz in 1988.[749] The most frequently reported pattern is striatal (caudate and putamen - see Figures 11-15) high signal.[569,663,682,691,707,709,710,712,713,715,718,724,725,727,750-754] Other patterns described are high signal in the 'basal ganglia';[683] striatum, globus pallidus and thalami;[682,755,756] striatum and thalami,[667,709,723,749,757-759] striatum and (often to a lesser extent) globus pallidus;[667,707,709,710,757,760] striatum and hippocampus;[712] caudate nuclei alone[761] and putamen alone.[710]

All of the above reports describe bilateral symmetrical abnormalities that are present on T2-weighted (Figure 11) or PD-weighted imaging (Figure 13) or both. Asymmetrical striatal (see Figure 12)[711,752,762] or globus pallidus[757] high signal have only rarely been described. Three patients have been reported to have only left caudate high signal.[691] Only four cases with high T1-signal have been described, in all cases in the pallidum (see Figure 16).[757,763,764]

Whether basal ganglia abnormalities can occur in patients presymptomatically has not been reported. However, basal ganglia and cortical high signal has been seen as early as three weeks after the onset of symptoms.[764] Several studies have reported on the appearances of the basal ganglia/thalami on serial MRI. Schröter found that 12 (32%) of 37 sporadic cases had normal basal ganglia on initial and follow-up scans and no other case had imaging that evolved from normal to hyperintense basal ganglia.[667] However, the latter occurrence has been described previously over a period as short as four weeks, either early[711,725,765] or late in the illness course.[752] Others have also shown similar evolution from normal to striatum (and globus pallidus or thalami) high signal in scans obtained two and five months apart.[710,759] Progression from normal to unilateral caudate high signal over two weeks,[764] and normal to bilateral putamen high signal over eight months[710] has also been described. In Schröter's series of 37 cases, 16 (43%) had high signal on initial imaging which did not increase over time and nine (24%) showed increasing hyperintensity over a period of follow-up that ranged from 4-20 weeks.[667] Two other articles report patients with striatal high signal on initial scans that became more prominent on scans performed three months later.[663,727] Three patients that underwent serial MRI up to six months apart showed persistent striatal high signal.[663,724,750]

Two cases demonstrated bilateral striatal high signal that was not seen on repeat MRI three or six months later.[715,753] A patient with a codon 210 mutation had increased T2 signal intensity in the striatum and thalami on initial MRI but a subsequent scan showed hypointense T2 signal within the basal ganglia and thalami.[766] Decreased basal ganglia signal on T2-weighted sequences has also been reported in a GSS case associated with a codon 198 mutation.[767] A decrease in basal ganglia hyperintensity over the later part of the clinical course was also documented in hamsters experimentally infected with scrapie.[744]

Patients with familial TSEs have been noted to have 'basal ganglia',[768] striatum and thalami,[534] or striatum[742,747,769] high signal. In some of these reports the basal ganglia high signal occurred relatively late in the clinical course. A case associated with a codon 180 mutation underwent serial MRI, with a total of four scans from 12 – 21 months out of an illness of total duration 30 months. Cortical high signal was seen on the first scan but striatal high signal was only present on a second and subsequent scans from 16 months into the illness.[769] A case with a codon 232 mutation had increased signal in the parietal and parietotemporal borders of the cerebral cortex bilaterally at eight months, four months later the high signal had spread to the temporal cortex, the following month to the whole cerebral cortex and at 17 months also involved the striatum.[747]

Despite numerous reports of MRI in iatrogenic CJD only a single case (hGH-related) has been described with basal ganglia high signal.[706] The reason for this apparent low occurrence is unclear, but this may reflect underreporting. No large study reviewing MRI films in iatrogenic CJD has been reported.

The first large series of CJD cases with detailed information on MRI was published by Finkenstaedt in 1996.[707] Twenty-three (79%) of the 29 patients had bilateral, symmetric areas of hyperintensity in the striatum on PD- and T2-weighted images. The abnormal increase in signal intensity was considered striking in 12 and moderate in 11 patients. Bilateral areas of hyperintensity in the globus pallidus were noted in seven (24%) patients, and hyperintense changes in the thalamus were found in four patients (14%). Another study, conducted by an Italian group and presented in a meeting abstract, reported that all of 31 cases studied had symmetrical bilateral striatal hyperintense signal.[757] Two large CJD case series have mentioned MRI findings, although in only limited detail. A French report of 53 cases included 19 patients in whom information on MRI was available.[770] The authors had personally reviewed about 30% of the scans (personal communication Professor Giraud). Only three (16%) were reported to have basal ganglia high signal on T2-weighted MRI. Lundberg commenting on MRI from 45 cases of sporadic CJD in Sweden stated that none showed any particular changes, but in some cases slight brain atrophy was seen as well as some non-specific MRI changes in white matter on T2-weighted images.

Two case-control series have ascertained the incidence of basal ganglia high signal in CJD patients. The first from the UK reviewed the MRI reports from 96 cases with definite or probable CJD.[706] Only four were noted to have basal ganglia high signal (three sporadic and one hGH-related). None of the six control patients (CJD suspects with a final alternative diagnosis) were reported to have basal ganglia high signal. The second study included 162 cases of definite or probable sporadic CJD identified through the German CJD surveillance system and 58 controls.[667] The films were evaluated blind to diagnosis and a positive scan was defined as one showing 'bilateral areas of increased signal intensity predominantly affecting the caudate nuclei and putamina on long repetition time images'.[255] This appearance was found to have a 67% sensitivity and 93% specificity for the diagnosis of CJD. Details of control patients with positive scans and the methodology of the radiological assessment were not described.

The large discrepancy between the low incidence of basal ganglia abnormalities seen in the UK, French and Swedish studies compared to the Italian and German reports is most likely to be largely explained by differences in methodology. The UK study relied entirely on the review of radiology reports from the patient's hospital and none of the scans were reviewed by the authors for the study. The French group only reviewed about one-third of the scans and the Swedish study relied on reports by neuroradiologists with no particular expertise in MRI in CJD (personal communication Dr Lundberg). In contrast, specialists with a particular

interest in CJD assessed all the MR images in the German and Italian studies.[667,757] The supposition that the MRI appearances were underreported in the UK, French and possibly also the Swedish series is supported by data from later studies comparing local radiology reports and subsequent review of scans at a specialist centre.[667,706,771] One possible explanation for the underreporting of basal ganglia high signal during primary reporting might be that MRI scans in CJD were usually performed to exclude other disorders usually affecting other areas of the brain, and abnormalities in the basal ganglia may not have been specifically sought.[667] Furthermore, the abnormalities may be overlooked because they are symmetrical and often subtle.

### Cortical high signal

Cortical high signal abnormalities (see Figure 12) are less frequently described than basal ganglia changes. The best estimate of the frequency of cortical high signal abnormalities on MRI in CJD patients comes from the Finkenstaedt's review of MRI films. These changes were only reported in four of the 29 patients (14%).[707]

A wide variety of patterns of cortical hyperintensity has been described in sporadic CJD including generalised cortical,[707,710,765] general cortical with unilateral predominance,[754] bilateral temporal and frontal,[710] unilateral or bilateral temporal lobe,[707,710] fronto-parietal,[759] unilateral cortical,[569] unilateral frontal,[772] unilateral parieto-occipital,[773] hippocampus,[757] cingulate cortex[709] and cerebellar cortex.[667,757] A number of cases have been reported with occipital cortex high signal[667,707,774] and several of these had prominent visual dysfunction clinically.[723] Correlation between the site of the cortical high signal and the distribution of EEG abnormalities has been described.[772]

Cortical high signal may[569,707,709,710,742,747,754,759,765,769] or may not[707,710,772-774] be associated with basal ganglia high signal. Cortical high signal may predate the development of basal ganglia high signal[710,747,769] but no report has described its first occurrence after the appearance of basal ganglia abnormalities.

Three familial CJD cases all initially showed high signal in the temporal lobe, either unilateral or bilateral, which extended on most of the cortex on repeat scanning[510,775,776]

### White matter high signal

White matter high signal has been described only rarely in sporadic and familial TSE cases.[563,715,727,729,758,777] It may show progression on serial scans,[563,715,727,729,742] be associated with basal ganglia high signal[715,727] and correlate with prominent white matter changes histologically, the so-called panencephalopathic form of CJD.[563] A single dura mater-related case with panencephalic pathology also showed MRI white matter high signal.[778] No other iatrogenic case with cortical or white matter high signal abnormalities has been reported to the best of my knowledge.

### Gadolinium enhancement

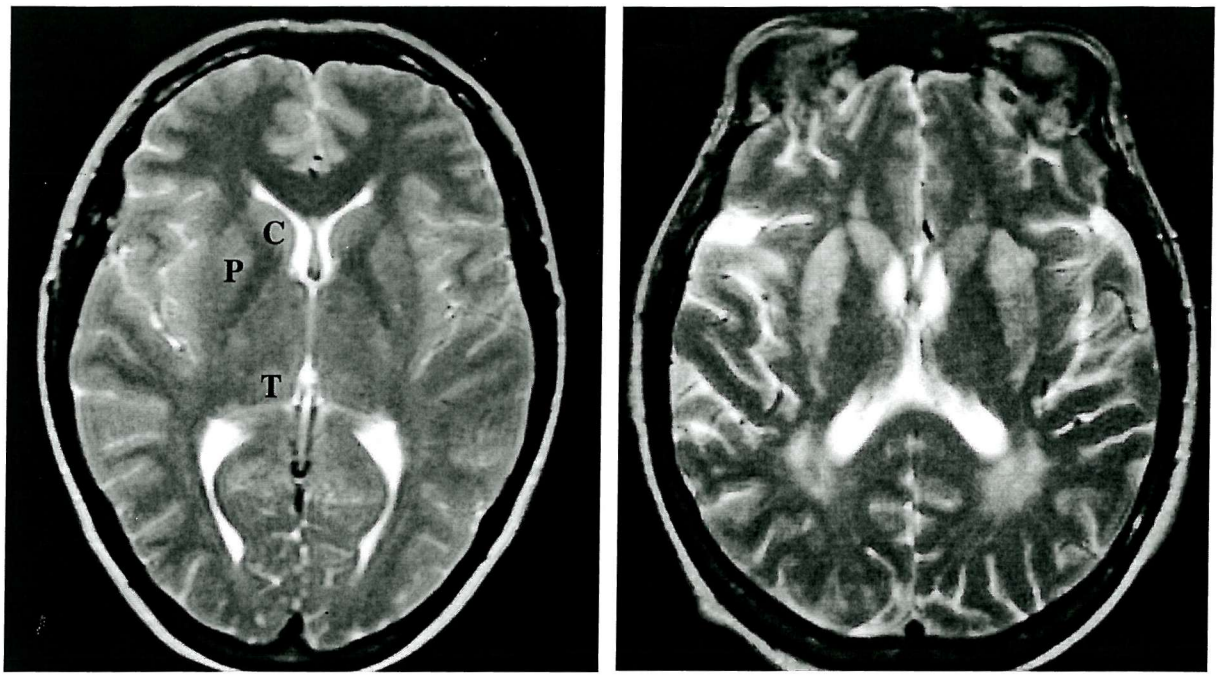
Only a single CJD case has been described with claimed basal ganglia enhancement with gadolinium.[712] The patient had bilateral striatal high signal on T2-weighted images and 'enhancement' in the left putamen on post-gadolinium T1-weighted images. However, the appearance of non-enhanced T1-images were not mentioned making this reported finding difficult to interpret. Multiple other reports and larger series found no enhancement with gadolinium.[707,710,751,760,765,769,774]

### FLAIR imaging

The low incidence of cortical involvement reported on MRI in CJD patients is perhaps surprising given that the cortex characteristically bares the burden of much of the pathological damage seen histologically. The basis of this discrepancy may be because of the fact that on T2-weighted MRI the high intensity of CSF interferes with the conspicuity of cortical signal due to partial volume artefact. The FLAIR sequence (see Figure 14) has been designed to suppress the high signal from CSF, whilst retaining the T2 characteristics of the brain parenchyma. Thus high signal changes in the brain substance adjacent to the CSF in the ventricular system and overlying cerebral sulci, are no longer 'masked' by the very high signal from CSF, and subtle pathological cortical grey matter changes are more conspicuous. FLAIR imaging is becoming increasingly utilised as a 'basic' MRI sequence in brain imaging due to its ability to demonstrate many varied pathologies (e.g. oedema, ischaemia, gliosis in mesial temporal sclerosis and multiple sclerosis plaques) with much greater clarity than on T2- or T1-weighted images.

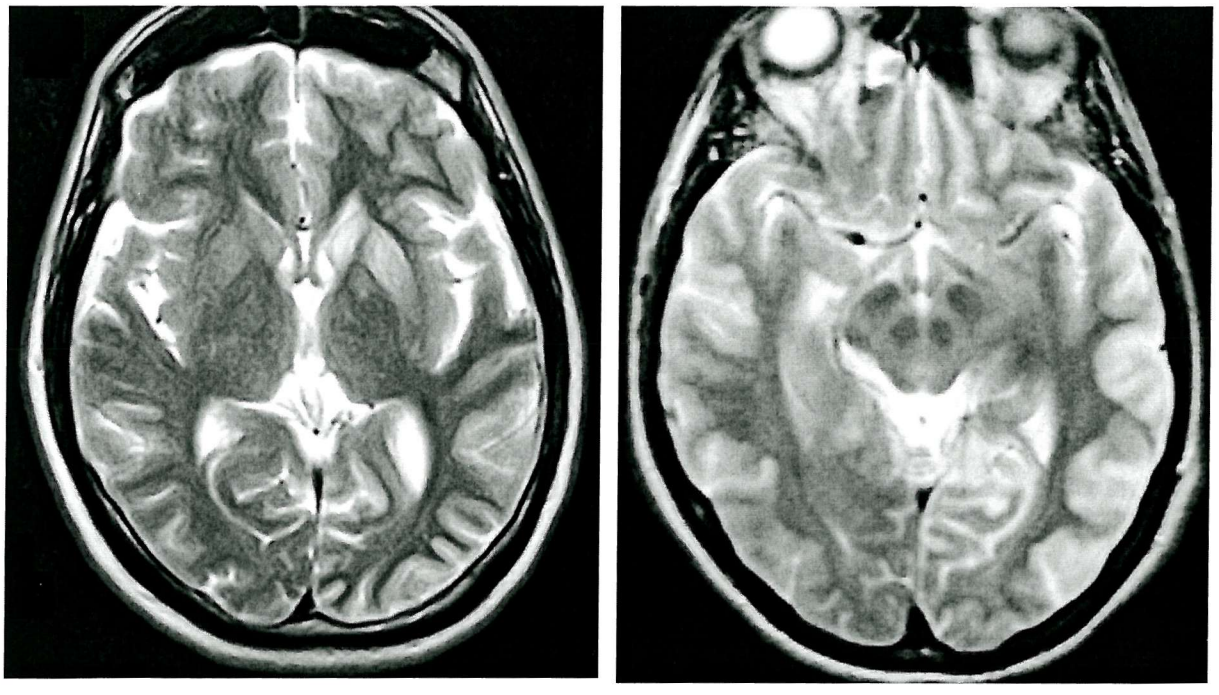
FLAIR has been reported to show areas of high signal which were not clearly identified on T2- and PD-weighted sequences in various distributions: 'cortical',[779-781] cortical (mainly parietal),[692] cortical (mainly right temporal and biparietal),[694] or amygdala, parahippocampal and cingulate gyri abnormalities,[756] and unilateral subtle right frontal and bilateral cingulate cortices.[780] FLAIR has also been reported to make cortical[772] or striatal[780] hyperintensity present on long repetition sequences more conspicuous. FLAIR did not show any added abnormalities compared to T2 imaging in one report.[780] Only a single patient has been described with normal FLAIR MRI,[781] although in this case diffusion-weighted images were abnormal.

**Figure 11: Axial T2-weighted MRI showing striatal high signal in a cases of sporadic CJD**



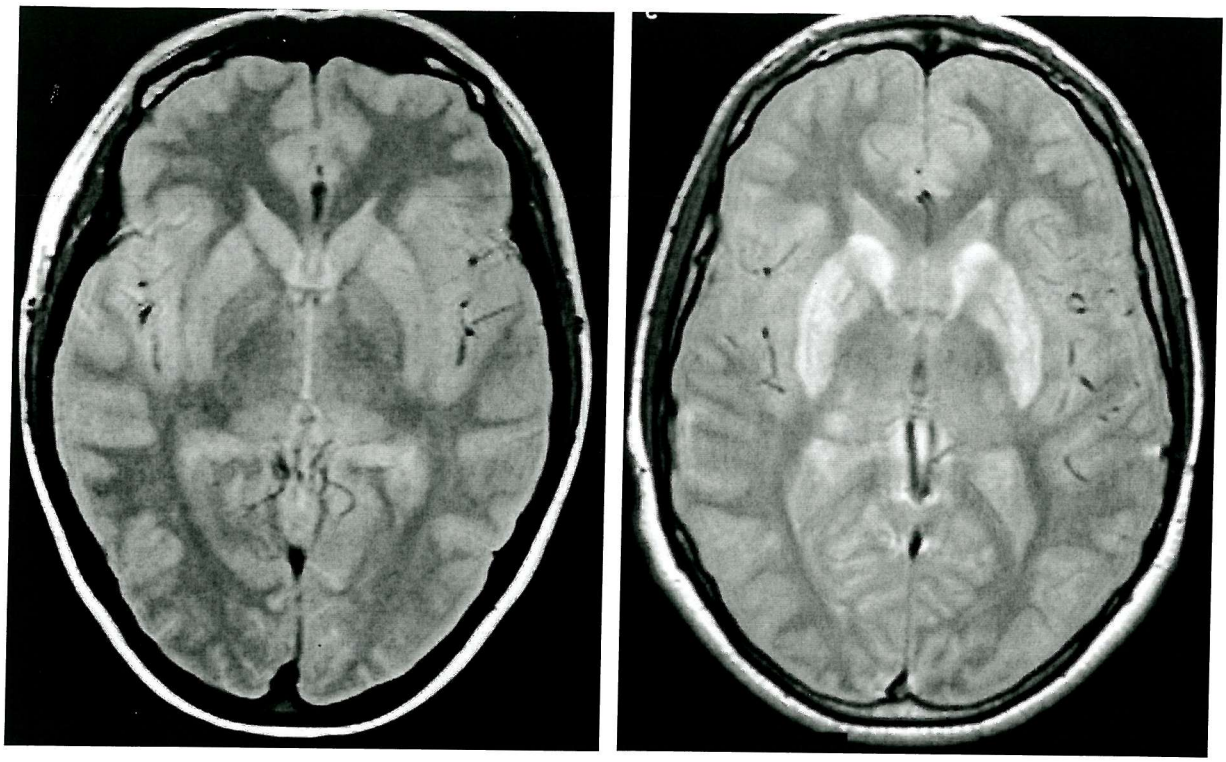

Left: Normal appearance, caudate (C), putamen (P) and thalamus (T). Right: sporadic CJD showing symmetrical high signal (relative to cortical and thalamic signal intensity) in putamen and caudate head. Focal white matter lesions, such as the periventricular white matter lesions seen here, are also seen in sporadic CJD, though may reflect coincident small vessel change.

**Figure 12: MRI in sporadic CJD showing asymmetrical striatal high signal and cortical high signal**



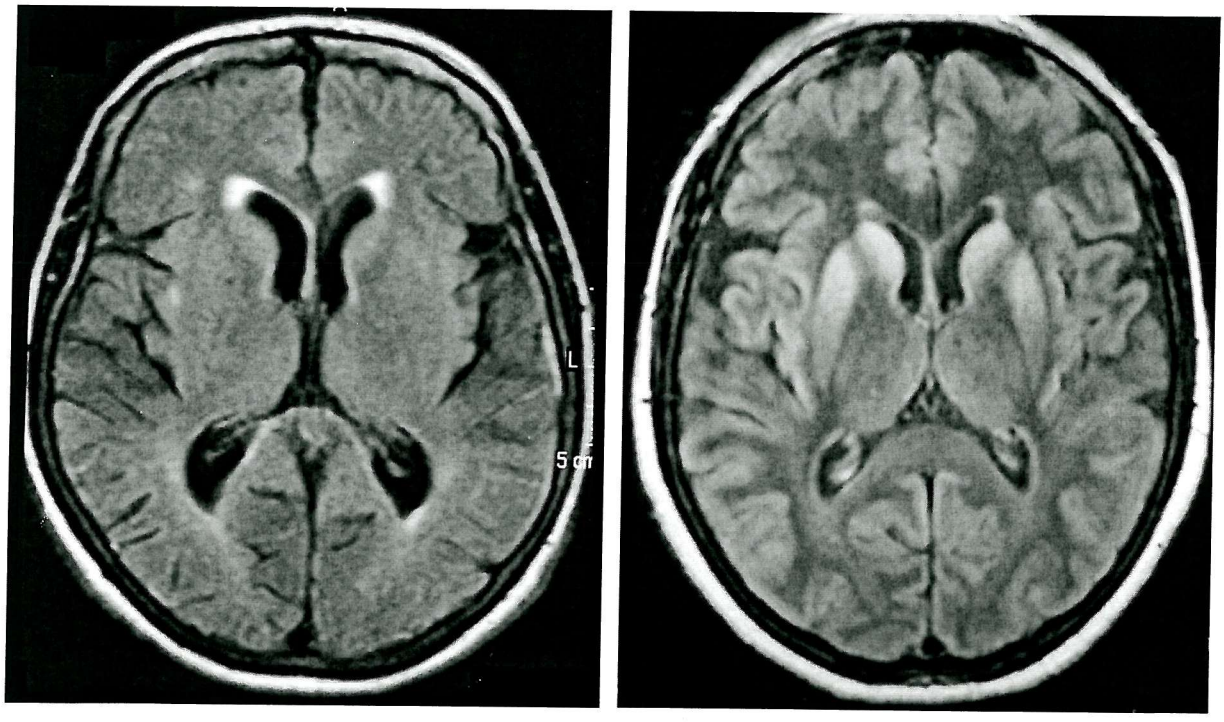
Left: An asymmetrical pattern of high signal in the putamen and caudate head. Right: hyperintensity of temporal cortical grey matter bilaterally, particularly on the right.

**Figure 13: Axial PD-weighted MRI**



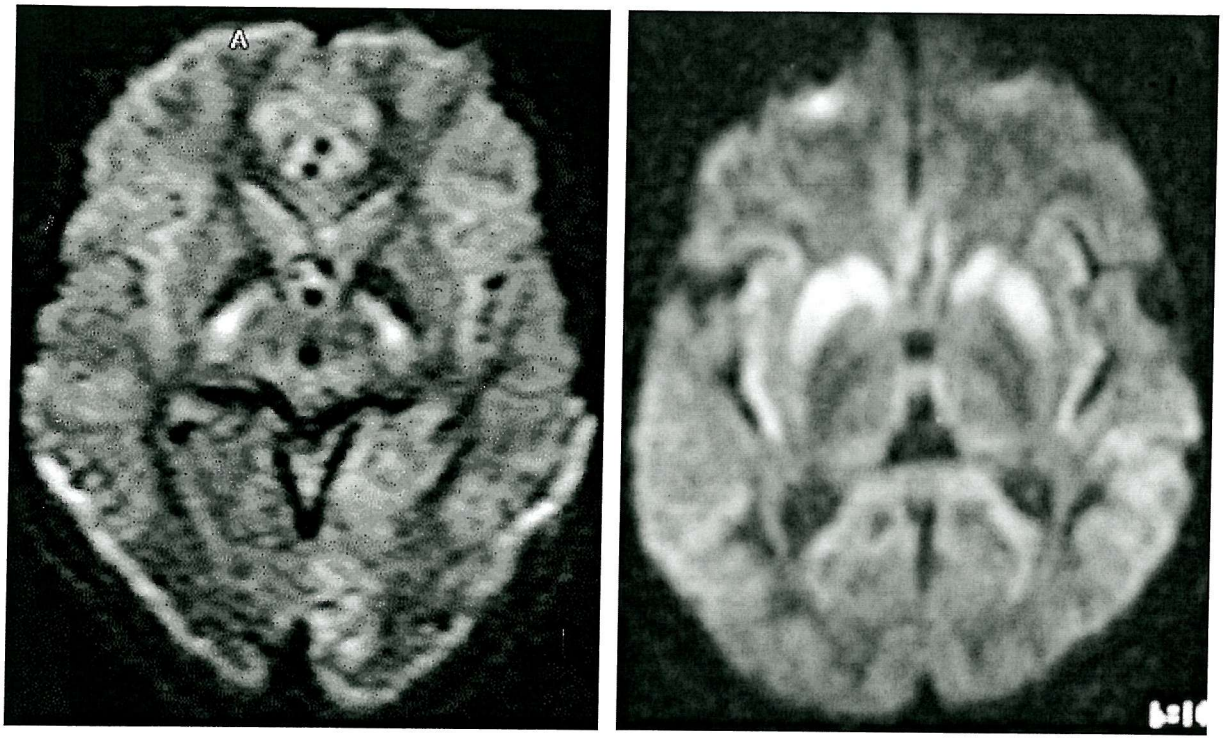
Normal appearance (left), sporadic CJD (right) showing homogeneous hyperintensity in the putamen and caudate head.

**Figure 14: Axial FLAIR MRI in control and sporadic CJD**



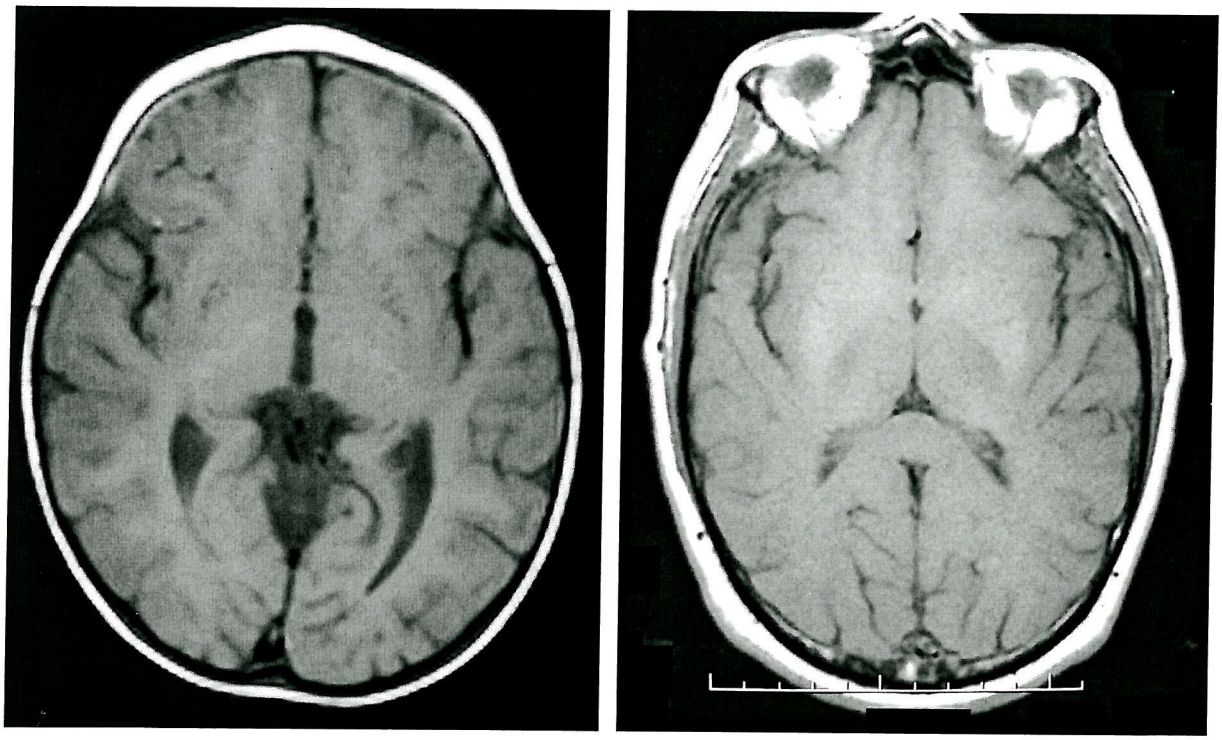
Normal (left) and sporadic CJD (right) with graded hyperintensity more marked in the anterior putamen relative to the posterior half of the nucleus, due to partial volume artefact from the white matter adjacent to the thinner posterior part.

**Figure 15: Axial diffusion-weighted MRI**




Normal (left) and showing high signal in the caudate and putamen bilaterally in sporadic CJD (right).

**Figure 16: Axial T1-weighted MRI**



Normal non-contrast T1-weighted axial image (left) and showing high signal in the putamen in a patient with sporadic CJD

**Diffusion-weighted imaging section**

Diffusion-weighted imaging (see Figure 15) highlights differences in water diffusability. It is most commonly used to detect cell swelling (such as in ischaemia) where the swollen cells impinge on the intracellular space, inhibiting diffusion. A number of other processes may also affect DWI.[782] The examination time is relatively rapid (about 30 seconds) compared to other sequences including FLAIR (several minutes).[780] It has been suggested that DWI is more sensitive in detecting grey matter abnormalities in CJD than conventional or FLAIR sequences.[783]

Abnormalities have shown on DWI which were not present on T2 or PD-weighted MRI sequences. These include high signal areas affecting the striatum, thalami, and cortex,[780] striatum and cortex,[692,714,764] striatum and cingulate gyrus,[784] thalami, unilateral frontal and bilateral cingulate cortices,[780] left temporal and occipital cortex,[764] occipital cortex,[764] caudate, temporoparietal areas, and unilateral frontal,[781] asymmetrical multifocal cortical,[785] asymmetrical occipital parasagittal region,[786] parietal and occipital cortices.[753] DWI has also shown basal ganglia,[692,781] thalamic[780] or cortical[780,781] high signal not seen on FLAIR sequences. In some cases cortical[780,781] or basal ganglia abnormalities[780,783] have been reported to be more prominent with DWI compared to FLAIR.[667] However, one case had grey matter high signal that was probably as conspicuous on T2 as DWI sequences.[759]

The earliest report of grey matter high signal on DWI is three weeks from the onset of symptoms.[764] Decreased basal ganglia hyperintensity with illness progression has been described on DWI, but concurrent with this change new cortical hyperintensities appeared.[753]

The distribution of DWI high signal has been reported to correlate with clinical features,[781,785] EEG changes[786] and functional imaging abnormalities.[781]

The pathological cause of DWI hyperintensity is unknown, but a positive correlation between the astrocytosis and high signal has been described.[764]

**Proton magnetic resonance spectroscopy**

Magnetic resonance spectroscopy (MRS) uses the principles of spectroscopy, developed initially for the analysis of pure chemical samples, to generate information about relative levels of a number of biological molecules in the body (including N-acetylaspartate, choline, creatinine and lactate). Although a number of clinical applications for MRS have been developed, such as for some metabolic diseases, most of the changes detected in MRS are very non-specific. Three reports have described the use of cerebral proton magnetic resonance spectroscopy in patients with CJD. All showed evidence of decreased activity of markers of neuronal activity, but this change was very non-specific,[766,787] and one study suggested that these were relatively late findings.[788]

### Histological substrate of MRI signal abnormalities

Various pathological causes for the MRI high signal seen on T2- and PD-weighted images in the grey matter in patients with TSEs have been postulated: neuronal loss or astrocytosis or both,[749] spongiform change or gliosis,[750,761] or neuronal loss.[752] Others have attempted to match abnormal MRI appearances with actual pathological findings. A correlation with gliosis and spongiform change[707,724] or solely spongiform change have been described.[776] Urbach reported increased T2-weighted signal and marked gliosis in the left caudate nucleus. No signal change, but prominent spongiform change and only slight gliosis were found in the right caudate nucleus suggesting astrocytosis as the cause of the MRI high signal.[691]

The above reports are difficult to interpret as the distribution of PrP deposition, spongiform change and gliosis at the time of scanning and autopsy may not be the same. Two studies of hamsters inoculated with scrapie and killed immediately after MRI scanning have gone some way to overcoming the limitations of previous reports. The first authors performed MRI on groups of animals at various time periods after inoculation. The earliest abnormalities were found 50 days after inoculation and 10 days prior to the onset of clinical signs. These consisted of moderate hyperintensity in the thalamus on both T1- and T2- images, and corresponded to a striking accumulation of PrP and moderate gliosis. The T2-hyperintensity in the thalamus increased as the disease progressed and, in later stages, the hyperintensity extended to the hypothalamus, striatum and septum, all sites of marked gliosis. Conversely, the T1-signal abnormality became undetectable 70 days after inoculation, when PrP accumulation spread across the entire brain. Spongiform changes were only observed in advanced stages. The authors concluded that their findings suggest that astrogliosis is likely to be responsible for T2 hyperintensity, and PrP accumulation for T1 abnormalities.[789] A report of CJD with high signal on T1-weighted imaging supports these findings, as the high signal seen in the globus pallidus also correlated with PrP deposition at autopsy.[763] The second animal study performed MRI just prior to the expected onset of clinical symptoms. Vacuolation of neurones/neuropil and gliosis were found to correlate with hypointense and hyperintense changes respectively on T2-weighted MR images.[744] Although the findings of these animal studies show differences, in particular with regard to the presence of spongiform change, both suggest that gliosis is the most likely cause of the T2-weighted grey matter high signal in CJD.

## Positron emission tomography

Positron emission tomography (PET) is an imaging technique that uses a positron-emitting radionucleotide, most commonly $^{18}$F-2-fluorodeoxyglucose (FDG), as a marker of glucose uptake and metabolism. PET is currently an expensive and relatively scarce imaging resource requiring access to a cyclotron for the production of the short half-life nucleotides used in the imaging process. However, it has a number of advantages over other isotope examinations in the depiction of functional differences in metabolism in different tissues. Characteristic patterns have been claimed for various neurodegenerative conditions including Alzheimer's disease, Pick's disease, progressive supranuclear palsy, and multi-infarct dementia.[790] The results of PET in only a small number of patients with TSEs have been reported, but it has been argued that there is a characteristic pattern in CJD[790-793] and FFI,[794] and that coupled with an appropriate clinical picture, PET can 'confirm the diagnosis' of CJD.[790] It has also been suggested that PET abnormalities may be helpful for targeting cerebral biopsy.[795,796]

The first description of PET in CJD was in a meeting abstract from 1982. The authors reported patchy hypofunction with loss of definition of subcortical structures in two patients and suggested that the appearances were significantly different from those of AD or other types of dementia that PET 'appears useful in confirming the diagnosis of subacute spongiform encephalopathy'.[791] Reviewing the subsequent literature indicates that the most common pattern documented is diffuse cortical hypometabolism,[378,793] which is most often heterogeneous and may be asymmetrical.[781,790,792,795-797] The cerebellum may[798] or may not[796] show abnormalities. The basal ganglia and brainstem have been reported in some cases to be relatively preserved or show no abnormality.[790,792,797] Similarly, white matter may[798] or may not show evidence of hypometabolism.[796]

The distribution of changes on PET may correlate with the clinical syndrome,[262,519,680,799-801] SPECT,[680] diffusion-weighted MRI[781] or pathological findings (in particular astrogliosis and neuronal loss),[795] although a discrepancy between PET findings and pathology has also been reported.[802]

PET in a series of seven FFI cases revealed reduced glucose utilisation of the thalamus and a mild hypometabolism of the cingulate cortex in all patients, a picture considered to be the hallmark of FFI. In six subjects the brain hypometabolism also affected the basal and lateral frontal cortex, the caudate nucleus, and the middle and inferior temporal cortex.[794] The PET abnormalities may correlate with clinical features, in that more widespread involvement was seen in patients with more diffuse neurological deficits.[802] It is interesting that the first report of PET in FFI related to a patient with a 178 mutation but who was considered to be suffering from familial CJD. Although PET showed a 10-25% reduction of activity in the thalamus the greatest abnormality (40% reduction) was seen in the medial frontal lobes.[456] SFI has also shown PET abnormalities identical to FFI with decreased thalamic activity.[459] PET has not shown any clearly distinct pattern in other forms of familial CJD.[460,510,519,528,799]

The cause of the hypometabolism seen on PET in CJD is unknown. One study suggested PET depicts neuronal dysfunction rather than actual neuronal cell loss, as the latter did not correlate with the PET abnormalities.[791] Goto postulates that as synapses are the most active sites of glucose metabolism in the brain the cause of the abnormality in CJD is PrP accumulation in the synapses causing neuronal dysfunction.[793] This hypothesis is supported by the finding of a correlation between PrP deposition and PET findings in FFI.[794]

The diagnostic value of PET in patients with TSEs is unclear. Claims of specific findings must be interpreted with caution and it is noteworthy that the first detailed report of PET in a CJD patient showed asymmetrical bilateral temporal lobe hypometabolism considered similar to that seen Alzheimer's disease.[803] No study has attempted to evaluate PET as a diagnostic tool using a suitable control group. Until this is done the utility of PET in CJD diagnosis remains debatable.

## Single photon emission computed tomography

Single photon emission computed tomography (SPECT) is a functional imaging technique that can be used to measure cerebral perfusion rate using a radioactive tracer agent detected by a conventional gamma camera.[804] Cross sectional images are produced which are similar to those seen on CT, MRI and PET, and SPECT is

cheaper and more widely available than PET, but produces less spatial resolution. As with PET, characteristic SPECT patterns are claimed to occur in various neurodegenerative conditions including CJD.[805]

The first report of SPECT use in CJD in 1987 described decreased perfusion of the left frontal and left temporoparietal using the radioisotope $^{123}I$, N-N-N$^{1}$ trimethyl-N- (2-hydroxy-3-methyl-5-iodobenzyl)-1,3 propanediamine.[668] Subsequent authors have reported various abnormal appearances in CJD patients, typically using the gamma emitting radiopharmaceuticals, $^{99m}$Tc-hexamethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) or $^{99m}$Tc-bicisate.

Patterns of decreased activity described include: entire brain,[683,806] heterogeneously throughout the brain,[807-809] cerebral cortex or 'supratentorial region',[674,711,715,762,810-812] unilateral cerebral cortex[569] or hemisphere (+/- thalamus),[261,681,682] fronto-temporo-parietal-occipital, basal ganglia, cerebellar,[813] fronto-temporo-parietal and thalami,[718] fronto-temporo-parietal,[210,683,781,814] fronto-parieto-occipital,[782] fronto-temporal and basal ganglia,[754] fronto-temporal,[715,815] frontal and thalamic,[458] frontal,[682], temporo-occipital,[816] temporo-parietal,[263,694] and occipital.[680] A similar diversity of SPECT abnormalities have been reported in familial TSEs.[210,281,495,535,540,545,742,769,817-819] The single hGH-related CJD case with reported SPECT findings had widespread cortical and basal ganglia hypoperfusion.[820] Only a single CJD case with a normal SPECT has been reported to the best of my knowledge.[808]

The SPECT abnormalities have been shown to correlate in some cases with clinical features,[261,680,681,816,820,821] EEG,[822] PET,[680] conventional[569,769] or diffusion-weighted MRI,[781] and pathology.[261,668]

In contrast to PET, a blinded study has assessed SPECT scanning in CJD. An unselected series of 27 dementia patients reported that the two CJD cases demonstrated a distinct irregular, dispersed 'mottled' pattern.[805] Although, this pattern was not clearly defined, the paper implies this refers to diffuse multifocal cerebral hypoperfusion. However, one of the CJD patients also had bilateral temporoparietal hypoperfusion (as seen more typically in Alzheimer's disease) and a case with Lewy body disease (LBD) was described as having in addition to bilateral temporoparietal hypoperfusion 'diffuse patchy abnormalities'. The difference between the latter and the mottled appearance of the CJD cases was not stated, raising the possibility that SPECT findings may be indistinguishable in CJD and LBD.

It has been suggested that SPECT scanning may be useful in CJD diagnosis by raising the possibility of an organic disease when other tests are unremarkable[820] or by providing information to help direct a brain biopsy.[813] As with PET, claims that there is a characteristic pattern that may be of diagnostic value[805] have to be interpreted with caution due to the current lack of an adequate case-control study. The multitude of SPECT patterns described above suggests that the detection of diffuse multifocal areas of cortical hypoperfusion may be a sensitive sign for CJD diagnosis. However, the differentiation from other degenerative dementias, like Alzheimer's disease or Pick's disease, is often impossible because of overlap of perfusion patterns, making SPECT a relatively non-specific test.[715]

### Conclusions

Computed tomography is of limited value in the diagnosis of CJD, showing progressive non-specific cerebral atrophy with considerable overlap with normal age-related changes and Alzheimer's disease. Nuclear medicine studies also show changes that are non-specific. In contrast, MRI can demonstrate fairly specific changes, in particular bilateral striatal high signal on T2 and PD-weighted sequences. This appearance is reported to occur in two-thirds of sporadic CJD cases. Similar changes involving cortical grey matter, the thalamus or globus pallidus are less frequently seen.

Basal ganglia changes are most conscious on DWI, FLAIR and PD MRI sequences, and least conspicuous on T1. DWI may be the most useful sequence in detecting cortical change and basal ganglia changes early in the clinical course.

## TREATMENT

Good nursing care to prevent the complications of immobility, such as pressure sores, is likely to be the most important treatment for a patient with CJD. Therapies aimed at palliation of any distressing symptoms, such as clonazepam or sodium valproate for myoclonus, are frequently successfully administered. Sedatives may be required for agitation, but such symptoms, if present, often abate naturally as the illness progresses.

No treatment has been proven to halt the course of the CJD, although a number of specific therapies have been tried including magnesium, cytosine arabinoside,[253] amantadine,[253,823,824] interferon[825] and other antiviral agents[210] and antibiotics.[826] There have been reports of improvement, although only temporary, with amantadine[827] and vidarabine.[828] Patients are frequently administered steroids,[253] acyclovir[829] or thiamine in the hope that they 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 in CJD.

Amphotericin B (an antifungal drug),[830,831] and iododoxorubicin (an anti-cancer agent)[832] have been found to delay death in hamsters or mice experimentally infected with scrapie. However, these drugs are potentially toxic and needed to be injected around the time of infection, or shortly afterwards (in the case of Amphotericin 80 days post inoculation),[833] to be most effective. Amphotericin B has been reported to prolong the clinical phase of experimental CJD in an African Green Monkey,[834] but had no effect in two human CJD cases.[835]

Administration of Congo red (a sulphonated amyloid-binding dye commonly used as a histological stain for amyloid) before or shortly after experimental scrapie infection, can significantly delay the onset of clinical disease in hamsters.[836] This compound has also been shown to inhibit the replication of scrapie infectivity in cell culture,[837] but has not been used as a therapy in humans.

Dextran sulphate 500 and pentosan polysulphate are members of a group of compounds known as polyanionic glycans. Dextran sulphate 500 prolongs the incubation period in mice inoculated with scrapie and reduces disease susceptibility, but to be effective has to be given within a month of infection.[838] Pentosan polysulphate (an agent traditionally used to treat interstitial cystitis) is less toxic in vivo[839] and is also more effective in lengthening incubation and reducing susceptibility when given at high dose weeks before scrapie inoculation.

Intraperitoneal pentosan polysulphate given seven hours after scrapie inoculation prolonged survival significantly in mice, with some animals living a normal lifespan with neither symptoms nor signs of a TSE.[840]

Short synthetic peptides that induce unfolding of β-pleated sheets termed β-sheet breaker peptides have been shown to significantly reverse the protease resistance of $PrP^{Sc}$ to a state similar to that of $PrP^{C}$ and decrease tissue infectivity.[841] The therapeutic effect of administering this compound to animals inoculated with scrapie is under investigation.

It has been shown that transgenic 'null' mice in which the PrP gene is absent (and which therefore do not produce PrP) may appear clinically well and are resistant to TSE infection.[842,843] This has led to the suggestion that ablative gene therapy, or the use of anti-sense oligonucleotides to 'turn off' the production of PrP, may be a useful treatment strategy. Although this is an interesting idea it is still unknown whether the neurophysiological damage associated with TSE infection results from the accumulation of abnormal PrP or the loss of the normal isoform. It has been suggested that if the latter is true abrupt cessation of PrP production may have a deleterious effect (theoretically not seen in null mice because of adaptation to hereditary PrP loss).

A number of other therapeutic strategies have been suggested, including the use of compounds to block agent replication sites,[844] counter the neurotoxic effects of $PrP^{Sc}$ or prevent conversion of $PrP^{C}$ into $PrP^{Sc}$,[6] for example by interfering with PrP glycosylation or binding of chaperon molecules such as the hypothetical protein X.[118,845,846]

Manuelidis[847] showed that administration of a long incubation strain of TSE agent could block expression of a virulent short incubation strain subsequently inoculated. This raises the possibility of vaccination with very long incubation period strains (exceeding the natural lifespan) and thus preventing the occurrence of clinical infection.

Despite the extensive list of therapies that have been studied in human and animal TSEs (see Table 13) there is no medication currently available that has any significant effect on the underlying disease process in symptomatic individuals.

**Table 13: Treatments tried for TSEs**

**Hormonal agents**

- Adrenaline
- Corticosteroids†[848]
- Insulin
- Oestradiol
- Propylthiouracil
- Testosterone

**Anti-neoplastic agents**

- Actinomycin D
- Cyclophosphamide
- Cytosine and adenosine arabinoside[826]
- Methotrexate
- Streptozotocin

**Reticuloendothelial system blockers**

- Carrageenan*
- Dextrans of various sorts*
- Silica
- Trypan blue

**Anti-parasitic agents**

- Chloroquine
- Diiodohydroxyquin
- Glycobiarsol
- Metronidazole
- Niclosamide
- Quinacrine†[849]

**Anti-fungal agents**

- Amphotericin B*
- Griseofulvin
- Sinefungin

**Anti-bacterial agents**

- Rifampicin[826]
- Sulphamethoxyizole
- Tetracycline
- Thiamphenicol[826]
- Trimethoprim

**Anti-viral agents**

- Amantadine†[823,824,826,827]
- Acyclovir†
- Dihydroxypropyladenine
- Ethyl-desoxyuridine
- Idoxuridine†
- Interferon†[825]
- Isoprinosine[826]
- Methisazone
- Methisoprinal†
- Phosphonacetic acid
- Sodium butyrate
- Sodium thocyanate
- Trisodium phosphonoformate
- Various heteropolyanions*
- Vidarabine†
- Virazole

**Miscellaneous**

- Blood transfusions†[848]
- Colchicine
- Congo red*[836]
- L-carnitine
- L-dopa†[850]
- Magnesium†
- Oubain*
- Polyanionic glycans*[840]
- Vitamin C
- Chlorpromazine†

* Some activity demonstrated. † Use reported in humans with CJD

## Scrapie

Scrapie is the prototypical TSE. It is an insidious neurodegenerative disease that naturally affects sheep, goats and moufflon.[851] The term 'scrapie' describes the tendency for affected sheep to scrape themselves against trees or bushes (see Figure 17). The disease was known as la tremblante (trembling disease) in France, as Gnubberkrankheit (itching disease) or Traberkrankheit (trotting disease) in Germany, rida in Iceland, súrlókór (brushing disease) in Hungary and by multiple other names in Britain. As a clinical entity it was recognised in sheep in England as early as 1730[88] and has subsequently been reported to have a virtually worldwide distribution, but is notably absent in Australia and New Zealand. A study of scrapie incidence in the UK suggested that about one-third of flocks were affected and that the incidence of the disease in these flocks was between 0.5 and 1.1 per 100 sheep/year.[852] However, this data may have over-estimated the true incidence of the disease.[6]

Scrapie was first experimentally transmitted by inoculation of a ewe with infected brain material in the 1930s.[328] At the same time as these experiments were being confirmed an outbreak of scrapie occurred in England affecting several hundred sheep that had been immunised against louping-ill using a vaccine prepared from brain, spinal cord and spleen of sheep that were belatedly discovered to have been exposed to natural scrapie infection.[323] The transmissible nature of the scrapie agent was thus established beyond any doubt.[109] Demonstration of transmission to mice in the early 1960s[853] permitted the disease to be intensively studied, but in spite of this the exact mechanism(s) of scrapie spread in nature remains uncertain. It is commonly accepted that the disease is infectious and contagious but that genetic factors are also important. Infection is probably most often transmitted from ewe to lamb, but whether this occurs in-utero, during parturition or afterwards when the ewe and lamb run together is unclear.[6] There is also horizontal spread of infection between unrelated adults and this may account for some of the scrapie cases in older sheep. The exact routes of infection are unresolved, but possibilities include transplacental, oral, nasal, optic or cutaneous. Hay mites from farms with scrapie have been shown to contain infectivity raising the possibility that they may play a role in horizontal transmission.[854] Complex genetic factors involving the PrP gene, and potentially other genes, are known to affect the incubation period and thus the apparent susceptibility of sheep to scrapie. The possibility of genetically engineered animals resistant to disease has therefore been raised.

Male and female sheep are affected equally. The average age at onset is about three-and-a-half years.[855] In natural scrapie the first clinical features are often insidious. Early signs are apprehension, restlessness, hyperexcitability and aggressiveness, and some animals even manifest apparent 'dementia'. Fine tremors of the head and neck are observed, and as the disease progresses these become more generalised, involving the whole body and producing a shivering effect. Fasciculations of superficial skeletal muscles may occur, and signs of cutaneous irritation, self-induced by rubbing and scratching, constitute one of the most characteristic clinical features, though do not occur in all cases, e.g. Icelandic scrapie (rida). As the disease evolves the gait becomes ataxic with severely affected animals unable to stand or walk without falling. In the advanced stages of scrapie animals become stuporous and manifest visual impairment, excessive salivation and wasting. The duration of the natural clinical course is usually less than four months.[851]

In keeping with other TSEs the neuropathological triad of spongiform change, neuronal loss and astroglial proliferation occurs in scrapie. Vacuolation of the neuronal cytoplasm is a marked and pathognomonic feature, being particularly evident in the brainstem and the ventral and lateral horns of the spinal cord.[851] Cerebral amyloidosis is seen in just over half of natural cases of scrapie. Another characteristic feature of scrapie and other TSEs is the presence of rod-shaped structures seen on electron-microscopy and known as scrapie-associated fibrils (SAFs). The SAFs are fibrillar forms of amyloid – the same amyloid which is contained in PrP plaques.

## Transmissible mink encephalopathy

Transmissible mink encephalopathy (TME) was first described in 1965 but had occurred on mink farms in Minnesota and Wisconsin as early as 1947.[91,856] The disease occurs as outbreaks, in farmed mink only, and has been recognised in Idaho, Russia, Finland, Canada and Germany.[857,858] The condition is rare and mortality high, with nearly all adult mink on an affected ranch succumbing to the disease during an outbreak. Affected animals present with behavioural changes such as hyper-excitability and aggressiveness, followed by incoordination and rapid death.[857] Evidence points to infected feed as the cause of TME and it has been suggested that scrapie is the likely contaminant.[6] However, experimental transmission of scrapie to mink via the oral route has not been successful to date, although TME may be caused by a different scrapie strain than those used experimentally. The possibility of a bovine origin of TME has also been raised. Products from fallen or sick cattle ('downer cows') were fed to a colony of affected mink in Stetsonville, USA, which it is claimed had been fed a diet free of any ovine material.[859] However, surveillance of cattle in the USA has not revealed a single case of BSE, thus arguing against a bovine origin of TME infection. Furthermore, although BSE has been experimentally transmitted to mink, the incubation period, clinical signs and neuropathology show significant differences from natural TME. No convincing evidence for maternal or horizontal transmission of natural disease exists.

## Chronic wasting disease of mule and elk deer

Chronic wasting disease (CWD) is a TSE of mule deer, white-tailed deer, black-tailed deer and Rocky Mountain elk. It was first recognised as a clinical syndrome in 1967 and has only occurred in limited areas in the western North America. A report from 1999 stated that approximately 500 CWD cases (including free ranging, privately owned, and research cervids) have been documented.[860] One study showed that about 5% of mule deer, 2% of white-tailed deer, and 0.5% of wapiti (elk) from endemic portions of Colorado and Wyoming showed evidence of pre-clinical CWD infection.[860]

There is no clear evidence to suggest that CWD is caused by exposure to any other form of animal TSE. Furthermore, other ruminant species, including wild and domestic cattle, sheep and goats, have been housed in facilities in direct and indirect contact with CWD-affected deer and elk, but no cases of CWD have been detected in these animals. Although the exact origin and mode of transmission of CWD is unknown, epidemiological studies suggest that transmission may be lateral, and possibly maternal. Transmission via feed is not believed to occur, affected animals have been fed a variety of foodstuffs with no common ingredient of animal origin being identified. It is of note that painstaking attempts to eradicate CWD from captive facilities,

including thorough decontamination and a 12-month period free of elk or deer, failed to prevent disease recurrence.

Deer and elk affected with CWD show progressive loss of body condition accompanied by behavioural changes. In the later stages of disease, emaciation, excessive salivation, increased drinking and urination, stumbling, trembling, and 'depression' may precede death.[860] Approximately 40% of deer diagnosed with CWD in surveillance studies show only accumulation of $PrP^{Res}$ in the brain by immunohistochemistry and do not have spongiform lesions.[860]

During 1997-8, three CJD patients 30 years of age or under were reported in the USA, two had been hunters and the other was reported to have regularly consumed venison.[861] The occurrence of these unusually young CJD cases created a concern about possible zoonotic transmission of CWD. However the clinicopathological features of these patients were consistent with sporadic CJD and none of the cases were reported to have consumed deer meat obtained from the known CWD-endemic areas of Colorado or Wyoming. Although the occurrence of these unusually young CJD cases suggest a possible relationship with CWD, clinicians from the US authorities who investigated these patients have concluded that the available information does not support a causal link.[860]

## Bovine spongiform encephalopathy

### The early years

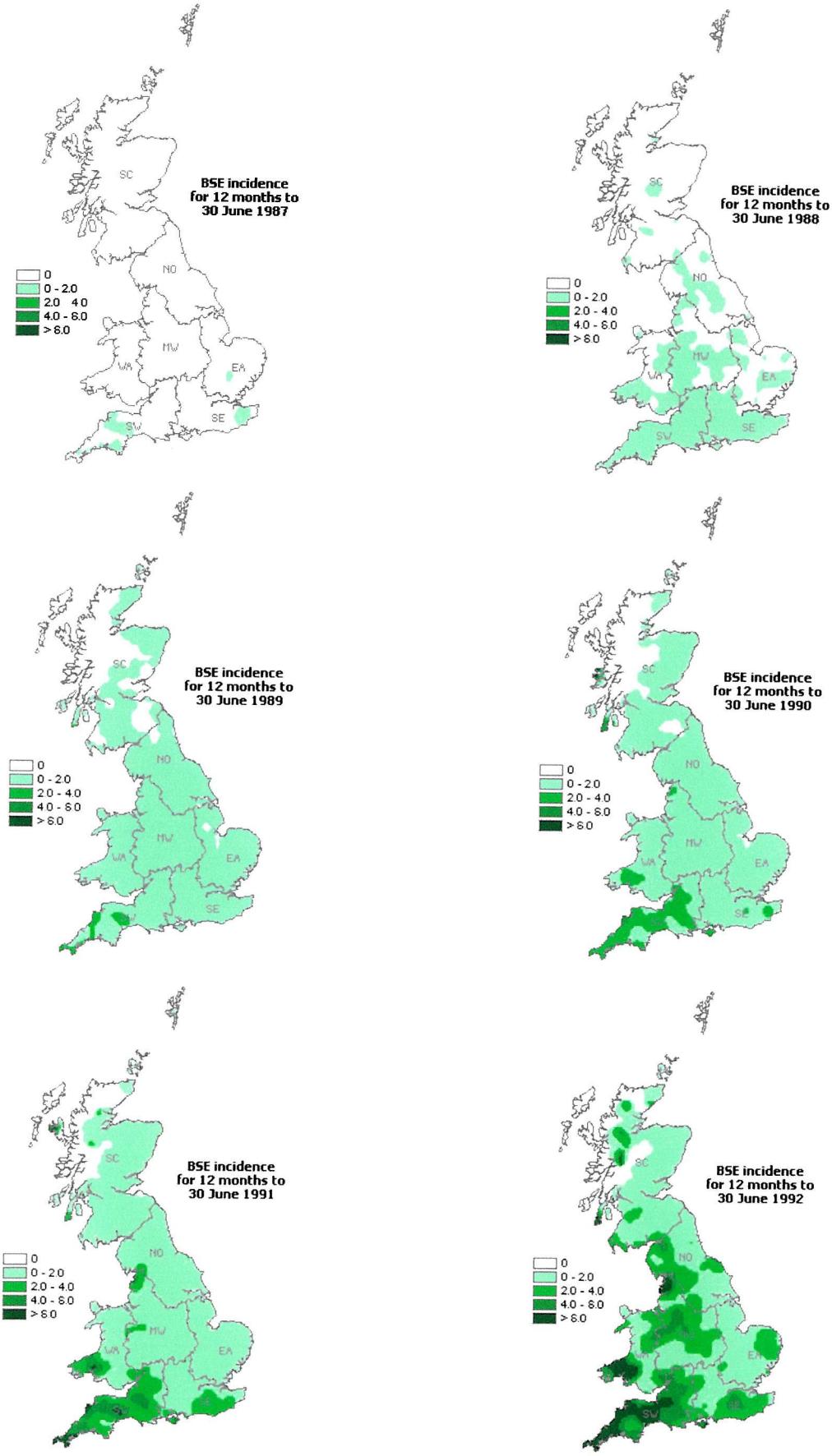
Prior to the mid 1980s no TSE had been reported in cattle anywhere in the world. The first case of confirmed BSE (see Figure 18) was a cow from Pitsham Farm in Sussex. Brain samples from this recently killed animal were first examined in September 1985 at which time the neuropathological changes observed were thought to be due to a toxicity of some description.[862] However, review of the pathology in June 1987 confirmed this as a case of BSE. There is anecdotal evidence from farmers and veterinarians to suggest that there may have been earlier, undiagnosed cases of BSE, e.g. between October 1983 and May 1985 five 'classic cases' of BSE are thought to have occurred on a farm in Malmesbury, Wiltshire.[6]

By December 1986 it had become apparent to the pathologists at the UK authorities Central Veterinary Laboratory (CVL) that they had discovered a new disease in cattle. Several brains from affected animals had been referred to them from farms in Kent and Bristol, and histopathological examination showed spongiform change in the brains which closely resembled scrapie in sheep. This suggested the possibility that, like scrapie, the disease could be a TSE. This hypothesis was supported over subsequent weeks by the demonstration of SAFs on electron microscopy.[6] The first paper to describe the clinical features and neuropathology of BSE was published in the Veterinary Record in August 1987.[93] This article concluded that the disease was probably due to an unconventional infectious agent, as in other well-recognised TSEs. The hypothesis that BSE was a TSE or prion disease was further strengthened by the identification of $PrP^{Res}$ in diseased cattle brain in October 1987. Transmission studies were first initiated using hamsters, but these were unsuccessful. Proof of transmissibility had to await inoculations into mice, which confirmed that BSE was a TSE in September 1988.[6]

**Figure 17: Sheep affected by scrapie**

**Figure 18: Cow affected by bovine spongiform encephalopathy**

**Figure 19: Incidence of BSE across Great Britain**




Incidence expressed as confirmed BSE cases per 100 adult cattle per square kilometre

EA = East, MW = Mid and West, NO = Northern, SC = Scotland, SE = South east, SW = South west, WA = Wales.

**How was BSE acquired?**

The mode of acquisition of BSE was initially unclear. Possible sources of infection from sheep and other species, especially animal protein in cattle feed, were considered, as well as genetic factors and semen used for artificial insemination. An epidemiological study of BSE was undertaken under the direction of Mr John Wilesmith in the latter half of 1987 and by December of that year information on approximately 200 confirmed cases of BSE had been compiled.[863] Vaccines, hormones, organophosphates, anthelmintics and other treatments were excluded as common factors. Autosomal dominant or recessive inheritance was also ruled-out, although genetic factors influencing susceptibility to infection were still a possibility. Sheep were not held on 20 per cent of the farms with BSE-affected cattle and thus direct or indirect contact with sheep scrapie was also excluded. A common factor to all affected farms was the use of commercial cattle feed and it was found that this had been fed at some time to all of the cases for which accurate records were available.

Since the 1920s animal protein in the form of meat and bone meal (MBM) had been incorporated into cattle feed to provide a rich source of protein.[864] MBM was produced by rendering discarded animal fat, bones, offal, whole carcasses, and other 'mixed material' from bovine, ovine, porcine, poultry, and other sources.[89] It was thus considered likely that MBM provided a vector for the transmission of scrapie from sheep to cattle. The fact that, compared to other countries, the UK had a high incidence of scrapie and a large proportion of sheep in the mix of carcasses rendered for animal feed supported this hypothesis.[865] Further anecdotal evidence that MBM was the vector for the agent responsible for the BSE epidemic was provided by the occurrence of a similar TSE in a nyala and a gemsbok from a wildlife park in the UK. Disease was diagnosed in these animals in 1986 and 1987 respectively, and it was established that both had been fed cattle feed containing MBM. The feed-borne hypothesis was additionally strengthened by the fact that BSE had occurred almost exclusively among dairy herds, an observation that correlated with the heavy feeding of cattle concentrates to dairy cows and their calves. Of interest, the practice of including MBM in the diet of calves for most of the first 12 weeks of their lives began in the mid 1970s in the UK, but does not appear to have been common in continental Europe and the USA.[866] A ruminant feed ban, which prohibited the use of ruminant-derived protein in ruminant feed in Britain, came into force on 18 July 1988. This led to a dramatic reduction after 1992 in the number of cases of BSE, indicating that the hypothesis that feed was the vector for the spread of the disease was almost certainly correct.

**Why did BSE first appear in the mid-1980s**

It is generally accepted that MBM-containing feed was the main source of BSE infection and that the recycling of affected cattle through the rendering process fuelled the BSE epidemic. However, the reason for the appearance of BSE in Britain during the mid-1980s and the origin of the BSE agent are unresolved and contentious issues. There are two main hypotheses, firstly that BSE arose as a consequence of a change in the rendering process allowing the widespread transmission of sheep scrapie to cattle,[865] and second that the BSE agent has a bovine origin, and that any changes in the rendering process may or may not have been relevent.[6] These theories are discussed below.

Analysis of Wilesmith's 1987 BSE data revealed that the disease was geographically widespread throughout Britain in a pattern that was considered characteristic of an extended common source epidemic. All cases

occurred in adult animals with an age range of two years nine months to 11 years, with the highest incidence in four-year-olds. As the first cases in the study were identified in 1985, this suggested that it was most likely that most of the cattle were first exposed to infection in 1981–82.[6] It was generally considered by those studying BSE in the later 1980s that contamination of MBM by an existing strain of scrapie was the most likely cause of BSE. An alternative explanation that the emergence of BSE might have been due to a new mutant strain of scrapie was discounted because the form of the epidemic and the geographically widespread occurrence of BSE would require the simultaneous emergence of this mutant scrapie strain in a large number of flocks (or cattle herds) throughout the country, an occurrence considered inconsistent with the data. However, at odds with the scrapie contaminated-feed hypothesis for the origin of BSE was the fact that BSE had been observed only after the mid-1980s, when it was almost certain that scrapie–affected sheep had been incorporated in cattle feed since 1900 at least. A number of potential explanations were considered:

1. A dramatic increase in the sheep population in Great Britain.
2. An increase use of protein derived from ruminants for cattle feed in the early 1980s, as world prices escalated for the then prevailing protein supplements, fishmeal and soybeans.[867]
3. A probable (but unproven) increase in the prevalence of scrapie-infected flocks.
4. A greater inclusion of sheep heads in material for rendering.
5. A greater inclusion of casualty and condemned sheep in material for rendering.
6. The introduction of continuous rendering processes during the 1970s and 1980s.
7. The decline in the practice of using solvent extraction of tallow in rendering since the mid-1970s.

Of the above, the most important factors were initially considered to be changes in rendering methods. The modification from batch to continuous rendering processes might have resulted in the treatment of animal material at lower temperatures and/ or for shorter periods, and therefore led to failure to inactivate the scrapie agent. A survey of all rendering plants in Britain revealed that the use of solvents to extract fat from greaves (the protein-rich solids from which MBM was produced) had ceased in all but two plants, and that the cessation was consistent with the estimated time of onset of exposure to the infective agent.[868] Furthermore, these two plants were in Scotland, where the incidence of BSE was relatively low. The decreased use of solvent extraction had occurred because the market for tallow had declined and also for safety reasons.[89] The impact of the solvent extraction procedure on the level of infectivity was thought to be two-fold: firstly, the use of the solvents themselves; and, secondly, the application of superheated steam to remove the traces of solvent from the defatted products.

The relevance of the changes in the rendering process to the occurrence of BSE has been challenged. The survey of all rendering plants in Britain established that the change to continuous rendering had not in fact generally resulted in lower time/temperature combinations. In addition, the introduction of continuous rendering took place gradually over a number of years and was not universal. Furthermore, during the 1960s and 1970s, some 30 to 50 per cent of MBM was produced by methods which did not use solvent extraction, so the absence of solvent extraction was not a universally new factor in the early 1980s. Moreover, Taylor[864] studied the effect of solvent extraction and showed that it was barely effective in reducing infectivity (although Brown argues that this minimal reduction may still be important[865]). Additionally, BSE was confined to Britain and the Channel Islands until 1989, but MBM was used in cattle feed in both Europe and North America and was prepared by

processes similar, if not identical, to those in the UK. Thus it is argued that changes in the rendering process could not have been solely responsible for the emergence of BSE.

The origin of BSE as an extended common source epidemic arising from exposure of cattle to scrapie in the early 1980s has also been challenged.[6] If BSE had originated from infection by sheep scrapie around 1980 then because of the species barrier effect subsequent cattle-to-cattle transmission of BSE would have been expected to have led to a decreased incubation period. This in turn would have led to an increased relative incidence in young cattle. However, this phenomenon did not occur, arguing that scrapie was not the source of BSE and/ or that several cycles of cattle-to-cattle infection had occurred prior the observation of the first BSE cases. This finding should not be over-interpreted however, as the mean incubation period of sheep scrapie inoculated intracerebrally into cattle is very similar to that on secondary passage between cattle (485 vs. 499 days respectively).[869] The BSE epidemic had first appeared in Southern England in 1985 and spread to the Midlands, Wales and Northern England, reaching Scotland in November 1987 and Northern Ireland late in 1988. This pattern lends support to the view that a new or modified agent might have arisen somewhere in Southern England, and then spread from there to other parts of the country (see Figure 19). A model based on a spatio-temporal analysis of the BSE epidemic reported to the BSE Inquiry is in-keeping with this hypothesis, as this suggested an intense focus of cases occurring in the Devon/Somerset region, and the subsequent spread of disease from this point to the surrounding areas.[6] However, although this suggestion is plausible, it is necessarily speculative because it deals with a putative undetected phase of the epidemic.[866]

The above suggestion that BSE arose from a point source with several cycles of infection occurring prior to the mid-1980s would be compatible with a bovine origin of the initial agent. This could have arisen as a result of rendering a cow that had developed or was incubating 'sporadic' or 'familial' BSE. If this event had occurred in the early 1970s it could have led to a unifocal epidemic of unidentified cases in 1975–77, which then precipitated the 1981 second wave, which in turn precipitated the third phase of cases from 1986. The report of the BSE Inquiry puts forward several other lines of argument in support of the theory that the BSE agent has a bovine rather than ovine origin: first, the observation that the BSE agent appears to exists as a single biological strain, that differs from those of scrapie;[870] second, the observation that the natural and experimental host range of BSE is markedly different from that of scrapie; and third, that the clinicopathological phenotype of experimental scrapie in cattle differs from that of natural BSE.[869,871] These arguments have been eloquently challenged by the Horn report, which reviewed the findings of the BSE Inquiry with regard to the origin of BSE.[866] Horn notes that the known strains of scrapie are based on a very small sample of sheep (surprisingly only 29) and that the number of strains in the whole population of scrapie-affected sheep (which number 5000-10,000 annually in the UK) is unknown. Furthermore, the difference in apparent host range between BSE and scrapie does not rule out a scrapie origin for BSE, as it is possible that repeated cycling of an existing scrapie strain through cattle selected a new strain with a different host range. With regard to experimental transmission of sheep scrapie to cattle, Horn highlighted the fact that this has been reported on only two occasions, both using scrapie sources from the USA, and thus the relevance of these results to the source of BSE in the UK was uncertain. Therefore, the origin of BSE remains controversial. Although unconventional theories relating to the use of organophosphates,[170] an autoimmune reaction,[172] endocrine or methyl bromide poisoning, zoo animals[872] or human tissues entering the rendering process and comets can probably be discounted, whether the agent arose from a sheep or bovine source may never be resolved.

**Further epidemiological and clinical aspects of BSE**

Most BSE cases were infected as calves; the modal age of disease occurrence is five years (range 20 months to 18 years)[89] and the average incubation period 60 months. Adult dairy cattle were predominantly affected, because as calves they had received the MBM.[89] Bull calves derived from dairy cows are nearly always castrated and slaughtered for beef at around two years of age, so, even if they were infected via MBM, disease would not usually have had time to be expressed.[89] The disease typically occurred in only a few animals in any one herd, but increased herd size increased the risk and some birth cohorts in large herds were very severely affected.[89] The low average within-herd incidence (<3% in any six-month period since the epidemic began) is attributed to a low average exposure of the cows to 'packets' of infectivity that were generally widely spaced in different batches of feed.[873] It has been calculated that the average exposure in affected herds may have been as low as 14 oral LD 50 per tonne of concentrate feed.[873]

The duration of the clinical course of BSE is typically one or two months, but ranges from seven days to 14 months. The most commonly observed signs are apprehension (see Figure 18), hyperaesthesia and ataxia, but affected animals may also show a decreased milk yield and loss of condition. A number of other bovine conditions can mimic the clinical phenotype of BSE, e.g. magnesium deficiency ('staggers'), and no practical and reliable laboratory diagnostic disease marker is yet available in live animals.

Pathological changes are similar to scrapie in many respects with vacuolar lesions largely confined to the brainstem and accompanied by neuronal degeneration and an astrocytic reaction. Sparse cerebral amyloid plaques are seen in a small proportion of cases. In contrast to scrapie, greater diagnostic importance is attributed to the neuropil vacuolation than neuronal vacuolation.

The bovine PrP sequence differs from that of sheep at seven or eight positions.[874,875] In contrast to the many PrP polymorphisms found in sheep, only one PrP polymorphisms has been found in cattle.[876] Though most bovine PrP alleles encode five octarepeats, some encode six.[874] PrP alleles encoding six octarepeats do not seem to be over represented in BSE.[876]

**Statutory measures and the evolution of the BSE epidemic**

The British Government made BSE notifiable in June 1988 and the following month a statutory ban on the feeding of ruminant-derived protein to ruminants was introduced. In August 1988 the compulsory slaughter and disposal of carcasses of all cattle suspected of having BSE was ordered, with 50% compensation paid (a figure that has been criticised[877,878] and which was subsequently increased to 100% in February 1990). In December 1988 all milk from suspect animals was ordered to be destroyed, but, for welfare reasons, an exception was made for feeding a cow's own calf. In November 1989 a ban was introduced on the use of certain specified 'high risk' bovine offals (SBO) for human consumption (brain, spinal cord, tonsils, thymus, spleen and intestines from animals >6 months old). The selection of which offals should be included in the SBO ban was based on the evidence of infectivity of tissues from scrapie-infected sheep. In September 1990 the use of SBO was further restricted, being prohibited for use in feed for all animals and birds.

At the end of 1992 BSE reached its peak incidence in the UK (see Figure 20) but thereafter declined rapidly, almost certainly in response to the statutory measures. However, new cases of BSE were being observed in

cattle that were born after the implementation of the feed ban. It has been suggested that most of these cases occurred because of the continued use of feed rations produced before the ban; cross-contamination of cattle feed by feed containing MBM intended only for pigs or poultry; and an incomplete compliance with the SBO ban. Further measures were instituted to address these particular issues.

**Tissue distribution of infectivity in BSE**

BSE infectivity has now been demonstrated in the brain, spinal cord and retina of naturally affected cattle and also in the trigeminal, cervical and thoracic dorsal root ganglia and bone marrow of those infected experimentally.[283] Infectivity has also been found in the distal ileum (the inoculum comprising the wall of the intestine including Peyer's patches) of cattle killed six months after oral challenge with 100g of BSE brain,[879] but not in naturally infected cattle,[880] although this may be due to the smaller dose received during natural inoculation and the fact that only three cattle had been tested.[283] Subsequent bioassay results provided evidence of infectivity in the distal ileum of cattle killed 14 and 18 months after experimental inoculation, and of a reduction in incubation period in the mice inoculated after each successive kill, at least up to 14 months after inoculation, suggesting that the agent replicated at this site.[881] A wide range of tissues and fluids from clinically affected cases of BSE have shown no detectable infectivity, using the mouse bioassay, and these include gut, lung, pancreas, bone, bone marrow, skin, muscle, cartilage, milk and serum. These negative results need to interpreted with caution as the RIII mouse bioassay used is estimated to be 1000 times less sensitive than titration in cattle.[283]

**Maternal transmission**

Although the pattern of the BSE epidemic remains consistent with the hypothesis that the vast majority of cases arose through infection with contaminated feed, it remains possible that other routes of transmission may occur infrequently, in particular maternal transmission from dam to calf. The most important piece of work carried out to test maternal transmission was a study in which 300 calves from affected dams were compared with 300 control calves from unaffected dams. BSE occurred in 14 per cent of the offspring of affected dams and in 4.3 per cent of those born to unaffected dams. The difference was statistically significant but unfortunately did not distinguish between maternal transmission of the BSE agent and maternal transmission of a genetic susceptibility factor.[882]

A further study to assess the possibility of maternal transmission used embryo transfer and was initiated in 1990. Two hundred suspect BSE cows were superovulated and artificially inseminated. Half were inseminated with semen from BSE-positive bulls and half from BSE-negative bulls. After seven days, embryos were flushed from the uterus by uterine lavage, washed, and implanted in surrogate dams, which were guaranteed free of BSE. Preliminary results at the end of 1996 found that none of 266 calves had developed BSE.[883] Studies to detect infectivity in milk, placenta, embryo tissue, uterine flushings and foetal membranes from BSE affected cattle have all proved negative so far.[6,884] Maternal transmission is discussed in more detail later.

**BSE outside of the UK**

By the end of 2000, over 180,000 confirmed cases of BSE had been reported in the UK. Over 2300 cases have also been reported elsewhere (see Table 14), and outside of the UK the incidence of BSE is still rising (see Figure 21). The first cases to be reported in countries outside the UK were in the Republic of Ireland in 1989, followed by Switzerland and Portugal in 1990 and France in 1991. It is likely that the European BSE cases were due to a combination of importing infected MBM or protein concentrates containing MBM, and importing infected cattle that were then recycled. The cases from Oman, Canada, and the Falkland Islands were animals imported from the UK. It has been suggested that it is surprising that there are not more cases of BSE in other countries in view of the large quantities (>71 kilotonnes) of MBM exported from the UK up to 1990 for use in pig and poultry feed (given the potential for cross-contamination).[89]

**Table 14: Numbers of cases of BSE in different countries**

| Country | Number of cases | Country | Number of cases |
|---|---|---|---|
| Great Britain | 177 853 | Spain | 42 |
| Northern Ireland | 1803 | Belgium | 27 |
| Guernsey | 695 | Netherlands | 14 |
| Ireland | 587 | Denmark | 3 |
| Portugal | 564 | Italy | 2 |
| Isle of Man | 437 | Liechtenstein | 2 |
| Switzerland | 374 | Oman | 2* |
| France | 272 | Luxembourg | 1 |
| Jersey | 149 | Canada | 1* |
| Germany | 57 | Falkland Islands | 1* |

Table accessed from www.oie.int, April 2000.
*Only in imported animals

## Spongiform encephalopathies of captive wild ruminants

In a British zoo in 1986 a nyala (an animal which belongs, like cattle, to the family 'Bovidae') died of a spongiform encephalopathy.[94] Thereafter additional cases of spongiform encephalopathy occurred in the following captive wild Bovidae in Britain: gemsbok (kept in the same premises as the nyala and fed the same diet – although had had no actual contact with the nyala),[98] Arabian oryx, greater kudu, eland,[96] and scimitar-horned oryx (see Table 15). As with FSE (see below), the temporo-spatial clustering of these novel spongiform encephalopathies would be consistent with a causative link to the BSE agent. Although it would seem likely that dietary exposure was of most importance, it is noteworthy that one of the kudu was the offspring from an affected mother,[885] thus raising the additional possibility of maternal transmission in this species. It is also hypothesised that some of the affected kudu acquired their disease via lateral transmission as it was considered very unlikely that they had been exposed to contaminated feed.[98]

**Figure 20: Number of cases of BSE in the UK per year**

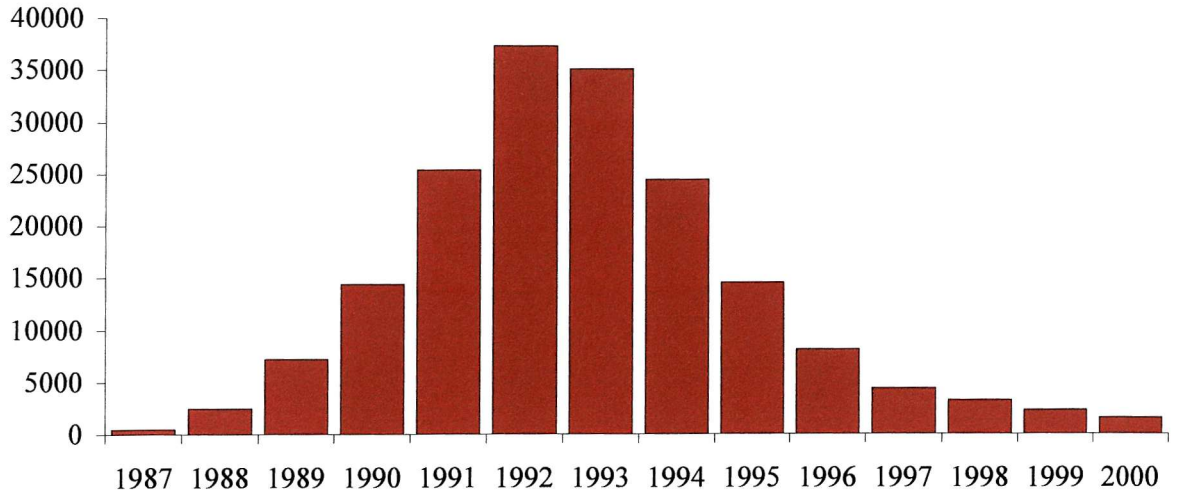



**Figure 21: Number of cases of BSE outside of the UK per year**

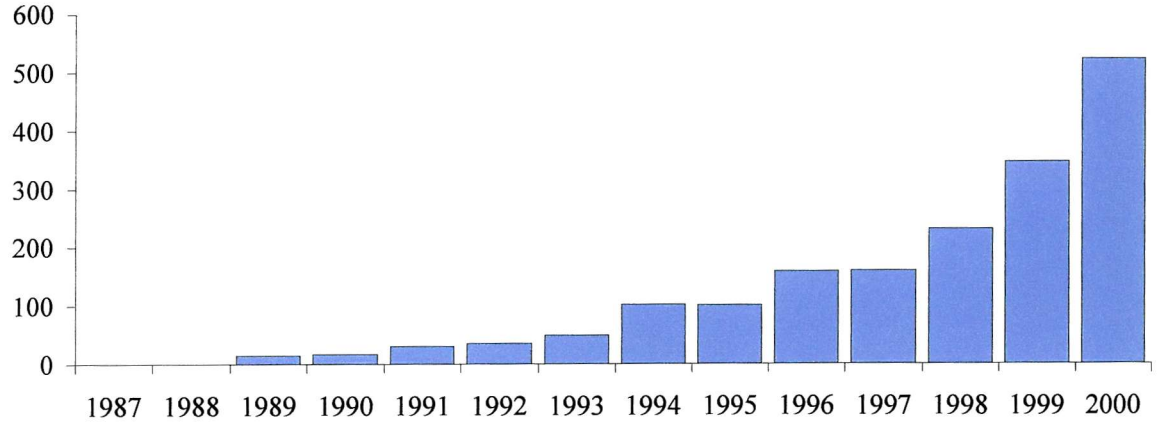
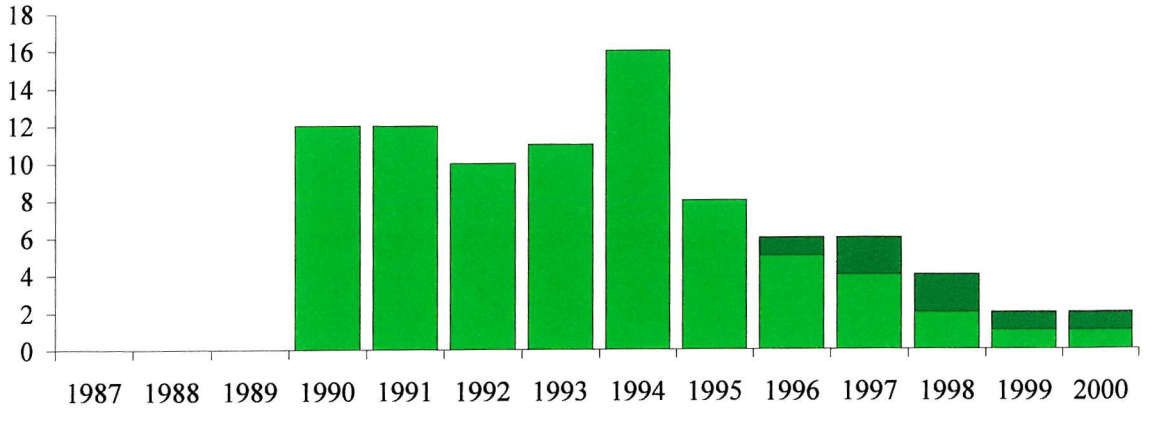



**Figure 22: Number of cases of feline spongiform encephalopathy per year**




Seven cases of FSE were born after the ban on the use of specified bovine offals was extended to any animal feed (dark green)

**Table 15: TSEs in exotic species**

| Species | Ref* | Date affected | Notes |
|---|---|---|---|
| **Ankole Cow** | 20 | March 1995 | Born 1987. Taken as normal BSE case |
| | 21 | Early 1991 | Born 1986. Taken as normal BSE case |
| **Bison** | 27 | October 1996 | Born 1989. (but possibly 90) |
| **Cheetah** | 15 | September 1992 | - |
| | 17 | October 1993 | Born May 1986 |
| | 28 | December 1996 | Born June 1987 but not in UK. Imported March 1988 |
| | 29 | Early 1997 | Born April 1991 |
| | 30 | February 1998 | Born June 1992 |
| **Eland** | 5 | December 1989 | - |
| | 7 | April 1991 | - |
| | 8 | May 1991 | - |
| | 10 | December 1991 | FSE also diagnosed in two domestic cats at this location |
| | 19 | November 1993 | Born December 1991. Same dam as case Ref. 10. Dam still healthy |
| | 24 | August 1995 | Born January 1993 |
| **Gemsbok** | 2 | June 1987 | - |
| **Kudu** | 4 | August 1989 | - |
| | 6 | November 1990 | Offspring of kudu Ref. 4. |
| | 9 | June 1991 | - |
| | 12 | April 1992 (culled) | Not clinically affected – culled for management reasons |
| | 13 | Date unknown | Not clinically affected – culled for management reasons |
| | 14 | December 1992 | - |
| **Lion** | 32 | December 1998 | Born November 1986 |
| | 33 | May 1999 (euthanased) | Born November 1981. |
| | 36 | Euthanased Aug 2000 | Born July 1987. Deteriorating hind limb ataxia. |
| **Nyala** | 1 | June 1986 | - |
| **Ocelot** | 18 | March 1994 | Born May 1987 |
| | 26 | October 1995 | Born July 1980 |
| | 34 | August 1999 (euthanased) | Born August 1991 |
| **Oryx (arabian)** | 3 | March 1989 | Confirmed at same time as Kudu Ref. 4. |
| **Oryx (scimitar)** | 16 | December 1992 | - |
| **Puma** | 11 | Early 1992 | - |
| | 22 | Early 1995 | FSE also diagnosed in one domestic cat at this location. |
| | 23 | May 1995 (euthanased) | Male 17 years. No clinical disease |
| **Tiger** | 25 | December 1995 | - |
| | 31 | October 1998 (euthanased) | Born February 1983 |
| | 35 | Euthanased December 1999 | Another elderly tiger from the same group as Ref. 31. History of arthritis, no clinical signs of SE. No histological lesions but PrP staining suggestive of SE. |

*Reference number indicates order of notification. Not included above are two cheetahs at zoos in Australia and the Republic of Ireland. Both were apparently litter mates and exported from premises in the UK where the cheetahs Ref. 15 and 29 were born. Two cases in cheetahs were also confirmed in France, one in January 1997, in an animal born in the UK in 1989. Details of the second case are not available, but it is reported to have been born in Britain.

## Feline spongiform encephalopathy

In 1990 the first case of feline spongiform encephalopathy (FSE) in a domestic cat was reported. The 6-year-old animal had been referred to the Bristol Veterinary School in England with a progressive neurological condition. It failed to respond to treatment and subsequent neuropathological examination revealed a scrapie-like spongiform encephalopathy.[99] Although no previous naturally occurring TSE had been documented in a feline, CJD had been experimentally transmitted to a cat in 1972[886] and several times thereafter. Since 1990 cats with FSE have been reported from most regions of the UK (a total of 86 up to the end of 2000 ) and single cases have been documented in indigenous cats from Norway[887] and Liechtenstein. FSE has also been documented in captive large cats (see Table 15).[101,888] Seven cases of FSE were born after the ban on the use of specified bovine offals was extended to any animal feed (see Figure 22). The results of strain typing experiments supports the hypothesis that these novel feline diseases and the spongiform encephalopathies of captive wild ruminants are caused by the BSE agent.[889] It is probable that the domestic cats have been infected through the consumption of infected feed, but the precise ingredient is not known. It is assumed that disease in the captive large cats arose through the consumption of uncooked infected bovine material, such as heads and necks containing CNS tissue.

## The possibility of BSE transmission to other species

The appearance of a number of novel TSEs, causally linked with BSE, in domestic and captive animals raises the question of whether BSE occurs, or will occur, in further animal species. Particular concern has been expressed regarding the possibility of BSE in sheep, pigs and poultry. BSE has been experimentally transmitted to sheep by feeding as little as 0.5g of infected bovine brain[890] and it is known that some sheep were fed MBM until this was practice was banned in 1988. Although there is no evidence of a BSE-related epidemic of sheep scrapie in the UK the early detection of such an occurrence may be difficult if ovine BSE is not easily distinguished from conventional forms of scrapie. Transmission studies of BSE to chickens have been negative,[89] but pigs have been shown to be susceptible to BSE by intracerebral inoculation of infected bovine brain homogenate.[891] However challenging pigs with a very large oral dose of BSE-infected brain failed to produce disease, at least up to 6.5 years post-challenge.

Tim Holt, a house officer from London, warned of the danger of BSE for human health in 1988 and raised the question of excluding brain from human food.[74] He noted that although brain was classified as a 'prohibited offal' and could not therefore be included in uncooked meat products, no regulation prohibited the inclusion of brain in cooked products, such as meat pies. He also noted that it had always been possible to buy raw brain over the counter in butchers' shops as an ingredient for stews and casseroles. Holt's concerns were not shared by many scientists with experience of TSEs. Baker expressed the commonly held view that 'There is no evidence that CJD results from eating tissue infected with scrapie, even though scrapie is common in British sheep. There is, therefore, little reason to believe that BSE will present any greater threat to humans than scrapie.'[892] The occurrence of FSE in the UK seriously challenged this reassuring view. Cats, like humans, had almost certainly been exposed to sheep scrapie in their diet without evidence of naturally acquiring the disease. The implication that the scrapie agent had now become pathogenic to cats following passage through cattle raised the question of whether this would also apply to humans. In fact the ability of a TSE agent to become pathogenic only after transmission via an intermediate host was not a new one. In 1979 Gibbs reported that kuru was only transmissible to ferrets after passage through non-human primates.[893]

# METHODS

## CREUTZFELDT-JAKOB DISEASE SURVEILLANCE IN THE UK

Surveillance of CJD in the UK was established in May 1990 on the recommendation of both the Southwood Committee and the Tyrrell Committee following the occurrence of the epidemic of BSE in the late 1980s.[93] The project is funded by the Department of Health and the Scottish Home and Health Department. The National CJD Surveillance Unit (NCJDSU) is situated in the Old Pharmacy Building of the Western General Hospital in Edinburgh. The basis for establishing CJD surveillance is the hypothesis that if BSE were to cause disease in humans this would most likely result in manifestations similar to those of CJD and that some or all of the following might be observed: (a) an increase in the overall incidence of CJD in the UK; (b) an excess of cases in groups most likely to have high exposure to the causative agent of BSE, such as farmers, abattoir workers and butchers; (c) a change in the epidemiological pattern of CJD, such as a change in the age distribution; (d) a change in the clinical or neuropathological characteristics of CJD.[7] In order to establish any such occurrences the NCJDSU attempts to identify the epidemiological, clinical and pathological characteristics of patients with CJD in the UK.

CJD surveillance had been undertaken in the UK prior to 1990, establishing a baseline of data to help identify any subsequent change in the pattern of the disease. Cases of CJD in England and Wales during the period 1970-79 were identified retrospectively at the end of that period[253] and a prospective surveillance system was instituted to detect cases during 1980-84.[233] Cases in the UK for the period 1985 to April 1990 were identified retrospectively. I was employed at the NCJDSU and involved in the collection of data from August 1994 up to the end of 1996.

### Method of case ascertainment

Since May 1990 neurologists, neuropathologists and neurophysiologists in the UK have been asked to refer suspect cases of CJD to the NCJDSU. Doctors in these groups are circulated on a regular basis to remind them of the study.

Although it would seem likely that if BSE were to transmit to man it would cause a CJD-like illness, there is no reason to believe that the clinicopathological phenotype would be similar to sporadic CJD. Indeed, it would perhaps seem more likely that BSE would mimic kuru if the mode of transmission were expected to be a major determinant of phenotype. Therefore, there has been no explicit referral criteria for a suspect case of CJD in an attempt to avoid missing atypical cases.

CJD has not been made a notifiable disease in the UK and experience of surveillance in Slovakia would argue that statutory referral may actually hamper detection of cases.[894] This is perhaps because in general people rather do things voluntarily rather than by enforcement. Furthermore, making a disease notifiable may discourage early referral and notification of unusual cases in which the diagnosis may seem unlikely. Clearly this could hinder the timely identification of an atypical form of CJD related to BSE.

The Office of Population Censuses and Surveys supplies all death certificates coded under rubrics relevant to CJD: 339.9 and 781.7 (International Classification of Diseases, eighth revision (ICD-8)) and 046.1 and 331.9 (ICD-9). Very few cases (less than 3 annually) are identified solely via death certificates. Some cases are referred by other routes, such as via physicians, psychiatrists or patients' families.

At the time of initial referral a judgement is made of the likelihood of the diagnosis of CJD based on an assessment of the clinical history, neurological features and results of investigations. The patient is classified as defined in Annex 3 and a decision is made whether a neurologist from the NCJDSU should visit the patient, based largely upon the perceived likelihood of a diagnosis of CJD. Suspect cases are only visited if the relatives (and, if possible, the patient) are informed of the possibility of the diagnosis. In some cases the referring physician defers a visit from NCJDSU staff until the diagnosis becomes more likely, and in some cases in which the illness evolution makes the diagnosis of CJD unlikely, no visit is made to see the patient.

Some patients are only notified after death and in these circumstances the patient's relatives are only contacted with the permission of the patient's clinician and general practitioner. When the result of a necropsy is pending a patient's family is only contacted if a pathological diagnosis of CJD is made.

As the main remit of the surveillance project has been to identify cases of CJD that may have arisen secondary to BSE, those cases with a known genetic or iatrogenic cause are not assessed in detail (i.e. the case and their family are not interviewed and the patient is not included in the case-control study). A small number of sporadic CJD cases are not assessed at the request of either the patient's clinician, GP or the family. Under these circumstances clinical information is obtained from the referring clinician and the patient's hospital records if available. The study received ethical approval.

## The collection of clinical and case-control information

Patients and their close relatives are usually seen in the hospital ward of the referring clinician. Less often patients are seen in their home or in a hospice. A detailed history is taken from the patient's relatives and where possible also the patient. Information is given about CJD, including a leaflet published by the Alzheimer's Disease Society and contact details for the CJD Support Network and CJD helpline. The closest relative is then interviewed separately, if possible, to collect information using the standard CJD questionnaire (see Annex 4). A neurological examination is performed and further information is obtained from the patient's case notes, including the results of investigations. If EEG tracings are available these are reviewed and photocopies are taken of relevant sections. Permission is sought from the patient's clinician before copying any of the patient's records.

After obtaining written informed consent from the patient, or more usually because of cognitive impairment a relative, blood is taken into EDTA tubes for DNA studies. A standard form outlining the implications of PrP gene analysis is used as part of the consent process (see Annex 5). Urine and CSF are also taken if samples are available.

A control patient is selected using the following criteria: age match +/-4 years, sex matched, inpatient in the same hospital as the index case, a relative of the same degree as the index case is available for interview and the patient is suffering from a condition clearly distinguishable from CJD. The first available control case fulfilling

these criteria is selected for interview, although in practice it is unusual for more than one control case satisfying the criteria to be identified. The control and their relative are interviewed separately using the standard CJD questionnaire. Often a control with a suitable relative available for interview cannot be identified and the patient only is interviewed. On other occasions only the control patient's relative is available for interview. When no control can be identified a further visit is planned for a later date.

Following the assessment of the patient and their hospital notes the patient's clinician is asked to inform the NCJDSU of the subsequent clinical course and the case classification is adjusted accordingly. In those cases subsequently confirmed to have vCJD the patient's GP notes were also requested and reviewed.

## Case definition

The diagnostic criteria for a case of CJD have been essentially the same since surveillance started in the UK in 1980. These criteria are modified from those produced by Masters[200] and have been published by Budka in 1995[895,896] (Annex 3) and validated.[255] In summary, classification as a definite case of CJD requires neuropathological confirmation of spongiform change *or*, since 1993, immunocytochemical confirmation of the presence of protease-resistant PrP *or* identification of scrapie-associated-fibrils on electron microscopy. In the absence of neuropathological investigation patients are classified as probable CJD if they present with progressive dementia of any duration, a typical EEG and at least two of the following: myoclonus; visual or cerebellar disturbance; pyramidal or extrapyramidal dysfunction; akinetic mutism. A further category of possible CJD, does not require a typical EEG, but the illness duration must be less than two years and the patient must have two of the above clinical characteristics.

With the increased incidence of iatrogenic CJD in recent years,[25] particularly among hGH recipients, criteria for the diagnosis of CJD have been updated to include a definition for iatrogenic cases. Cases have been classified as 'iatrogenic' when they have a known risk factor for accidental transmission. The criteria for 'familial' CJD have also been updated in order to include information available from genetic analysis. Cases are now classified as inherited if they carry a disease-specific PrP genetic mutation or have a family history of probable or definite CJD in a first degree relative. All other cases are classified as 'sporadic'.

## The assessment of clinical and diagnostic features

The date of onset of illness is defined as the time when unexplained progressive neurological or psychiatric symptoms first occurred. The results of investigations, including brain imaging and EEG, are, unless otherwise stated, based on laboratory reports from patients' notes. Since April 1996, CSF from suspect cases has been tested blind for the presence of the 14-3-3 protein. This testing was performed by colleagues from the United States National Institute of Health and/or the National Hospital for Neurology and Neurosurgery, London. The method of 14-3-3 analysis has been published by Hsich et al.[573] PrP gene analysis was performed either at the NCJDSU or by Professor Collinge's Prion Disease Group in London using published methods.[517]

## Analysis of groups with potentially high occupational exposure

If BSE was to transmit to humans the following are hypothetical occupational route of transmission:

1. Contact with BSE-contaminated MBM.

2. Contact with live cattle infected with BSE.

3. Contact with brain or spinal cord from dead cattle infected with BSE.

Individuals most likely to be exposed via route 1 includes those working on farms with cattle, pigs, poultry, sheep, or goats (as ruminant-derived MBM may have been fed to these animals) and workers in feed mills producing MBM. Occupational groups at high risk of exposure via route 2 would include workers on farms with cattle, veterinarians and abattoir workers. Individuals most likely to be exposed via route 3 includes veterinarians, abattoir workers, workers in rendering plants and butchers.

The numbers of workers on different types of farms during 1990-4 were obtained from the annual agricultural censuses for England and Wales, Northern Ireland, and Scotland (1990-5). Data on the numbers of workers on farms affected by BSE were obtained by linking agricultural census data with the database on the disease held at the CVL. The number of veterinarians, butchers, and meat cutters in Great Britain was estimated from the 10% sample of the 1991 census by using the recommended conversion factor of 10.16.[7] The number of abattoir workers could not be derived from published census data for Great Britain, but information on these workers was provided by MAFF.[7]

Data for Northern Ireland on veterinarians and on workers employed in the slaughtering of animals and the production of meat for 1993 were provided by the Department of Economic Development, Belfast. The number of workers in rendering plants or feed mills producing MBM could not be derived from published census data for Great Britain. However, the numbers of workers in these groups are small compared with the number of farm workers and their exclusion from calculation of the expected number of cases is therefore of no consequence. The number of feed mill workers is estimated at about 3000.[7]

## Expected numbers of cases in different occupational groups

Expected numbers of deaths from CJD between 1 May 1990 and 31 December 1996 were calculated by multiplying age-specific and sex-specific death rates for the UK population by the estimated numbers of people in the different occupational groups. The age and sex distribution of farm workers was assumed to follow that reported for farm workers in England and Wales in the European Commission's structure survey of 1990. The age and sex distributions of veterinarians and of butchers and meat cutters were assumed to follow those of the wider occupational groups within which they were classified in the 1991 census. Observed and expected numbers of cases were compared in the assumption that the observed number of cases followed a Poisson distribution.

## Pathology

Whenever possible, neuropathological examination was carried out on cases referred to the NCJDSU. Such examinations have been performed on over 70 percent of patients notified since May 1990, either by referral for necropsy in Edinburgh or in cooperation with neuropathologists in other centres who refer cases after biopsy or post-mortem.[897] Brain biopsy specimens from Edinburgh were fixed in 10% formalin overnight; paraffin blocks were obtained from brain biopsy cases from other regional neuropathological centres. Tissues from autopsy cases had been fixed in 15% formalin for a minimum of three weeks prior to brain dissection. The brains were sampled extensively to allow accurate neuroanatomical investigation, including material from the frontal, parietal, temporal and occipital cortex, the hippocampus, hypothalamus, thalamus, basal ganglia, midbrain, pons, medulla and spinal cord (when available). Other organs were examined histologically if appropriate permission was obtained and material was available. Blocks were immersed in 96% formic acid for one hour before routine processing into paraffin wax. Sections were cut at five micron intervals and stained by conventional histological techniques (haematoxylin and eosin and some cases also had a myelin stain such as luxol fast blue) and PrP immunocytochemistry with two monoclonal PrP antibodies (KG9 and 3F4)[376]. Pretreatments for immunocytochemistry included incubation in 96% formic acid for five minutes, then four mol/L guanidine thiocyanate for two hours, and hydrated autoclaving at 121$^0$C for 10 minutes. Neuropathology from all of the vCJD cases was reviewed by Professor Ironside at the NCJDSU.

## MRI STUDY

In 1995 Finkenstaedt reported striatal high signal on MRI in 79% of a series of 29 sporadic CJD cases.[707] This raised the possibility that MRI may be a useful tool in CJD diagnosis. Two cases of vCJD published in the Lancet in April 1996 were reported to have bilaterally increase signal in the posterior thalamus (pulvinar) on MRI.[343,898] This raised the hypothesis that the identification of bilateral pulvinar high signal might be a sensitive and specific test for the diagnosis of vCJD in patients with suspected disease. To address this question the NCJDSU attempted to obtain MRI brain examinations from all patients in the UK with suspected vCJD who were referred up to the end of June 1999. Control MRI examinations were obtained on two groups of patients: patients suspected of having vCJD who were later diagnosed with other conditions; and patients known or suspected to have other forms of CJD.

The scans were labelled with a reference number and then read by two experienced neuroradiologists from the Department of Clinical Neurosciences at the Western General Hospital in Edinburgh (Dr Donald Collie and Dr Robin Sellar) who were not aware of clinical information, including the diagnosis. When a patient had more than one set of scans available the most recent was assessed. Any earlier scans were later reviewed openly to assess any radiological change with time. The neuroradiologists were aware of the study hypothesis and the potential for basal ganglia (including thalamus) signal changes in sporadic and vCJD, but were not aware of the proportion of cases with the various forms of CJD or the number of controls.

All scans were reviewed on two separate occasions by each radiologist under normal scan-reporting conditions to assess intra-observer and inter-observer variation. Films were reviewed in random and in different orders on

each occasion. Each scan was assessed for the distribution of all abnormalities and the most likely radiological diagnosis. The presence and degree of signal abnormalities in the caudate, putamen, globus pallidus, and thalamus (with the distribution of changes within the thalamus if present) were noted. Scans with bilaterally increased signal predominantly in the pulvinar were diagnosed radiologically as vCJD. Scans considered to have increased signal predominantly in the putamen or caudate head were diagnosed radiologically as sporadic CJD. The degree of certainty of the radiological diagnosis based on the prominence of any abnormalities were subjectively graded on a three-point scale: possible (minimal/equivocal changes), probable (moderate changes), and definite (marked changes). All other scans were diagnosed as either normal, or as another diagnosis if other specific changes were present.

All films from patients with histologically confirmed vCJD and scans falsely diagnosed as vCJD were subsequently reviewed with clinical information available to obtain a consensus on the radiological abnormalities and the reasons for any discordance between the radiological and final diagnosis. The following data were recorded from the scans at this time: presence, distribution, and degree of abnormal signal in thalamus, globus pallidus, putamen, caudate head, and brainstem; presence and degree of focal or generalised atrophy; and the presence and distribution of any other changes. The degree of signal hyperintensity was scored with a five-point scale (0 to ++++), based on a subjective assessment of signal change in the area of interest relative to the other basal ganglia. If there were changes throughout the basal ganglia, cortical grey matter signal was taken as the reference area. Scan quality (including presence of movement artefact), the MRI sequences available, and the sequence on which abnormalities were most clearly identifiable, were also noted. In patients who had undergone more than one scan, differences between these scans were assessed. The original MRI reports for the histologically confirmed vCJD cases were reviewed to assess abnormalities noted at the referring hospitals.

Thalamic pathology was reviewed in the first 26 patients with vCJD identified for which necropsy material was available, and in 20 control patients with sporadic CJD matched for sex, PrP codon 129 genotype, and age (as far as possible). Paraffin-embedded blocks taken at different levels to include all major thalamic nuclei, including the pulvinar, were stained by conventional histological techniques and by immunocytochemistry for PrP and glial fibrillary acidic protein, as previously described.[376] Sections were examined and scored by two independent observers (Mr Selwyn Selvendran and Professor James Ironside) for the anatomical distribution and severity of spongiform change, neuronal loss, PrP deposition, and astrocytic gliosis.

# RESULTS

Between 1 January 1970 and 31 December 1996, 708 definite and probable cases of CJD were identified. Five were patients alive on 31 December 1996. In all, 540 (76%) cases were classified as definite. Fourteen cases were vCJD. Twenty-three cases were classified as iatrogenic and 23 as inherited, both of which groups were excluded from analysis below unless otherwise stated.

## DISTRIBUTION OF CJD CASES BY AGE, SEX AND TIME PERIOD 1970-96

In England and Wales the yearly number of deaths from CJD increased from around 10 at the beginning of the 1970s to around 40 in the 1990s (Figure 23). No increase in yearly numbers of deaths was evident for Scotland and Northern Ireland after 1985 (Figure 23).

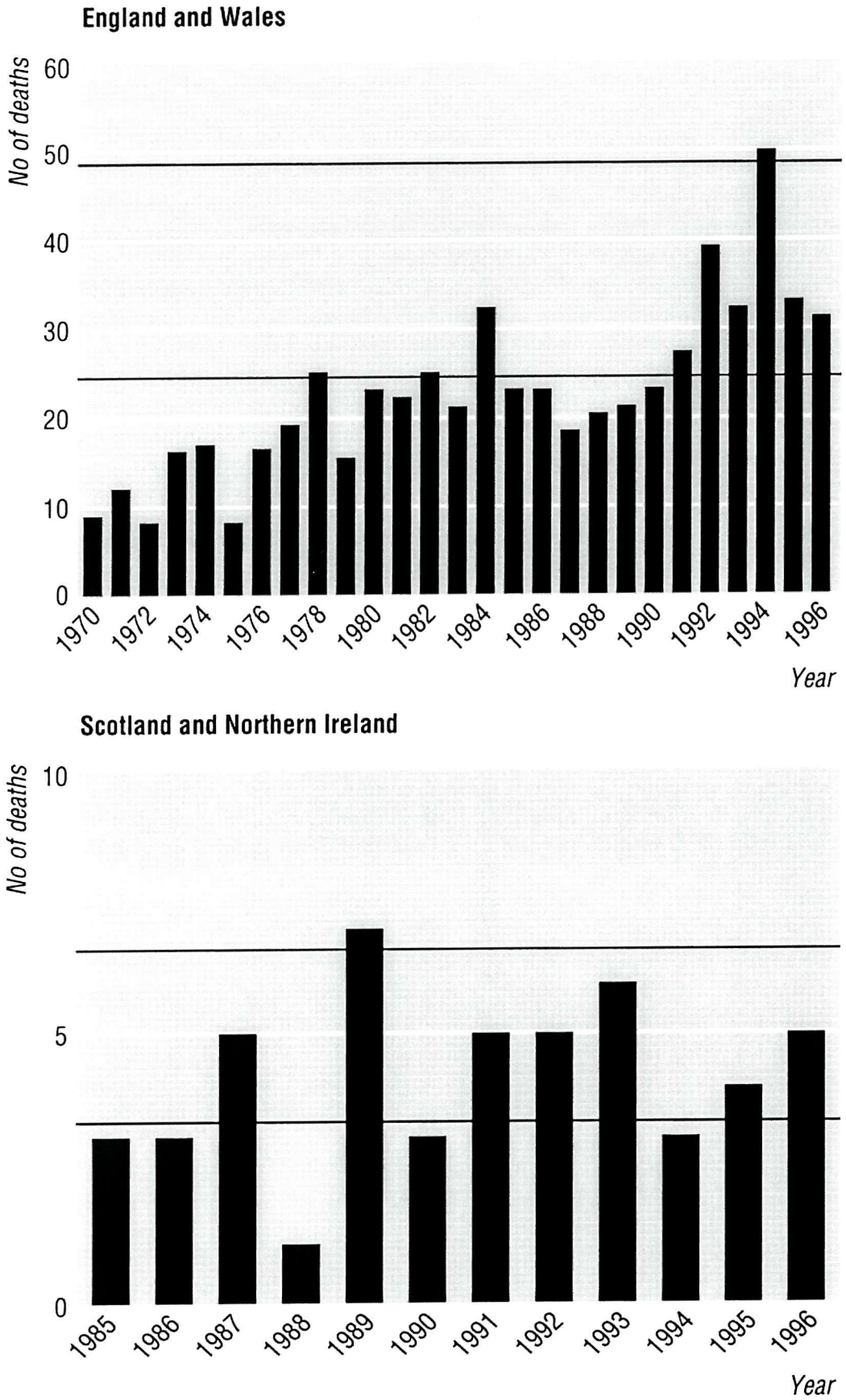
Figure 24 shows the average yearly age-specific and sex-specific death rates over the study period. Below 40 years of age, death rates were extremely low (<0.1/million yearly). Death rates increased substantially in the 50-59 year age group and reached a peak of around 2.0/million yearly in the 60-69 year age group before declining in people aged 70 and over.

Table 16 shows the numbers of deaths in 10-year age groups for each two-year period from 1970 to 1996. Mortality from CJD increased substantially over the period in patients aged 70 and over and by successively smaller proportions in those aged 60-69 and 50-59 (Figure 25; Table 17). The increase in patients aged 40-49 years was not significant (P = 0.27). Under the age of 40 numbers of cases were small, but of the seven patients who died under the age of 30, six had onset of the disease after 1 January 1994. All six died in 1995 or 1996.

## SPORADIC DISEASE IN GROUPS WITH POTENTIAL OCCUPATIONAL EXPOSURE

Six cases ( see Table 18) of sporadic CJD (five definite, four in men) were identified between 1 May 1990 and 31 December 1996 in people whose occupation at disease onset was in one of the groups identified in the Methods section 'Analysis of groups with potentially high occupational exposure'. Four of the six patients lived or worked on dairy farms (on three of which there had been confirmed cases of BSE), and the other two lived or worked on farms with beef suckler herds (one with a confirmed case of BSE). Two of the six cases were in spouses of farmers. All patients had lived or worked on farms throughout their working lives. Table 19 shows the distribution of these cases by occupational group. There was a significant excess of cases among animal farm workers (six observed, 2.4 expected; P = 0.03). All these cases were in cattle farmers, four of whom worked on farms with a case of BSE. This excess of cases was highly significant (four observed, 0.58 expected; P = 0.003). Table 20 shows the distribution of observed and expected cases by hypothesised route of transmission. There was an excess of cases among workers potentially exposed to ruminant-derived MBM and among workers exposed to live cattle infected with BSE. Two cases among farmers were on farms without a case of BSE compared with an expected number of 1.78 (P = 0.5).

**Figure 23: Annual mortality from CJD in England and Wales and in Scotland and Northern Ireland**




Yearly numbers of deaths from CJD in England and Wales during 1970-96 and in Scotland and Northern Ireland during 1985-96 (data exclude known iatrogenic and inherited cases). Horizontal lines represent numbers of deaths corresponding to yearly death rates of 0.5/million (lower) and 1.0/million (upper)

**Figure 24: Age- and sex-specific average yearly death rates from CJD in the UK during 1970-96**

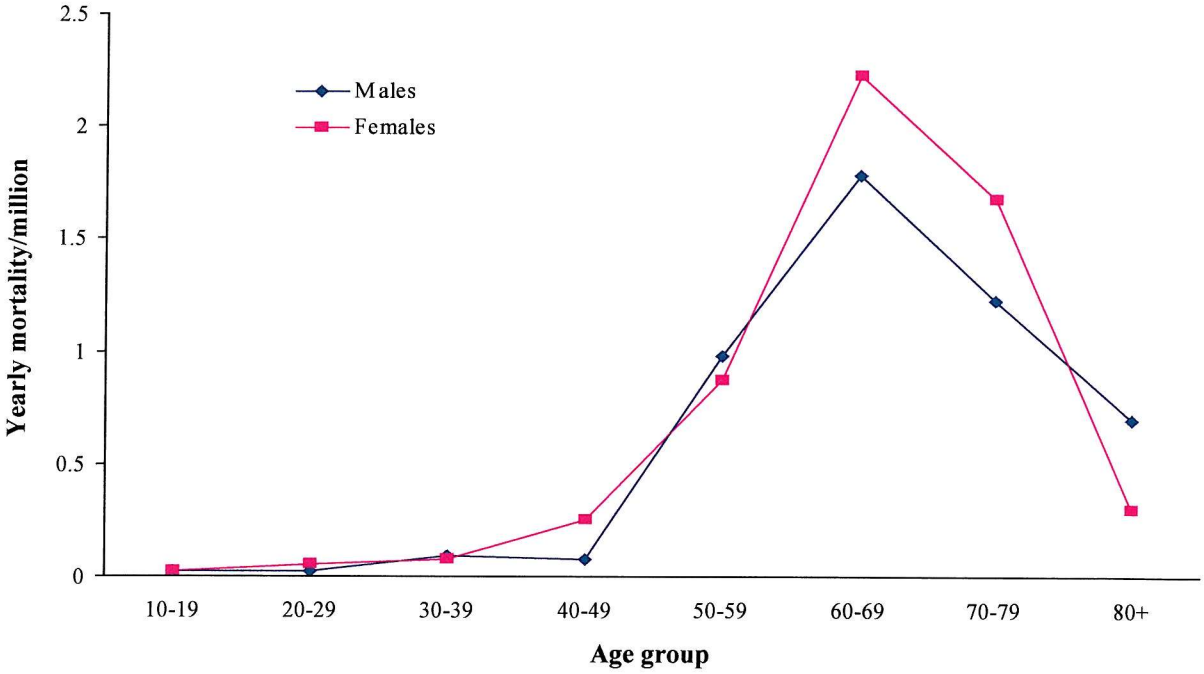
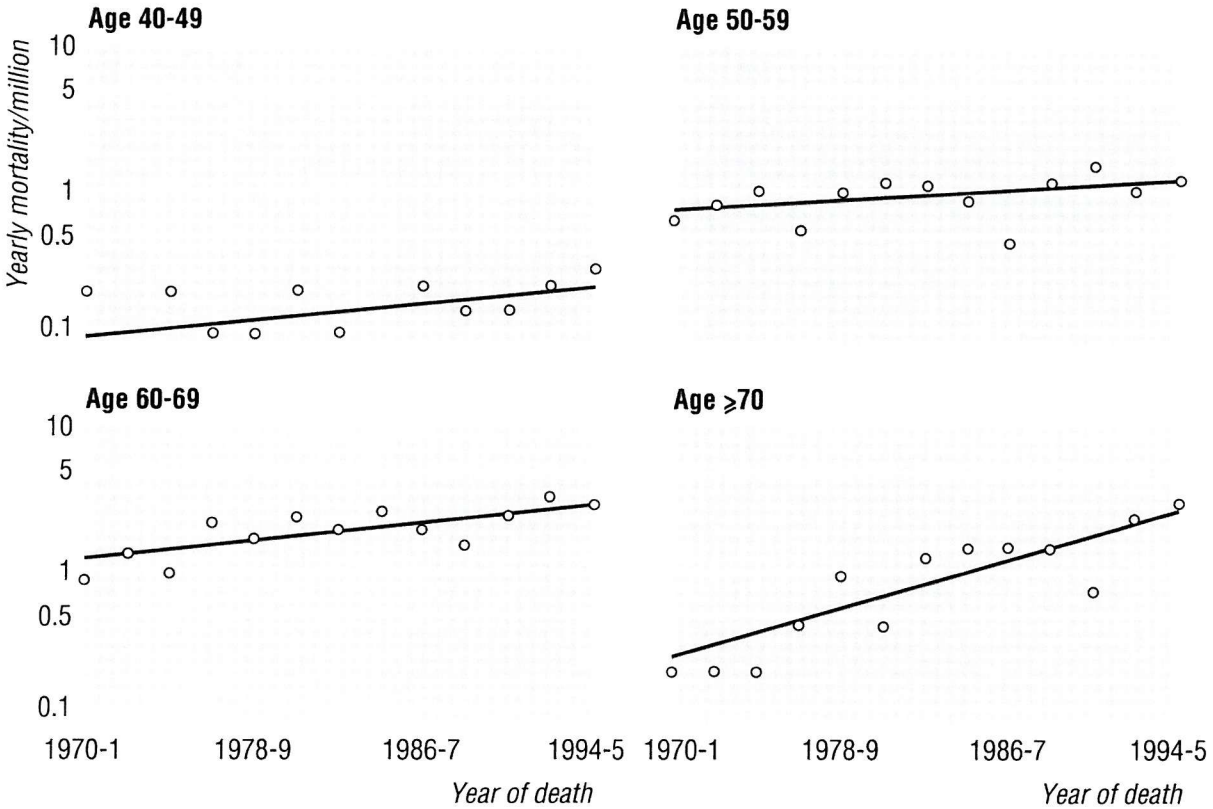



**Figure 25: Trends in age-specific death rates from CJD in England and Wales during 1970-96**




*Mortality trends by age group*

For age group 40-49 years death rates for 1972-3 and 1984-5 are not plotted as there were no deaths in this age group in the periods

**Table 16: Deaths from CJD by age groups in England and Wales (from 1970) and UK (from 1985)**

| Age (years) | Two-year period | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1970-1** | **1972-3** | **1974-5** | **1976-7** | **1978-9** | **1980-1** | **1982-3** | **1984-5** | **1986-7** | **1988-9** | **1990-1** | **1992-3** | **1994-95** | **1996*** | **Total** |
| **10-19** | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | **3** |
| **20-29** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | **4** |
| **30-39** | 1 | 0 | 0 | 2 | 2 | 1 | 1 | 4 | 1 | 0 | 1 | 0 | 1 | 4 | **18** |
| **40-49** | 2 | 0 | 2 | 1 | 1 | 2 | 1 | 0 | 3 | 2 | 2 | 3 | 4 | 4 | **27** |
| **50-59** | 7 | 9 | 11 | 6 | 11 | 13 | 12 | 10 | 5 | 13 | 18 | 12 | 14 | 4 | **5** |
| **60-69** | 9 | 13 | 10 | 22 | 17 | 24 | 20 | 28 | 22 | 17 | 28 | 38 | 32 | 8 | **288** |
| **70 +** | 2 | 2 | 2 | 4 | 9 | 4 | 12 | 16 | 18 | 17 | 9 | 29 | 37 | 12 | **173** |
| **Total** | **21** | **24** | **25** | **35** | **40** | **45** | **46** | **58** | **49** | **49** | **58** | **82** | **90** | **36** | **658** |

Table excludes cases known to be iatrogenic or inherited.

*Data for a single year only

**Table 17: Rate of increase in mortality from CJD by age group**

| Age group (years) | Average % increase over a two-year period | 95% confidence interval | P value |
|---|---|---|---|
| **40-49** | 6.6 | -4.8 to 19.5 | 0.27 |
| **50-59** | 4.1 | -0.1 to 8.8 | 0.08 |
| **60-69** | 7.3 | 3.0 to 10.8 | <0.001 |
| **70+** | 21.3 | 15.4 to 27.5 | <0.001 |

Table excludes cases known to be iatrogenic or inherited

**Table 18: Farmers with CJD in the UK, 1 May 1990 to 31 December 1996**

| CASE NO. | Age at death | Year of death | Sex and reference | Clinical features | Typical EEG? | Duration (months) | Codon 129 | Pathology | BSE CASES (YEAR) | Other details |
|---|---|---|---|---|---|---|---|---|---|---|
| **148** | 61 | 1992 | Male[899] | Progressive dysphasia and memory impairment. Thereafter rapid dementia, myoclonus, pyramidal, extrapyramidal and cerebellar signs | No | 7 | MM | Typical of sporadic CJD | 1 (1989) | No contact with internal organs/tissues. Drank pooled milk from animals including BSE case |
| **201** | 54 | 1993 | Female | Withdrawn, progressive ataxia followed by apraxia, myoclonus, dysphasia, dysarthria, paratonic rigidity and primitive reflexes | Yes | 17.5 | MM | Typical of sporadic CJD | Nil | Farmer's wife. Helped on farm |
| **256** | 64 | 1993 | Male[900] | Rapidly progressive dementia, behavioural disturbances, hallucinations, dysphasia and myoclonus | Yes | 3 | MM | Typical of sporadic CJD | 3 (1992-3) | Drank pooled milk, including from BSE case and assisted with veterinary procedures (dehorning & injections) |
| **356** | 54 | 1994 | Male[901] | Forgetfulness, odd behaviour, dysarthria, ataxic gait and myoclonus. Developed dementia, primitive and pyramidal signs, and akinetic mutism. | No | 5 | VV | Typical of sporadic CJD | 3 (1988-92) | Assisted with calving, but no other operative procedure. Drank unpasteurised milk, but not from affected animals |
| **463** | 64 | 1995 | Female | Behavioural change, rapidly progressive dementia, myoclonus, rigidity, frontal signs and akinetic mutism | Yes | 3 | MM | No autopsy | Nil | Farmer's wife, had not helped on farm past eight years. Drank unpasteurised milk until 1990. Previously would have assisted with calving |
| **470** | 59 | 1995 | Male[684] | Visual disturbance - distortions and hemianopia. Subsequent rapid decline with dementia and myoclonus | No | 3 | MM | Typical of sporadic CJD | 1 (1991) | Had assisted with caesarean sections. Did not drink unpasteurised milk |

**Table 19: Expected and observed cases in occupational groups at potential increased risk of BSE infection**

| Occupational group | Number of individuals in group* | Number of cases of CJD 1-5-90 to 31-12-96 | | Incidence /million/year | P-value† |
|---|---|---|---|---|---|
| | | Expected | Observed | | |
| **Farm workers on farms with cattle, pigs, poultry, sheep or goats** | 447162 | 2.36 | 6 | 2.01 | 0.03 |
| **Farm workers on farms with cattle (dairy or beef)** | 354242 | 1.87 | 6 | 2.54 | 0.01 |
| **Farm workers on farms with dairy cattle** | 149436 | 0.79 | 4 | 4.02 | 0.01 |
| **Farm workers on farms with a confirmed case of BSE** | 109643 | 0.58 | 4 | 5.47 | 0.003 |
| **Veterinarians** | 8225 | 0.03 | 0 | 0 | 1.0 |
| **Abattoir workers/ butchers/meat cutters** | 68300‡ | 0.15 | 0 | 0 | 1.0 |

* Numbers given for farm workers represent an average over the period.

† P values indicate the probability of obtaining the observed number of cases or more under the null hypothesis that there is no occupational risk of CJD

‡ Includes an estimated 48,300 butchers and meat cutters in Great Britain (1991 census), 12,400 staff involved in red meat slaughtering (MAFF) and 7600 staff involved in slaughtering of animals and production of meat in Northern Ireland (Department of Economic Development, Belfast)

**Table 20: Expected and observed cases of CJD by hypothesised route of exposure to BSE agent**

| Type of potential contact with BSE agent | Number of individuals with exposure* | Number of cases of CJD 1-5-90 to 31-12-96 | | Incidence /million/year | P value† |
|---|---|---|---|---|---|
| | | Expected | Observed | | |
| **Contact with ruminant derived meat and bone meal (farm workers on farms with cattle, pigs, poultry, sheep or goats)** | 447162 | 2.36 | 6 | 2.01 | 0.03 |
| **Contact with live, BSE-infected cattle (farm workers on farms with a confirmed case of BSE, veterinarians, abattoir workers)** | 132408 | 0.64 | 4 | 4.53 | 0.004 |
| **Contact with brain/spinal cord of dead cattle (butchers/meat cutters)** | 68300 | 0.15 | 0 | 0 | 1.0 |

* Numbers given for farm workers represent an average over the period.

† P values indicate the probability of obtaining the observed number of cases or more under the null hypothesis that there is no occupational risk of CJD

## HISTORY OF THE IDENTIFICATION OF A POSSIBLE NEW VARIANT OF CJD IN THE UK

On 20 March 1995 the NCJDSU received a brain biopsy for pathological review taken from an 18-year-old patient (case 417). The sections showed no spongiform change on conventional staining and although PrP immunocytochemistry was positive using two antibodies it was negative with a third. The pathology was therefore considered non-diagnostic. The referring centre was asked to inform the NCJDSU of further clinical details and whether there was any relevant family history as it is known that familial forms of TSE can present at a relatively young age and have an unusual (including minimal) pathological appearances. It was later established that the patient had no relevant family history or risk factors for iatrogenic CJD. Following the patient's death on 21 May 1995 an autopsy was performed and the diagnosis of CJD was subsequently confirmed.[894,902]

In August 1995 a further young case (case 433) was notified to the NCJDSU. The patient, a 17-year-old girl, had recently had a brain biopsy which was diagnostic of CJD.[903] Only four cases of sporadic CJD in teenagers had been reported in the literature at this time[212,904-906] (see Table 21) and the occurrence of these two young CJD cases added fuel to the speculation, first started by the occurrence of CJD in farmers, that there was a connection between BSE and CJD. Consumers reacted strongly to this concern and the sale of beef fell markedly.

In September 1995 two more young patients (cases 466 and 467 - both aged 29 years) with suspected CJD were notified to the NCJDSU. The following month a further two young individuals were referred: case 474 a 29 year-old women who had had a recent biopsy confirming CJD[898] and case 476 a 29-year-old women who had developed a rapidly progressive dementia during pregnancy. The presence of PrP-positive amyloid plaques was noted as a factor in addition to age which linked the three patients that had a pathological diagnosis at this time (cases 417, 433 and 474). The significance of this was unclear.

At the end of October cases 417 and 433 were reported in the Lancet,[902,903] and the following month a series of articles in the British Medical Journal (BMJ) discussed the implication of the identification of CJD in teenagers and farmers in the UK.[907] At this time the first of a series of tables was drawn up by the NCJDSU detailing the recent known and suspected young CJD cases. This table was regularly undated over subsequent months (see example Table 22). Table 23 summarises the information collected from the tables up to 11 March 1996. Case 467 was not included until a pathological diagnosis and PrP analysis were available because it was believed prior to this that the patient was most likely to be suffering from a familial form of dementia.

A crucial issue at this time was whether the occurrence of these cases could be explained by improved ascertainment of CJD in young persons. The identification of the young CJD cases led to intensive review over the next few months of the clinical and pathological features of previous young patients identified by the NCJDSU and published cases from elsewhere (see Table 21).[894] No cases similar to the recent young patients were discovered (see Discussion section).

**Table 21: Known young patients with sporadic CJD around the world prior to 1996**

| Age | Onset | Residence | Duration* | Clinical Features | EEG | Pathology | Other details |
|---|---|---|---|---|---|---|---|
| 14 | <88 | Canada[212] | 24 | Clumsy and forgetful, truncal and limb ataxia, labile mood and myoclonus | Not typical | No plaques | Born in England. No family history (FH) of neurological illness |
| 16 | 6/76 | USA[906] | 28 | Forgetful, behavioural disturbance, choreoathetosis, seizures and myoclonus | ?Typical | Plaques not mentioned. White matter involvement | No FH of neurological or psychiatric illness |
| 19 | 6/82 | France[904] | 4 | Lethargy, insomnia personality change, ataxia and myoclonus | Typical | No plaques | No FH |
| 19 | <9/91 | Poland[905] | 10 | Loss of memory, confusion, dementia, spasticity and tremor. No myoclonus | Not typical | Typical spongiform encephalopathy (SE) Plaques not mentioned | No FH of CJD |
| 20 | 10/78 | USA[655] | >3 | Behavioural changes, increasing forgetfulness, clumsy, cerebellar signs and myoclonus | Not typical | Biopsy. No plaques | Had performed pathology on rhesus monkeys |
| 23 | 70-80 | Australia[908] | Unknown | Unknown | Unknown | Unknown | Unknown |
| 23 | <9/91 | Poland[905] | 3 | Loss of memory, confusion, dementia and cerebellar ataxia No myoclonus | Not typical | Typical SE. Plaques not mentioned | No FH of CJD |
| 23 | ~94 | Holland | Unknown | Unknown | Typical | No histology | Unknown |
| 24 | <65 | Japan[909] | 55 | Visual hallucinations, psychosis, peripheral sensory disturbance, ataxia, dysarthria, dysphagia, rigidity and hyperreflexia | Unknown | Typical SE No plaques | No FH |
| 25 | <80 | Japan[910] | 20 | Initially hand tremor, dysarthria and unsteady gait, followed by amnesia, dyscalculia and dysphasia. Thereafter developed myoclonus, hallucinations, delusions, primitive reflexes, rigidity and finally and an apallic state | Unknown | Typical SE. No plaques. White matter changes | No FH |
| 26 | 73 | New Guinea[319] | 18 | Forgetful, hemianopia, hemiparesis, cerebellar and parkinsonian syndromes, epilepsy and myoclonus | ?Not typical | Biopsy. Plaques not mentioned | - |
| 26 | 10/75 | France[911] | 34 | Depression, ataxia, pyramidal and cerebellar syndromes, forgetful, rigidity and coma. No myoclonus | Not typical | Typical SE. Plaques not mentioned | Mother died young |
| 27 | 69 | Austria[912] | 8 | Initially 'nervous spells', dizziness and ataxia. Later anxiety, paranoid symptoms, motor restlessness, spastic paraparesis, progressive ataxia, recurrent falls and increasing dementia | Not typical | No plaques | - |
| 27 | 84 | Poland[905,913] | 3.5 | Loss of memory, confusion, dementia, rigidity and myoclonus | Not typical | Typical SE. Plaques not mentioned | No FH of CJD |
| 29 | 69 | Japan[914] | 51 | Presented with progressive ataxia and dysarthria. Thereafter progressive dementia, alexia, auditory hallucinations, emotional instability, masked facies, hyperreflexia and muscle atrophy | Typical | Typical SE with kuru plaques | Transmitted. No FH |
| 29 | <69 | Japan[915] | 5.5 | Diplopia, dysarthria, euphoria, tremor, ataxia, forgetful, cerebellar signs, myoclonus and akinetic mutism | Unknown | Typical SE. No plaques | No FH |
| <30 | 92-95 | France | Unknown | Unknown | Unknown | Unknown | Unknown |

*Months. Table produced at the end of 1995. Several of the cases were unpublished and information was very limited. At least one other case had been reported prior to 1996 that we were unaware of.[916]

**Table 22: Young cases of CJD in the UK 12 November 1995**

| Age | Name | Hospital | Onset* | Dod | Duration† | Clinical Features* | EEG | Genetics | Pathology | Other details* | Class‡ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **17** | | | 1/5/94 | Alive | >18 | Facial and limb dysaesthesia, tremor. Behavioural change, ataxia, dysarthria, progressive cognitive impairment and myoclonus | Not typical | MM °mutation | Biopsy Classical Plaques | Possibly ate bovine brain in Cyprus five years ago. | **1** |
| **19** | | | 6/94 | 21/5/95 | 11 | Behavioural change. Global dementia plus spasticity. Cortical blindness, pyramidal & cerebellar signs. ?No myoclonus | Not typical | MM °mutation | Classical Plaques | Visited Aunt's farm and drank unpasteurised milk. | **1** |
| **29** | | | 1/4/95 | Alive | >7 | Progressive memory impairment, & ataxia. Supranuclear gaze palsy, akinetic mute, myoclonus, cortical blindness, frontal and pyramidal signs. | Not typical | Awaited | Biopsy Classical Plaques | | **1** |
| **29** | | | 1/1/95 | Alive | >11 | Behavioural change, ataxia and memory impairment. Mute, myoclonus, unresponsive, frontal and pyramidal signs. Pregnant | Not typical | Awaited | Nil | Butcher '85-87. FH of dementia, parkinson's and psychiatric illness | **3** |
| **29** | | | 15/2/94 | Alive | >20 | Limb dysaesthesia, ataxia, wt loss, lethargy, dysphasia & dysarthria, memory impairment, & chorea. Now AM, myoclonus. frontal and pyramidal signs. | Not typical | Blood in CJD Unit | Nil | Grandfather had dementing illness in his 70s. | **3** |
| **30** | | | 20/3/95 | 5/11/95 | 7.5 | Behavioural change, dysarthria, ataxia, lethargy, & memory impairment. Myoclonus, frontal and pyramidal signs | Not typical | Blood in CJD Unit | PM performed | Visited abattoir for 2 days '87 . Grandmothers demented over 5-10 year period aged ~80 | **3** |
| **38** | | | 7/5/95 | 3/11/95 | 6 | Ataxia, dyscalculia, tremor, progressive memory impairment & myoclonus. AM, startle, paratonic rigidity and grasp. | Typical | DNA in CJD Unit | PM performed | Worked in butcher's shop for 1 year but in ~1975 | **2** |
| **42** | | | 5/5/95 | Alive | >6 | Behavioural change, progressive memory impairment, dysphasia, tremor, myoclonus, & ataxia. Paratonic rigidity & frontal signs. | Not typical | DNA in CJD Unit | Nil | Maternal aunt demented in 80s over 5years | **4.1** |

*Some of these details were later updated in the light of further information. ‡Months †Classification: 1=definite, 2=probable, 3=possible, 4.1=other.

**Table 23: Summary of tables of suspect young cases with eventual classification**

| Date of table | No. of suspects | Classification at date of compilation | | | | | | Final classification post 20 March 1996 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Definite | Definite + plaques | GSS | Probable | Possible | Other | vCJD | CJD | GSS | Other |
| 27-09-95 | 5 | 2 | 0 | 0 | 1 | 0 | 2 | 3 | 2 | 0 | 0 |
| 12-11-95 | 8 | 0 | 3 | 0 | 1 | 3 | 1 | 6 | 2 | 0 | 0 |
| 14-12-95 | 10 | 0 | 3 | 0 | 1 | 3 | 3 | 7 | 2 | 0 | 1 |
| 07-01-96 | 9 | 1 | 3 | 0 | 0 | 3 | 2 | 7 | 2 | 0 | 0 |
| 16-01-96 | 10 | 1 | 5 | 2 | 0 | 0 | 2 | 7 | 2 | 0 | 1 |
| 29-01-96 | 11 | 1 | 7 | 1 | 0 | 0 | 2 | 7 | 2 | 1 | 1 |
| 07-02-96 | 12 | 1 | 7 | 1 | 0 | 0 | 3 | 8 | 2 | 1 | 1 |
| 11-03-96 | 14 | 1 | 8 | 1 | 0 | 0 | 4 | 10 | 2 | 1 | 1 |

In November 1995 a further suspected young case was identified after a presentation on young CJD cases at a neurology meeting. A neurologist in the audience mentioned that he was looking after a 29-year-old women (case 480) with an undiagnosed dementing illness that was similar to the patients described.[10] The following month details were obtained about another suspect case when an immunology laboratory made an inquiry to the NCJDSU regarding the handling of a CSF sample from a 41-year-old women (case 485).

At the end of January 1996 I saw a 31-year-old man (case 497) in a general neurology clinic (not connected with CJD surveillance) who had been referred with cognitive impairment. It was clearly concerning to identify a further suspect case in this manner as it raised the possibility that there might be a considerable number of young CJD cases now emerging.

By the end of January 1996, six 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 had hereditary forms of CJD, and analysis of the PrP genotype in these patients therefore became a high priority.

In February a further young suspect case of CJD was identified following inquires made as a result of a newspaper report. At this time ten cases of atypical CJD in young people had been identified, eight of which had been neuropathologically confirmed. A crucial issue was whether similar cases were being identified outside of the UK, 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 aged less than 45 years. Seven such individuals were identified and were reported to the NCJDSU over the subsequent weeks, but only one, from France, possibly had a similar clinical phenotype,[343] and in this case neuropathological information was not yet available. On 7 March 1996, details of full PrP gene sequencing became available for 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 for 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 patients were presented at the UK Spongiform Encephalopathy Advisory Committee (SEAC) 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, consultation with senior neurologists and neuropathologists provided support for the hypothetical novelty of these cases and two further cases were confirmed neuropathologically. 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 PrP gene 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 NCJDSU was announced in the UK Parliament on the 20th of March,[917] 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'.[84] The history of the identification of vCJD has been scrutinised by the BSE Inquiry and is meticulously documented in Volume 8, Chapter 5. A summary of the evolving assessment of the criteria used to link BSE and vCJD during early 1996 is given in Table 24.

Up to the end of 1996 14 cases of pathologically-confirmed vCJD had been identified by the NCJDSU. The dates of onset, notification death and confirmation of these patients are given in Figure 26. The section below describes the 14 cases in detail.

**Figure 26: Dates of onset, notification, death and confirmation for the first 14 cases of vCJD**

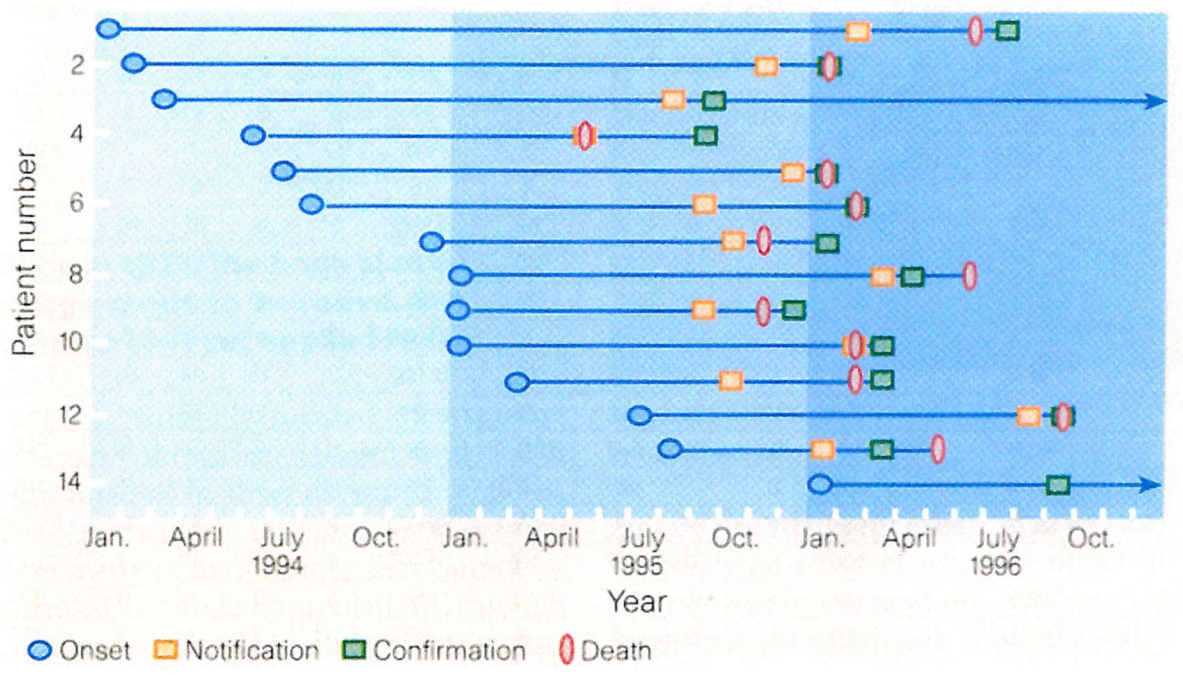



**Table 24: Evolving assessment of criteria used to link BSE and variant CJD**

| Criteria | Assessment through early 1996 | | | | |
|---|---|---|---|---|---|
| | **January 1** | **February 1** | **March 1** | **March 8** | **March 20** |
| **Novel clinical phenotype** | Uncertain | Possible | **Probable** | Probable | Probable |
| **Novel neuropathologic phenotype** | Uncertain | Possible | **Probable** | Probable | Probable |
| **Distinct from pre-1980 UK cases** | Unknown | Possible | **Probable** | Probable | Probable |
| **No associated PrP gene mutation** | Uncertain | Uncertain | Uncertain | **Probable** | Probable |
| **Distinct from cases outside UK** | Unknown | Unknown | Unknown | Possible | **Probable** |

Table modified from a review of the history of the identification of vCJD by Brown.[918]

## Case 417

In June 1994 an 18-year-old student performed poorly in his sixth-form exams. He had previously been a high academic achiever, obtaining 15 O' levels. He was usually outgoing and talkative but he became quiet, withdrawn and passive. Two months later he was the driver in a road traffic accident that occurred for no apparent reason. At the beginning of October his exam results were again poor and he gave up his Saturday job because of the pressure of school work.

In mid-November he attended a psychologist at the recommendation of his school for counselling and the following day decided to leave school and get a job. He subsequently told people that he had found employment when he had not. His GP diagnosed depression and started treatment with sertraline. He was referred to a psychiatrist who saw him at the end of November and noted that his main complaint was that he thought he was going 'nutty'. He said that he had been forgetful and his mother mentioned disorientation in time and odd behaviour such as lifting invisible cups to his lips and trying to change the TV with an invisible remote control. He reported his mood was low most of the time and that he had a broken sleep pattern, poor concentration and weight loss. Examination revealed poor concentration and he was disorientated for day and month, but his registration and recall appeared normal. The psychiatrist agreed with the diagnosis of depression and advised an increase in the dose of sertraline.

He was reviewed by the psychiatrist a week later, and his parents reported that their son had described 'seeing a little fireman flying in the air called Matthew' and had said that he was looking after a little boy in his room. He had become frightened of water and stopped washing, found brushing his teeth painful and was afraid of cutting himself shaving even though he used an electric razor. He was sleeping excessively, including in the middle of meals, was having difficulty coordinating feeding and started to become unsteady on his feet. He was unable to explain his bizarre utterances and reiterated that he felt he was 'going round the bend'. Examination revealed no focal neurological signs but he did walk with a limp and was noted to be 'fidgety'. The psychiatrist was concerned that the patient's illness may be organic and arranged some investigations. An EEG showed an excess of posterior slowing and a CT brain scan was normal.

The patient was reviewed a week later and was noted to have developed increasing phobias, including spicy foods, hot tea and having his toenails cut. He appeared tense and frightened and was prescribed chlorpromazine and advised to slowly reduce the sertraline.

At the beginning of January 1995 he was seen once more and was admitted to a psychiatric ward. The patient now said that he felt his body was humanoid and that his mind was not his own. He also complained of having microscopic people inside his body. On examination his cognitive function, including memory, was poor and his speech was incoherent. Hyperreflexia with ankle clonus was noted, he had cerebellar signs in the upper limbs and an unsteady broad-based gait. Although he had not complained of any sensory symptoms, he appeared to have hyperaesthesia when touched.

He became hostile, aggressive and disinhibited at times with continuing fleeting delusions such as believing his eyes and internal organs had been removed. During the following month his medication was changed to dothiepin and sulpiride without any improvement. A trial off all medication led to a more disturbed and irritable state so the dothiepin and sulpiride were reinstituted. A further EEG showed generalised slowing although it was unclear if this was related to his medication.

At the beginning of February he developed a seizure-like episode during which he was noted to become unconscious with twitching and cyanosis. All his medication was stopped but he again became disturbed and was commenced on zuclopenthixol. A further seizure-like episode occurred, all medication was again stopped and he was referred to a neurologist. By now he was occasionally incontinent, very ataxic and needed help with washing, dressing and feeding. He was drowsy and at times agitated and visually hallucinating. The neurologist noted myoclonic jerks and arranged further investigations. A repeat EEG showed generalised slowing, routine haematology and biochemistry was unremarkable apart from transient elevation of bilirubin and white cell count. Cranial MRI and routine CSF examination were reported to be normal.

In mid-February he was referred for a second opinion to another centre where he was noted to be disorientated in time and place, had poor concentration and decreased verbal fluency. His upgaze was limited, he had spasticity in all limbs and extensor plantar responses. A skin biopsy and repeat biochemistry and haematology were unremarkable. CSF was acelluar with a slightly elevated protein of 0.5 g/l and no oligoclonal bands. Nerve conduction studies and visual evoked potentials were normal but somatosensory evoked potentials were bilaterally abnormal, and of a degree that was thought to favour a central conduction problem. A frontal brain biopsy was performed and considered non-diagnostic: although there was a strongly positive reaction with two of three PrP monoclonal antibodies there was no spongiform change.

He subsequently became increasingly incapacitated with a fluctuating level of consciousness and persisting myoclonus. A further EEG again slowed generalised slowing with no periodic complexes. He became unable to communicate and bedbound just prior to his death, 11 months after the onset of his symptoms. An autopsy was performed.

## Case 433

During the first week of March 1994 a 16-year-old student slipped and fell when carrying a vacuum cleaner down some stairs. She did not sustain any significant injury. The next day she complained of pain in the left heel and went to her GP who prescribed ibuprofen. She took three doses and the following day developed a rash over her forearms and, over the next few days, swelling of the hand, feet and eyebrows. This resolved over the subsequent two weeks. Her face and hands however now started to feel continually numb and 'sticky'. By mid-April she started to complain of knee pain and over the next month she had low back and then neck pains. Her family had noted a mild shaking of her hands when holding a cup of tea.

In early June she 'fainted' in the bathroom after getting out of the bath and hit her head, but did not lose consciousness. She said that she felt that she had been pushed over. Although she had initially claimed that her first fall was due to slipping she had started saying in May that she had been pushed. She had also been claiming since March that someone was trying to touch her and wake her up at night. All of these claims were untrue.

In July she took and passed four GCSE exams. Her parents noted that she was passing urine every half-an-hour at night, and more frequently during the day (this persisted throughout her illness). She became uncharacteristically nervous, lacked her usual maturity and would 'cry over little things', e.g. when her mother combed her hair and a month later when she was not allowed to sit next to a window in an aeroplane. By the end of July she stopped keeping a diary as her writing had deteriorated due to her shaking.

From the first week in August she became increasingly unsteady on her feet and was having to hold on to walls and furniture to get around the house. She became forgetful and her speech became slow. She was having difficulty doing up shirt buttons because of her shaking. At the end of the month her family took her to casualty because of her increasingly unsteady gait and abnormal speech. On examination she was fully orientated, had a slight tremor of her arms and clumsiness of the limbs, but was not considered to have any convincing neurological signs. A CT brain scan was normal and she was referred to a neurologist.

At the beginning of September she was seen by the neurologist and admitted for two weeks assessment. She was talking less and was very tearful and emotional at this time. Using a knife and fork was becoming difficult and she started choking on fluids. When examined she was fully orientated and knew she had taken four GCSEs. However she was only able to recall three of the subjects and could not recall the grades. Her premorbid intellectual ability was thought to be average but at this time her verbal IQ, performance IQ and full scale IQs were 69, 86 and 62 respectively. She was unable to recall any of three items after five minutes but could give her address and telephone number. She was unable to perform serial sevens or any but the simplest mental arithmetic. Her response time appeared slowed and she had a slightly impassive face with apparent inability to close her eyelids tightly, smile broadly or open her jaw. Eye movements were normal. She had an intention tremor, cerebellar dysarthria and limb and gait ataxia. Generalised involuntary movements of limbs, head, face and trunk were present and described as either myoclonus or chorea. She had thalassaemia trait but other haematological, biochemical, immunological and microbiological investigations were unremarkable. CSF contained no white cells, normal protein and glucose and no oligoclonal bands. Cranial MRI and EEG were reported to be normal. Nerve conduction studies and EMG were unremarkable. Rectal and skin biopsies were normal and a muscle biopsy was reported to show minimal non-specific features on light microscopy and a suggestion of regeneration on electron microscopy.

Over the next four months she continued to deteriorate: by November she was needing help to mobilise and feed and in January 1995 she became more apathetic and was talking less. She was sleeping poorly. When examined in December she was partially disorientated in time and place but registration and recall was normal.

She was readmitted for further investigation in February 1995 and was still complaining of pains in her neck, arms and knees. Her dysarthria made her speech at times incomprehensible and she communicated by writing, holding her right hand with left to keep it steady. Her tongue movements were slow and her swallow weak. Involuntary movements included myoclonic and choreiform components, which disappeared during rest and sleep and were increased markedly on action. Walking was now difficult even with assistance. Further EEG and CSF examination were unremarkable. Another brain MRI was performed but because of movement artefact had to be repeated. The repeat scan was reported to possibly show minimally prominent CSF pathways throughout but no other abnormality.

When examined at the end of March 1995 she had generalised chorea and marked dystonic posturing of her feet. Repetitive stereotyped movements of the hands, comprising rubbing and grasping of the right hand with the left, were noted, and at times the appearance, particularly of the left arm, was considered to be that of an 'alien limb'. She had a positive snout reflex, bilateral palmo-mental responses and bilateral grasp reflexes, most pronounced on the left.

By the beginning of May she was mute, would only intermittently obey commands and was having difficulty swallowing saliva. Three months later she was again readmitted. At this time she was incontinent, mostly bedbound and had no obvious recognition of people or sounds. She was generally hyperreflexic when examined. Endoscopy and duodenal biopsies were normal. A right frontal brain biopsy confirmed the diagnosis of CJD. This procedure was complicated by the development of a cerebral abscess that was successfully drained.

She continued to deteriorate, becoming akinetic mute and developing generalised paratonic rigidity and sustained ankle clonus. Several episodes of respiratory infection were actively treated with antibiotics. She died on 23rd May 1997 and did not undergo necropsy.

## Case 466

At the beginning of January 1995 a 29-year-old engineer developed persistent left foot pain. Over the next six weeks he had difficulty concentrating and keeping up with routine paperwork. His sleep was poor and he was anxious about his inability to cope at work. At times he appeared to laugh for no reason. From mid-March his speech was slurred and he was feeling very fatigued and would fall asleep during the day. Six weeks later his walking became unsteady. This was initially attributed to persisting foot pain. He was unable to recall a familiar P.I.N number and was forgetting to pay bills. His driving became erratic and he had difficulty coordinating the use of the clutch pedal. A change in personality was noted: he was generally apathetic, flat and emotionless but at times unusually over-affectionate. He had episodes of confusion and on one occasion did not recognise his wife.

In mid-June a rheumatologist suggested that the continuing foot pain was 'mechanical'. Around this time he also saw a neurologist. Examination was unremarkable and he was fully orientated. However, he was unable to recall how many brothers he had or the name of the Prime Minister. Routine biochemistry, haematology and cranial CT scanning were normal. Over the following month he became aggressive and deluded: he was convinced the All Blacks rugby team were in his house. His gait deteriorated rapidly, he was falling and having difficulty rising from a chair.

At the beginning of July he was admitted for further investigations. He was disoriented in time and mildly dysphasic but his concentration and bedside testing of short-term and semantic memory were not obviously abnormal. However he showed impairment of episodic memory. He was fidgety, had generalised hyperreflexia with ankle clonus and a brisk jaw jerk, and had mild limb and gait ataxia. An EEG showed mild generalised non-specific slow wave abnormalities. Brain MRI was reported to be normal and CSF was acellular with a normal protein and no oligoclonal bands. He was assessed by a psychiatrist who considered that he did not have a frank psychosis but was very suggestible: when asked if he was aware of the presence of the devil, he said the

devil was trying to make him worship snakes, although moments later he denied this. The psychiatrist felt that the patient was suffering from an organic rather than a functional illness.

He was discharged but continued to deteriorate, becoming more unsteady, requiring help to wash and dress, getting lost around his own home and developed perseverative behaviour. Urinary incontinence and a startle reaction were first noted. A neuropsychological assessment revealed a generally poor intellectual ability with striking deficits of long and short-term memory but intact reading ability.

He was readmitted in September and by now was very confused and needing assistance to walk. Mini Mental State Examination score was 5/30, his speech was quiet with perseveration and neologisms and he had micrographia. He had eyelid retraction, generalised mild rigidity, bradykinesia, primitive reflexes and persisting bilateral pyramidal signs. Repeat investigations revealed normal routine blood tests and normal CSF constituents apart from a slightly elevated protein of 0.78g/L. A deterioration in his EEG has occurred, with no background alpha and dominant widespread theta activity. During the admission be became bedbound and developed spontaneous, action and touch-induced generalised myoclonus. Over the next month he became mute and unresponsive. He died 10 months after the onset of his symptoms. An autopsy was performed.

His paternal grandmother had a six-year history of dementia prior to death age 84 and his maternal grandmother had a 10-year history of dementia and also died aged 84.

## Case 467

A 29-year-old clerical officer was admitted to a psychiatric hospital in September 1994 following a one-month history of increasing depression, anxiety, agitation and paranoid delusions. She claimed that the tannoy at her work and voices at home had stated that she would be murdered and that voices at work had repeatedly called her a 'bitch' and said she was ugly. She believed that people were driving around to find and murder her because she was reputed to be related to Lord Leverhulme (untrue). Other paranoid delusions included the belief that her work colleagues had 'bugged' her house and telephone and 'know' of her plans. Examination revealed no evidence of cognitive or neurological impairment but she expressed delusional ideas and occasionally laughed inappropriately. She retained some insight, saying that there was something wrong with her and that she was 'cracking up'. Routine blood tests were unremarkable. She was diagnosed as having 'a paranoid illness with possible first rank symptoms' and treated with trifluoperazine. Within two weeks the delusions and auditory hallucinations had stopped, her mood was considered to be normal and she was discharged, still taking the trifluoperazine.

She was described as usually quiet and introverted and two years previously she had been successfully treated with an antidepressant for an episode diagnosed as 'paranoid depression'. This had been characterised by withdrawal, weight loss, depressive delusions and auditory hallucinations. She had no other risk factors for the development of a psychosis and the possibility was raised that the current episode may have been precipitated by the stress of commencing a new job.

In the month following discharge she was a little withdrawn and would occasionally laugh inappropriately, but was otherwise almost back to her normal self. Around this time she started to complain of pain below both knees and occasionally in her thighs, symptoms that persisted throughout her illness. Over the next two months

no further psychotic symptoms were noted and the trifluoperazine was stopped. However during this period she lost interest in her personal hygiene and became uncharacteristically aggressive and emotional. She was sleeping excessively and moved her bed downstairs. In February 1995 she was noted to be eating poorly, losing weight and having difficulty concentrating. Her family discovered that for the previous three months she had uncharacteristically been missing GP appointments and failing to claim unemployment benefit. She became increasingly lethargic and apathetic, losing interest in television and newspapers, and would not bother to answer the telephone. Loud noises, such as dogs barking, upset her and she adjusted the telephone so that the ring was quieter. The phonophobia persisted subsequently.

In March she became unsteady on her feet and occasionally fell, her memory started to decline, e.g. she forgot a previously familiar computer password, and she was now unable to manage simple word processing. Over the next two months her weight loss and anorexia continued and she became occasionally confused, not knowing the day of the week and making tea with cold water.

Because of her deterioration she was readmitted to the psychiatric unit in May. She was by now clearly confused: putting on other patients' clothing, looking for people in the plants and saying she was going to find 'the Hovis man'. Although she denied hearing voices she appeared at times to be possibly responding to auditory hallucinations. On examination she was restless, tense and nervous. She was disorientated in time and place and had grossly impaired concentration and memory. Heel-shin testing was impaired and her gait was markedly unsteady. She was treated with diazepam and zimovane and restarted on trifluoperazine. Routine blood tests were again unremarkable and an EEG was reported to show no abnormalities. Because of her physical signs she was referred to a neurologist who additionally noted mild generalised chorea, slow eye movements with hypometric saccades, dysarthria, increased tone in the legs and weakly extensor plantars. A neuropsychological assessment revealed global intellectual impairment, with a predicted decline in IQ of over two standard deviations. A particularly marked deficit of short-term memory was apparent.

It was felt that she had a progressive neurological disorder and she was transferred to a neurological centre for further investigations. CSF examination and CT brain scan were reported to be normal. Brain MRI showed some fullness of the lateral and third ventricles but no other definite abnormalities were noted, although the images were degraded by movement artefact. A further EEG in early June was abnormal demonstrating non-specific slow-wave activity over both hemispheres. She continued to deteriorate, and over the following two months becoming incontinent of urine, more emotionally labile and increasingly forgetful. She was now unable to walk unaided and needed help to wash and dress. Her family noted an occasional startle response to noise but myoclonus was not apparent at any stage of her illness. A further EEG showed a deterioration with more generalised slowing and a lack of normal resting rhythms. The question of a hereditary neurodegenerative condition, including familial CJD, was raised at this stage as the patient's mother had died age 66 of an undiagnosed rapidly progressive dementing illness (an autopsy was not performed). By November she was virtually bedbound, mute and had become rigid. Over the next three months she became blind and unable to swallow. She died in February 1996, 18 months after the onset of her symptoms. An autopsy was performed.

Her mother had died age 66 and was said to have suffered from 'dementia' for four to five weeks before death. She had had poor balance, speech difficulties and parkinsonian-like tremor, but no myoclonus and no autopsy was performed. The patients' maternal grandmother was also said to have had 'dementia' for two months prior to dying aged 84.

## Case 474

In October 1994 a 28-year-old solicitor changed job to what was considered a more stressful position and for two weeks she experienced mild insomnia. Her GP prescribed temazepam and her symptoms settled. At the beginning of November she was standing in the street selling poppies when a retired GP noted that she had exophthalmos. She visited her own GP who found that she also had a goitre and seemed thyrotoxic (tremor, tachycardia and lid lag). He described her as 'like a cat on hot bricks, very anxious'. She was prescribed propranolol and referred her to a physician.

Her past medical history was unremarkable except for a diagnosis in 1981 of primary generalised epilepsy. At that time she had experienced episodes of brief jerking of her limbs associated with loss of consciousness, an EEG showed bilateral atypical spike-and-wave activity with photosensitivity and she had been commenced on sodium valproate. Treatment was stopped in 1989 when she had been seizure-free for two years.

She saw the physician at the end of November 1994 and was found to be biochemically thyrotoxic with a moderately high thyroid microsomal antibody titre. A thyroid scan showed diffuse uptake consistent with Graves' disease. She was started on carbimazole and the dose was increased a month later when she was still biochemically thyrotoxic, although she reported feeling better at this time. Her husband described his wife as being completely well by Christmas of 1994 and she was working as a solicitor without difficulty.

In February 1995 she awoke in the middle of the night and punched her husband. She said she thought he was a burglar. During early March she complained of forgetting things e.g. the pass number of the door at her work. She was assessed again by a physician on the 10th of March, who noted her history of memory problems and that she was unable to remember the dose of tablets she was on, but could recall having a blood test the previous week. Although her TSH was still very low her T4 was now at the upper end of normal. It was suggested that the propranolol might be causing the memory impairment and the dose was reduced. Five days later she became very uptight about a court case and later the same day about losing her car keys. She phoned her husband in 'floods of tears', which was considered very out of character. Her family were so concerned about her behaviour that she was taken to casualty from where she was admitted with a presumptive diagnosis of a thyrotoxic crisis. She was found to be alert and orientated in time, place and person. Treatment was commenced with hydrocortisone and fluids and she was discharged the next day. Thereafter she stopped working.

On 24th of March she was reviewed by a consultant in nuclear medicine who noted that she could not remember any of the events of the previous few weeks and had difficulty remembering when her thyroid problems had started. Biochemical assessment revealed a suppressed TSH but normal free T4 and T3. The consultant considered that the patient's memory disturbance was not related to hyperthyroidism or its treatment. He suggested that it may be due to 'a functional change secondary to depression, or ... continuing epilepsy affecting the temporal lobes, or whether there has been damage to the temporal lobes'. Brain CT and MRI were performed and reported to be normal. She was referred for a neurological assessment.

Over the next month her memory problems persisted and were said to fluctuate. On one occasion she locked herself out of her flat and had to get a locksmith to help her get in, forgetting that she actually had a spare key in a near-by flat. Her husband was needing to help feed her at times because 'she just didn't seem to be bothered'. Her menstrual periods completely stopped in early April. At the end of this month she was readmitted and

treated with radioactive iodine, although she was clinically and biochemically euthyroid. Routine biochemical and haematological testing were unremarkable, other than a transiently and minimally elevated alkaline phosphatase level. The day after admission she was assessed by a consultant psychiatrist who noted 'her mood was labile with frequent tears interspersed with more controlled jocular phrases. Speech content was at times bizarre, for example she insisted that she flits between here and "another world". Her views seem provocative and without any conviction. There are no definite psychotic features on examination of her mental state; nor is she severely depressed...She has no acknowledged past psychiatric history. Personally I think that the amnesia may well be hysterical rather than organic.' The psychiatrist suggested that formal psychometric testing and an EEG should be performed. The EEG showed a dominant background activity of symmetrical, reactive, occipital alpha rhythm. There was also irregular background theta activity, some episodic, showing varying localisation and associated with sharp waves. Examination by a neurologist at the end of April found no neurological abnormality. A PET scan at this time was normal. It was considered that her memory impairment might be secondary to epilepsy and she was restarted on sodium valproate and discharged home.

She subsequently became increasingly forgetful and confused, e.g. asking her husband who he was, not knowing her occupation and thinking she was still at school. She would sit and stare at magazines without reading them. At the beginning of May she was readmitted for further assessment to a local general hospital, where her behaviour deteriorated. At times she would become aggressive and on one occasion she ran away from the hospital in her night clothes. The nurses noted that she seemed unable to register even simple messages. She was treated with chlorpromazine. On the 11th of May she was again assessed by a consultant psychiatrist. She stated that for six months she had been hearing auditory hallucinations. It was noted that she heard two sets of voices - the most frightening being the devil, who did not talk directly to her, but talked to other people. She also said she heard two voices which came from badges of Mickey and Minnie Mouse. The voices would tell her to do things including running away from the hospital. It was felt that her manner was plainly psychotic, '...possibly a functional illness, possibly schizophrenia'. However, it was noted that some aspects of her story: the loss of memory and confusion, were not typical of a functional psychosis. On the 18$^{th}$ of May she was transferred to a psychiatric hospital and at this time she was needing assistance with all activities of daily living and was becoming unsteady. She was very confused, forgetful and disorientated, getting lost around the ward and believing her husband was dead. Restlessness and anxiety were noted at night and she was sleeping very little. On the 24$^{th}$ of May she was again assessed by a neurologist and it was noted that her neurological examination remained unremarkable. A video EEG with sleep deprivation was performed and showed abnormalities that were considered compatible with the previous diagnosis of epilepsy and sodium valproate was continued. Her confusion appeared to fluctuate and at times she was quite lucid and able to hold a rational conversation.

On the 31$^{st}$ of May she became intermittently unrousable, being described as having 'absences attacks' every 15 minutes and was transferred to a medical unit. At this time her medication was sodium valproate, diazepam, chlorpromazine and procyclidine. She was reviewed by a neurologist who noted that she was disorientated in time and place, had poor concentration, but a 'reasonable' memory. There were no abnormal neurological signs. He felt that her fluctuating confusion and absence attacks were due to increasing and decreasing her valproate and that her psychosis was the predominant problem. She refused admission for further neurological investigation at this time. Chlorpromazine was stopped and risperidone commenced and she was transferred back to the psychiatric hospital on the 8$^{th}$ June. However, she continued to deteriorate, becoming increasingly

unsteady and expressing intermittent bizarre thoughts. She said she had killed a man but his mother did not believe her, that the staff should not take away her wooden leg, she was on a plane, that trees would come into her room and rustle their leaves and that her giant ate to make himself smaller. On one occasion she became angry and tried to leave the ward because, she said, she had seen a dwarf who was going to kill or attack her. On another occasion she stated that she felt there were spiders in her mouth and that she could see spiders in the room. She tried to give the psychiatrist her toy dog as she said that a voice told her that if she did not she would murder it. One day she said she was on an all-girl ship and the next that she wanted to kill herself by jumping overboard. She was not noted to express suicidal ideation on any other occasion. Because of the hallucinations she was started on haloperidol and a week later on 27th June moclobemide was started.

Over the following four days she became lethargic and drowsy and was transferred back to the general hospital. Her T4 was raised and she was recommenced on carbimazole and her 'sedative' medication was reduced. She become unable to walk without assistance, dysphagic and was still having hallucinations and 'absence attacks', during which she would develop a fixed stare for 2-5 minutes, become rigid with facial grimacing and unresponsiveness. In mid-July she was transferred to the neurology unit and treated with benzodiazepines. At this time she said very little and was unable to get up from a chair or bring a beaker to her mouth. She became incontinent of urine. Neurological examination revealed bilateral ankle clonus, extensor plantar reflexes and a best motor response of flexion to pain. A second MRI showed minimal enlargement of the ventricles, although the temporal horns were not dilated. No other abnormality was noted. CSF was acellular, with a normal glucose and total protein and no oligoclonal bands. CSF albumin and IgG were slightly raised. An EEG showed a lack of generalised epileptiform abnormalities and was diffusely slower than the previous record. These changes were considered to possibly reflect the administration of an adequate dose of sodium valproate and her neuroleptic medication respectively. It was thought that she may be over-sedated and the benzodiazepines were decreased. Her carbimazole was stopped and she was given a further dose of radio-iodine on the 24th July. This was followed by a course of intravenous acyclovir.

She became less responsive on the 31st July, developed signs of conjunctivitis with a pyrexia and a leucocytosis, and was treated with antibiotics and i.v. fluids. The following day the possibility of CJD was first raised and because of her continued deterioration she was transferred to another neurological centre. On admission she was mute, with sluggish corneal reflexes and exaggerated deep tendon reflexes, ankle clonus and extensor plantars. At best she could obey simple commands and follow with her eyes. A further EEG showed a background dominated by theta and slow (2-5Hz) activity over both hemispheres admixed with some fast activity. A third brain MRI showed mild generalised atrophy and abnormal high signal in the postero-medial thalamus. She was empirically commenced on a five-day course of intravenous methyl prednisolone followed by oral steroids.

Over the next month her speech minimally improved to become comprehensible, although quiet and slurred, but she remained disorientated in time and place. Dystonic posturing of the arms and back and limb hypertonia were apparent. She had a paucity of spontaneous eye movements, with decreased upgaze. In September she again became virtually mute, at best would localise to pain and now had bilateral grasp reflexes, a pout reflex and a brisk jaw jerk. Repeat CSF analysis was again acellular with a normal glucose but the protein was elevated at 0.94. There was no abnormality of the IgG pattern. At the beginning of October a right frontal brain biopsy was performed and showed characteristic features of CJD and PrP-positive amyloid plaques. By the 12th October she was akinetic mute, had developed myoclonus of the face, limbs and trunk and failed to respond to visual

menace. A gastrostomy feeding tube was inserted and she was transferred back to a local hospital. Intermittent infections were treated with antibiotics, but she continued to deteriorate and died at the beginning of February 1996.

## Case 476

A 28-year-old housewife uncharacteristically did not send any cards prior to Christmas 1994. She referred herself around this time to a local psychologist for 'support'. Although the reason for this is not known she had experienced a number of family problems in the previous year and her mother was unwell with dementia.

In January she became irritable and aggressive, threatening to throw things and hit her husband. She also became poorly motivated and couldn't be bothered to do the cooking or washing. She lost interest in gossip and stopped going out to bingo as often. At this time memory difficulties were also becoming apparent, e.g. she would forget to pick up her husband from his work.

In April she found that she was pregnant. She was noted to have became very quiet and fidgety. Since Christmas she had been saying less and less and by now she was mainly giving only yes or no answers. She was failing to keep up her appearance and personal hygiene and lost interest in keeping her children looking tidy. Her daughter won a medal for dancing but her mother didn't bother to go to the ceremony. On one occasion she sent her four year-old child shopping and let her keep £4 change from a £5 note. Her memory problems were getting worse, e.g. would forget that she'd put food in the microwave oven and was forgetting to pay bills.

In May she became unsteady and was described as 'walking like a drunk'. Her dog was soiling the house but she didn't appear to care. By the end of the month she was sleeping excessively during the day. She would dial phone numbers at random and speak to anyone. In June she was phoning her relatives and not knowing why. She was feeling tired and gaining weight. At times she said bizarre things e.g. claiming her mother had come to visit her (in fact her mother was house-bound), insisting falsely that when she was out shopping with her aunt that they had had a drink in Boots that they hadn't paid for. She had also told her GP that another doctor had knocked a child down outside her home.

At the beginning of July she consulted her GP who considered that she was depressed and referred her to a psychiatrist. She was seen again by her GP at the end of the month and was noted to be 'very low, but not suicidal' and amitriptyline was prescribed. Around this time she was becoming increasingly confused, saying she had seen relatives when she had not, calling her husband by the name of a former boyfriend and using onions instead of meat in a meal. Her driving became very erratic and she was hitting fences and bollards.

Toward the end of August she saw a psychiatrist who noted that she had been 'fed-up and miserable' for the previous six months with no sleep disturbance, obvious precipitant or diurnal variation. She denied recent feelings of worthlessness or uselessness and denied guilty feelings or persecution. He reported that she had experienced occasional fleeting thoughts of self-harm but had made no serious plans. It was noted that her concentration was very poor and that she was finding reading very difficult. She was said to have improved slightly since commencing amitriptyline a month previously. On examination she appeared dishevelled, ill-kempt, restless, agitated and occasionally appeared perplexed. At times she looked vague, at other times smiling and she only became tearful when talking about her mother's death (she was actually alive). Her speech was

normal in rate and form but its content was sparse. Subjectively and objectively her mood was low. The rate and form of her thought were normal. She was orientated for day of the week and place and had good concentration and attention. The date and month were given incorrectly. Her short-term memory was intact (recalling all of six items after five minutes) though her long-term memory was inaccurate, being unable to recall the Prime Minister's name and only giving the Queen's name with prompting. No abnormality was detected on neurological examination. The psychiatrist concluded that the most likely diagnosis was a severe agitated depression and advised that the amitriptyline should be increased. However, he noted an acute confusional state characterised by fluctuating levels of consciousness was also a possibility given the episodic inappropriate content of her speech.

At around this time she was becoming increasingly disturbed in relation to her children: grabbing and shouting at them, searching the house for them when they were at school and accusing relatives of stealing them. She was also becoming increasingly clumsy, knocking food off her plate, spilling drinks and being unable to place a cup down on the arm of a chair. Her handwriting became illegible and she was unable to cope with simple arithmetic. She needed to be washed and dressed. Her house was burgled but she didn't appear to care.

She was reviewed by the psychiatrists in mid-September and it was noted that her family felt that she had not improved on the increased dose of amitriptyline, although the patient said that she had felt less miserable. She was noted to have been increasingly irritable and was still confused, e.g. telling people that she had just discovered she was pregnant. During the interview she admitted to certain suspicious ideas that the interview room might be bugged, but there did not appear to be any other abnormal beliefs. She was alert and orientated. There was some involuntary twitching in her right hand. She walked with a wide based gait and had clumsy movements and inaccurate finger-nose testing. The psychiatrist's primary diagnosis remained that of a severe agitated depression and recommended that the treatment should continue with amitriptyline and that thioridazine should be added. A referral was made for a neurological opinion.

At the beginning of October she was reviewed again by the psychiatrist and noted to still be severely depressed with a continuously low mood and frequent severe irritability and intermittent confusion. She appeared to be sleeping and eating fairly well and was considered less agitated than previously. It was recommended that she should stop her thioridazine and commence chlorpromazine.

Around this time she started to experience auditory and visual hallucinations, hearing voices in the kitchen and seeing people that were not there. She thought incorrectly that a picture on the wall was a family portrait and would send people to check on a baby that didn't exist. Incontinence of urine was first noted.

In mid-October she saw a neurologist and was found to be disorientated in time and place and was confabulating and weepy. She was fidgety, generally hyperreflexic, had a variable grasp reflex, mild asterixis and non-specific limb clumsiness and a markedly unsteady broad-based gait. It was felt that her illness was compatible with either a diffuse encephalopathy or a focal structural lesion, and she was admitted for further investigation. A brain MRI scan was reported as normal, although this was severely degraded by patient movement. Her EEG was reported to show generalised and persistent low voltage delta activity.

Over the next two days she became increasingly distressed and aggressive, shouting and screaming, throwing drinks at her relatives, attacking members of staff and intimidating other patients. She appeared to be

responding to auditory and visual hallucinations and had delusions regarding staff and patients. The level of confusion and disorientation were noted to be variable during day. She had mumbled, nonsensical speech, and was giving inappropriate answers, such as 'River' to questions about how she feels. At this time she was mostly bedbound, was unable to use cutlery and was having difficulty recognising people. On examination she was disinhibited, showing her abdomen, was very restless and agitated and distractible by surrounding noises. She was unable to communicate any recent news or recall a name and address after five minutes. Her verbal fluency was severely diminished. She was able to reasonably reproduce intersecting pentagons. Cellulitis was noted on her foot, her WCC was 25.7, CRP 90 and ESR 42. Routine CSF examination was normal and there were no oligoclonal bands. She was thought to have an acute confusional state and an infection and was treated with flucloxacillin and sedated with droperidol and diazepam.

A week after admission she had an episode where she was found on the floor with a reduced level of consciousness, not opening her eyes and at best localising to pain. The cause of this was unclear.

She continued to deteriorate over the following week and a possible diagnosis of CJD was first raised. Her oral intake was poor and intravenous fluids and thiamine were commenced. At best she would open her eyes spontaneously, visually fixate, obey simple commands and speak in a rambling and confused manner. She had myoclonus, grasp reflexes and bilateral spasticity with a brisk jaw jerk and extensor plantars. A further brain MRI scan was normal, although again degraded by movement. Daily pulses of intravenous methylprednisolone were given for three days in the hope that she may have a steroid responsive encephalopathy.

At the end of October she had another episode of unresponsiveness during which her eyes were deviated to the left, all her limbs became rigid and her back was arched, but there was no convulsion or tongue biting noted. This episode was thought most likely to be a seizure and carbamazepine was commenced.

The following day she experienced multiple tonic seizures lasting minutes at a time and was treated with sodium valproate and transferred to the intensive therapy unit.

An elective caesarean section was performed and she was transferred back to the neurology unit where her deterioration continued. She became akinetic mute with decerebrate posturing and a pronounced startle response to visual, auditory and tactile stimuli. Myoclonus became more marked and bilateral palmomental reflexes were present. EEGs had been performed on approximately a weekly basis since mid-October and had shown a progressive deterioration, with increasing prominence of diffuse slow wave activity and decreased faster components. The 4th EEG of the series on the 8th of November was also reported to contain low voltage bilaterally synchronous spike elements which occurred predominantly over the frontal areas with a left sided emphasis.

She became pyrexial, with yeast being grown from her blood cultures, and she died on the 25th November 1995. An EEG taken two days prior to death showed a further deterioration, being of extremely low voltage with widespread irregular slow wave activity. An autopsy was performed.

Her mother had a four-year history of 'multi-infarct dementia' and was alive aged 56. The patients' maternal grandfather had a seven-year history of confusion and 'parkinson's' before dying aged 67.

## Case 480

At the beginning of November 1993 a 26-year-old civil servant saw an ear, nose and throat specialist because of a left facial weakness that had developed 3-4 weeks previously. She was diagnosed as having a Bell's palsy and was treated with steroids. By February 1994 she no longer had any facial weakness but started to experience a cold/pins and needles/tingling sensation in her right arm and a few days later in her right leg. This sensation was continuous and 2-3 weeks later spread to the left side. Her main concern was her legs which she found difficult to get warm at night. She started using bed-socks and a hot water-bottle. Her GP noted in April that the paraesthesia involved the right side of her face and right shoulder and referred her for a neurology opinion. Over the next month the sensory symptoms spread to involve the whole head.

In early June she saw a neurologist who found a subjective alteration of sensation down the right side of her body on examination. Additionally, her finger-nose test was unsteady and her eye movements were possibly ataxic. The neurologist raised the possibility that the patient may have a demyelinating condition. At about this time she also developed walking difficulties, and would take rests when walking long distances. Her family considered this to be a result of the sensory symptoms in her legs.

In mid-July she saw the neurologist again because she was getting increasingly concerned about her condition, particularly the coldness in her legs. She was admitted for investigation of possible multiple sclerosis, but cranial MRI and routine CSF examination were normal and she was discharged.

Over the next two months she developed unexplained weight loss of about two stone and became very fatigued, going to bed at 7pm rather than after midnight as she had previously. She complained that her reactions and speech had 'slowed up'. Her GP felt that she was anxious and depressed and she was referred to a clinical psychologist, whom she saw in mid-November. The psychologist considered that her psychiatric problems were secondary to her persistent sensory disturbance.

Her family noted in November that she was having difficulty getting her words out, although she knew what she wanted to say. At the end of this month she again saw a neurologist. She was found to have great difficulty outlining her various physical complaints and gave a recent history of forgetfulness, with difficulty recalling dates and names of people who had visited her. She was unable to hop on either foot and had brisk deep tendon reflexes but no other abnormal neurological signs. The neurologist concluded that the patient had an undiagnosed 'acquired movement disorder with cognitive impairment', and made arrangements for further investigations.

By mid-December she was becoming unsteady when walking and her speech was slurred at times, particularly when she was tired. Because of her symptoms she stopped working. In January 1995 she started to occasionally fall and needed support when walking outside. She was unable to do domestic tasks and sometimes required help to wash and dress. Holding a pen became difficult and her handwriting was very scrawled. She developed nocturia and at times was incontinent of urine.

In mid-January she was admitted for investigation. Her speech was noted to be dysarthric and she was found to have subtle choreiform movements of the limbs. An EEG was reported to be normal (although in retrospect, the background rhythm of 8Hz was considered abnormal when the tracing was reviewed three months later). Visual

evoked responses were reported to show no significant delay although 'the configuration of the responses were considered to be marginally abnormal'. EMG and nerve conduction studies were normal. Thermal thresholds obtained from the dorsum of the right foot revealed grossly elevated perception of warm stimuli to greater than $10^0$C, with cold threshold elevated to $8^0$C. The patient was rather inconsistent in her responses however, and the significance of this result was therefore questioned. By the time of discharge at the end of January she required a stick to walk. She was considered to have a psychiatric element to her illness because her neurological dysfunction varied considerably and she had admitted to feeling depressed. On one occasion said that she had 'thoughts about other patients,' and when asked to elaborate her speech became almost inaudible and her involuntary movements became quite striking. The neurologist considered that she had a neurodegenerative condition with a 'fairly florid behavioural component' and referred her for a psychiatric opinion.

In mid-March she saw the psychiatrist. She reported having become more withdrawn with intermittent spells of low mood, but was not suicidal. She described her sleep as poor and admitted to waking early, but said her appetite was good and her weight had been stable since December. It was noted that she had been emotionally labile and prone to emotional outbursts, including both anger and tearfulness. It was documented that she had consulted her GP because of tearfulness and had been commenced on a small dose of amitriptyline with, she felt, some benefit. On examination she was fully orientated but had difficulties with immediate recall and short-term memory. Her concentration appeared slightly impaired but she clearly had insight into her problems and displayed a degree of distress about this. A slight tendency to perseverate was also noted. The psychiatrist concluded that her psychological and behavioural changes were secondary to the underlying neurodegenerative disorder rather than being the primary problem. He recommended treating her depression with an SSRI.

She was reviewed by a neurologist a month later and was found to have limb and gait ataxia, dysarthria, a pout reflex and positive glabellar tap, a right extensor plantar response and a combination of choreic, dystonic and apraxic movements. Neuropsychological assessment revealed a marked impairment of general intellectual ability, including very significant memory impairment. Her IQ was 73 using the Raven's Progressive Matrixes. She was disorientated for year, day, month and year. She could not name the present Prime Minister or recall any current events. The results of verbal memory from the Wechsler Memory Scale was in the range of memory retardation (Index 67). Frontal executive function appeared to be intact.

At the end of April she was admitted for further investigation. Routine haematology, biochemistry, microbiology were unremarkable except for a slightly low blood albumin, vitamin E level, lactate and zinc. Routine CSF examination was again normal with no oligoclonal bands detected. A repeat EEG was reported as showing an alpha rhythm at the lower end of the normal range but remained responsive to stimuli input. It was suggested 'this feature might suggest a mild degree of diffuse cerebral dysfunction but, overall, there has been no significant change since the last recording'. Brain MRI was degraded by patient movement but was considered to show a suggestion of some atrophy but no other abnormality. A SPECT scan showed an extensive region of reduced flow in the left temporo-parietal region. Muscle and liver biopsies were normal. The possibility of CJD was first raised.

At the beginning of May she was transferred to a rehabilitation ward. She was walking mainly with a Zimmer-frame at this time. Her speech became increasingly sparse, quiet and less intelligible. She was losing weight. In June her family noticed jerking movements for the first time. In mid-July she was reviewed by a neurologist and now she had brisk deep tendon reflexes and bilateral extensor plantars responses. She subsequently went home

for a week but swallowing difficulties became apparent, she was feeling extremely fatigued, and was unable to manage stairs. At the end of July she was transferred to a hospice. Her mobility deteriorated over the next three months and she became bedbound. A gastrostomy tube was inserted for feeding and her speech declined to either yes or no answers. In August she was saying only four or five words a day and by mid-October she was mute and appeared to no longer recognise her family.

When examined in November she was akinetic mute, had generalised spasticity, primitive reflexes and florid widespread myoclonus, but no startle reaction. Her best motor response was flexion to pain and she had no eye-opening to command but did react to visual menace. She died on the 5th of January 1996. An autopsy was performed.

Her maternal grandfather had been confused (unknown how long), paranoid and was not able to recognise her family before dying in his 70s.

## Case 485

In the summer of 1994 a 39-year-old care assistant developed backache and a burning sensation on the soles, and later the dorsum, of her feet. She became depressed and lost interest in doing the housework, cooking, keeping the household accounts and socialising. Her libido was also diminished and she stopped disciplining her son for any bad behaviour. She was feeling tired and cap-napped during the day but slept poorly at night.

At the end of the year her memory started to deteriorate and she was forgetting to make bank payments. In January 1995 she saw a physician because of her sensory symptoms. Nerve conduction studies were performed and were reported to show very mild denervative changes in the left tibialis anterior and absent left peroneal F waves. It was stated that these abnormalities indicated a proximal pathology although it was not possible to say whether is was a root or a lumbo-sacral plexus lesion.

In April her speech became slurred and she was making errors when taking down phone numbers. Her behaviour was increasingly odd, e.g. she started hoarding lighters and cigarettes, and she stopped working. At the end of the month she saw the physician urgently because of her painful sensory symptoms were now making walking difficult. Physical examination did not reveal a significant abnormality, a bone scan and X-rays were unremarkable and it was thought that her symptoms were due to mild root compression. Analgesia and a knee support were prescribed and it was noted that 'there may well be a considerable psychogenic element'.

In May she gave up driving because she had had difficulty gauging distances and had bumped her car. At the end of the month she attended a pain clinic. It was noted that in addition to the burning sensation in her feet and shins she was experiencing a very localised right-lumbar sacral pain and shooting pains in her back on movement. She also complained of soreness of the skin of her face and tongue that was stopping her from eating properly. Her teeth were described as feeling 'crowded' and she had been putting paper between them to stop them pressing together. She had taken amitriptyline for three weeks without benefit and she had also failed to respond to carbamazepine or voltarol. It was noted that her concentration had diminished and she had been feeling mentally slowed and 'muddled'. She stated that she was very depressed and had been crying a lot in the previous four months. Her appetite was poor and her weight had decreased by a stone in the previous month. On examination she was anxious, walked with a limp and had tenderness in the localised area over the right L5/S1

facet joint. However, neurological examination of her lower limbs was normal, back movements were good and straight leg raising was 80 degrees bilaterally. The pain consultant considered that the burning pain could have a dermatological, neurological or, less likely, a psychological basis. She was subsequently referred to a neurologist. A dermatologist concluded that her symptoms were not due to a skin problem.

She saw the neurologist in mid-June. Her history showed inconsistencies and she appeared quiet and withdrawn. She had a 'bizarre' unsteady gait with stiff legs. Her lower limbs were thought to be possibly slightly ataxic but the rest of the neurological examination was normal. It was felt difficult to know whether the illness was psychogenic or organic, but the possibility of CNS demyelination was considered and brain and spine MRI was arranged. This was reported to be normal.

At the end of June her GP referred her to a psychiatrist because of 'marked anxiety, depression and agoraphobia'. The psychiatrist saw her four days later. She admitted to being anxious and irritable and terrified of being on her own. Going outside her house made her feel shaky, sweaty and panicky. She said her concentration was poor and that she thought she might be having a breakdown. Her walking difficulties, sensory symptoms, slurred speech and memory loss were still causing her problems. She had been sleeping more than usual and although she had been taking fluvoxamine for three weeks her depression had not lifted and was like 'a black cloud'. On examination she was described as being very tense, rather disassociated, clearly anxious and at times a touch depressed. She appeared to have little insight or concern about her problems. Her cognitive state was considered essentially intact although she gave the wrong day of the week and date of the Second World War. The psychiatrist concluded that she appeared to have a depressive illness with significant anxiety symptoms, but that her presentation had been almost that of hysteria, as she was rather indifferent to her plight in many ways, with marked physical complaints and little recognition of any psychological factors. He added that it was important, however, not to dismiss the possibility of an organic diagnosis. It was suggested that her fluvoxamine should be stopped and lofepramine started and it was arranged for her to be taught anxiety management techniques.

Her family reported that around this time she started falling and required support to walk. She had outbursts of aggression and was hallucinating: seeing spiders, cats and dogs and hearing dogs barking and babies crying. She was loosing her train of thought and her memory was deteriorating: she was losing her purse and keys every morning, she would forget that she'd eaten and she kept asking what the time was. On one occasion she asked when her son was coming home from school, when he had actually left school years previously. She also started knocking on neighbours' doors even though she didn't know them and on one occasion wandered into an someone else's house and sat down.

In mid-July her family were finding her difficult to manage and she was admitted to a psychiatric hospital. Cognitive testing and neurological examination were reported to be normal. It was noted that her gait was inconsistently abnormal and it was wondered if she was possibly hysterical. During the admission she was restarted on fluoxetine and her level of anxiety decreased and her mood improved. The diagnosis at this time was a depressive illness with somatisation disorder and she was discharged home in early August.

Her family reported that her odd behaviour and ideas continued. On one occasion she picked up a nappy and thought it was an animal. She thought her child (aged 20) was a baby and that her dead mother was alive. She was able to only walk a few steps and became uncoordinated when feeding and was having difficulty putting a

cup down. She was reviewed over the next couple of months by the psychiatric services and it was noted that her symptoms seemed to vary according to the situation, with her gait, behaviour and confusion being much better when on the ward without her family than that reported in her own home.

Her family however, noted a continuing decline and by mid-October she was having difficulty recognising people, needed to be fed and could barely stand. She was re-admitted to the psychiatric hospital following a fall at this time. When asked to describe her mood she replied 'not bad, OK - can't walk'. She denied delusions or any abnormal perception. On examination she looked anxious and was 'fidgety'. On testing of cognitive function she got the day, date, month and year all incorrect. She knew the name of the Prime Minister, but not the president of the USA and gave her date of birth as 1934. She was grossly out with serial sevens (100, 634, 537, 503). When asked to name five cities she was only able to name one. Her speech was slurred at times, her gait was wide-based and staggering and she was falling 'randomly'. Routine blood tests were normal. Again her abnormal gait showed evidence of variability. In early November she was referred back to the neurologist.

Over the next two weeks she became incontinent of faeces with coprophagia. Bizarre content of her speech was noted:

Patient: My mother died 10 days ago.

Doctor: When did you last see her?

Patient: Three days ago.

Doctor: Are you sure she died 10 days ago and you saw her three days ago?

Patient: Yes.

Doctor: Do you mean she was a ghost or a spirit?

Patient: Spiritual

Doctor: How did she look?

Patient: Spiritual

When re-examined in mid-November she was unable to register three items, her writing was completely illegible and when shown a toothbrush she was unable to say what it was used for. She was able to read several sentences and had no constructional or dressing apraxia. An EEG at this time was dominated by generalised slow activity. A week later she was assessed by a neuropsychiatrist. She was found to have both an expressive and receptive dysphasia. A startle reflex was noted and there were constant involuntary movements of her head and limbs and intermittent truncal movements. She had limited upgaze, and her eye movements were jerky with some beats of nystagmus on looking up. Tone was asymmetrically increased in the arms and she had generally increased reflexes. She was incoordinated with intention and resting tremor in the hands and dysdiadochokinesia. A gross truncal ataxia was evident when she tried to walk. It was felt that her condition was not hysterical and the possibility of CJD was first raised.

She was reassessed at the end of November by a neurologist who noted myoclonic jerks of the limbs. She was admitted to a neurology ward in early December for further investigation. Routine blood tests and CSF

microbiology were normal. CSF chemistry was not performed. EEG showed diffuse and marked slow wave changes.

She was discharged home and over the following month continued to deteriorate. She didn't know where she was and became very suspicious, believing that her family were trying to poison her. Her weight had fallen by three stone over the course of her illness. When re-examined in mid-December she was able to say only a few words, but could smile appropriately. Primitive responses in the form of a unilateral grasp reflex and a pout reflexes were noted. She was neither rigid nor blind. By the beginning of January she was bedbound with prominent myoclonic jerks. She died on the 5th of January 1996. An autopsy was performed.

## Case 497

In August 1995 a 31-year-old process engineer became mildly aggressive and unusually critical e.g. he said to his mother that people should be run off the road if their driving was bad. On one occasion when his car window was dangerously dirty he insisted, uncharacteristically, that it be ignored and carried on driving. A month later his workmates noticed that he had become forgetful, being unable to recall people names and how to use some of the machines. He complained of cold feet and took to wearing two pairs of socks. He was described as a quiet person but in October he became unusually more talkative. In mid-October his GP received a letter from the principal engineer at the patient's work expressing concern that he had become 'extremely forgetful, not knowing if he has had lunch etc. and cannot remember instructions or discussions held 10 minutes beforehand'. It was also noted that he was 'worrying about issues which he would normally cope with'. The patient saw his GP the next day and said that he felt under pressure at work as he was very busy. He denied sleep problems and his concentration was normal on testing serial sevens. A few days later he cried with joy when his niece was born, an emotional display that was considered very out of character. He had become apathetic and couldn't be bothered to cook meals and help around the house. His personal hygiene was poor and he was losing weight. He constantly wanted to be with his mother, which was unusual for him, and at times he would cry and say 'I don't know what the hell's going on'. His mother reported that he seemed restless and occasionally in a world of his own.

Early in November visual hallucinations first occurred. He saw monsters chasing him and when looking through a window saw people that weren't there. Using a TV remote control had became difficult. His GP was concerned that he was suffering from the pressures of his job and made a referral to a psychologist. By the middle of November his memory had deteriorated to such an extent that he had become unable to work and he was referred to a consultant physician. His GP noted at this time that his behaviour was childlike. When examined his reflexes were symmetrical and his plantars flexor. By the end of November he was found to be driving his car on the wrong side of the road and at the beginning of December he was getting lost on the way home from local shops. He stopped reading books as he couldn't remember what he'd just read.

On the 4th of December he attended the medical outpatient department. He was noted to have non-specific involuntary movements and impairment of long and short-term memory. He was unable to give the day and date or recall an address after two minutes, but he knew the month, his date-of-birth, address and the name of the Prime Minister. No focal neurological signs were found on examination. A brain CT scan was considered to be normal although there was 'some slight elongation of the posterior horn [of the lateral ventricle] on the right

side and some slight enlargement of the body of the lateral ventricle on the right side which appears to be a longstanding finding as is the slight increase in the interhemispheric cistern in the cerebellum.' In view of the 'normal' scan, a psychiatric opinion was sought.

On the 20th of December he saw a psychiatrist and denied any disturbance of appetite, sleep or mood. He was unsure how long or why he had been off work. Although he clearly realised he was not well, he did not appear to be unduly concerned about this. When trying to answer questions he would frequently look away and he appeared restless. His talk was normal in rate but he had difficulty finding answers at times. He was emotionally blunted and his mood at times was incongruous. He was slightly distractful and perplexed but he denied any auditory hallucinations or interference with his thoughts. On cognitive testing he correctly gave his address, date-of-birth, and telephone number and knew where he was and why he was attending the OPD. However, he thought it was the 27th of December and seemed to have great difficulty with the time scale of events, e.g. he stated that his father died 17 years ago when in fact he had died only seven years previously. He had difficulty with short-term memory, but performed well on tests of concentration and was able to repeat four digit numbers forwards and backwards. He was a little slowed up and his gait was slightly abnormal. The psychiatrist concluded, 'This man presents with a very odd picture with both organic and functional symptoms... I think the likelihood is that he is developing psychosis'. He was admitted to a psychiatric hospital for assessment a week later. Toward the end of December his mother noted that he had started to become unsteady.

His family found that he became increasingly confused in the psychiatric hospital, thinking initially he was in a boat and that dead relatives were still alive. He subsequently became obsessed with the war, believing he was in a POW camp and telling his family to 'get out while its safe'. He was unable to make simple decisions, such as a choice of foods, and would eat anything that was placed in front of him. Help was required with washing and dressing. He became agitated and irritated by loud noises. Cognitive function tests were performed between the 5th and 19th of January and revealed global intellectual dysfunction: full scale, verbal and performance IQs were 35%, 25% and 42% below estimated pre-morbid values respectively. Weschlar Memory Scale (Index Scores) were all severely impaired: general memory (<62), verbal memory (<50), visual memory (53) and attention/concentration (74). An EEG showed generalised slow background with intermixed alpha components. An MRI scan was reported to show cerebral atrophy moderately abnormal for the patient's age but no other abnormality.

He was referred to a local neurology clinic and was seen (coincidentally by myself) on the 24th of January 1996. On examination his mental test score was 20/30. He knew his name, address, date-of-birth, the name of the Prime Minister and the Queen, but did not know his age and thought he was he was in Australia. Difficulties were noted with abstract thought and planning, compatible with frontal lobe dysfunction. His ability to read, write and draw a clock face appeared intact. Cranial nerve examination was normal. He had an ataxic gait and poor coordination in all limbs with possibly some fine postural myoclonus of his outstretched fingers and strong bilateral grasp reflexes but no other primitive reflexes. Deep tendon reflexes were generally hyperreflexic and plantar response bilaterally flexor. Tone, power and sensation were normal. The consultant who ran the clinic and myself were concerned that the patient had CJD and he was admitted for further neurological investigation. Subsequently choreiform movements of his limbs and trunk were noted and he was getting lost around the ward. All routine haematological, microbiological, immunological and biochemical investigations were normal or

unremarkable. CSF examination revealed no leucocytosis, normal glucose and protein levels and no oligoclonal bands. A further EEG again showed a mild generalised abnormality with an excess slow-wave activity.

In early February he was transferred back to the care of the psychiatrists, and continued to deteriorate with increasing confusion, ataxia with occasional falls and the development of dysarthria. He was intermittently agitated, restless and emotionally labile. In the middle of February he developed episodes of unresponsiveness and sweating lasting about two minutes that were considered more likely to be vaso-vagal attacks than seizures.

He returned for further neurological assessment at the end of February at which time he was needing full assistance with washing and dressing and was displaying disinhibited behaviour. Examination now additionally revealed dressing dyspraxia, constructional apraxia, dysphasia, upgaze paresis, broken-up ocular pursuit and a shuffling element to his gait. Routine haematological, microbiological, immunological and biochemical investigations were again normal or unremarkable. A further EEG was reported to again show moderate generalised slow-wave abnormalities. A right frontal brain biopsy was performed and was complicated by the development of a large extra-dural haematoma that was successfully drained. Pathology confirmed the diagnosis of CJD.

He continued to deteriorate and by mid-March he was unable to stand without help, required total nursing care and was doubly incontinent. When examined at the end of March he would not obey commands and would only intermittently answer questions. He had developed a few myoclonic jerks of his limbs and auditory and tactile startle reactions. By the beginning of April be was mute and later that month was completely bedbound. A further lumbar puncture was performed at the end of April for the 14-3-3 assay which was positive. He subsequently development dysphagia, akinetic mutism and died at the end of May 1996. An autopsy was performed.

His paternal grandmother had been 'confused' for at least a year prior to death aged 84.

## Case 499

Around Christmas 1994 a 19-year-old student was noted by a friend to be quieter than usual. Unusually, he would stand on the sideline and not join in with a game of football. In early January his family described him as being 'down in the dumps', he became withdrawn, was not talking very much and lost interest in his appearance. He started coming home from university increasingly often and was not playing his guitar (his main hobby) as much as usual. One night in February his parents found him crying, which he said was because he was behind with his university work. He was eating less at this time, possibly losing weight, and his speech became a quiet whisper, he was saying very little and would answer questions but not initiate conversation. His concentration was poor and he became frustrated at times e.g. stamping on TV remote control. By early March 1995 be started to have difficulties playing computer games and setting up the video, both of which he had previously been very adept at. Later that month his parents found a notebook with comments that had been written at sometime since the previous Christmas 'Why me. why?.....RIP'. He had also written the poem below at some time before his birthday on 17 February.

Blackness fills the sky
Your face it fills my mind
The rain pours down
Like tears from an eye
Ice on the sheet
Inside I feel so cold
Help me, I'm trapped
Trapped in myself I have to get out
Why me, what did I do
Can you see me struggling can you hear me shout
Looking for answers of what made me this way
Feel so helpless got to live another day
Feeling the rage, inside it builds so fast
Got to strike out how long will it last
And if I die who will care?
I know my relatives will
In fact they're the only reason that I left it that long
'Cos recently I fail at everything I do
And do you know how low that makes you
I'm a failure, a born loser
The only way to stop this pain is by ending it
If I do it on my birthday, will that cancel me out
As if I never existed, so no memories are left
Then I think of my parents
What an embarrassment it would be to have a son suicide
Will they ever be treated the same?
And how long will I be remembered for?
Will February the 17th be a day marked on the calendar
I don't know and don't care
I'm not doing this for sympathy
All I can say is sorry for what I've done
I guess I've always been a disappointment to you
And I know you would not want to see it end this way

In mid-March he saw his GP who diagnosed depression and treated him with two different antidepressants, lofepramine and clomipramine, for about a week each (stopped due to side effects) and then started fluoxetine. He was noted to be emotional labile at the beginning of April: when watching 'Robocob' on the TV he became very upset by the violence and cried. During the day he would be excessively sleepy and he developed nocturia. There was minimal improvement on the fluoxetine and from the middle of April he started to become increasingly forgetful and at the end of the month he started to stagger and bump into doorways. When he went to a swimming bath with friends but just clung onto side of the pool. After this date he spent most of his time lying on a couch at home because of his ataxia. His writing became very poor. At the end of April his GP was called, he noted a history of poor balance/increasing dizziness and nervousness/anxiety and stopped the fluoxetine. The next day the GP was again called to see the patient who had become 'shaky and weak since this morning'. On examination he was described as being cooperative, laconic and anxious, with a rather depressed affect. He denied any introspective thoughts or hallucinations. A tremor of both hands and fingers was noted, he was rather sweaty and had an ataxic heel-shin test and gait. His reflexes were brisk and Romberg's test was negative. The GP discussed the patient with a local psychiatrist and neurologist, and an appointment was arranged with the neurologist. The GP stated in his referral letter that his initial impression was that the patient was suffering from depression or a schizophrenic-type illness with a preponderance of negative features, but that the abnormal signs had raised the possibility of a primary neurological illness.

In early May the patient was assessed by the neurologist who noted a two month history of tremulousness in addition to his other symptoms. He denied any Schneiderian first rank symptoms and did not appear to be preoccupied or hallucinating. On examination he was generally tremulous and made no eye contact. He was fully orientated but could not remember any of four items after five minutes, although it was suspected that this was primarily due to a retention rather than a short-term memory problem. The rest of the neurological examination was generally unremarkable, except for generally brisk tendon reflexes and a mild degree of gait ataxia. There were no mirror movements and no primitive reflexes.

His memory continued to deteriorate, he was losing things and was unable to remember what he'd just seen on TV. A brain CT scan was considered to be normal although high slices showed some convexity dural calcification with what was probably additional parafalcine calcification. On the 5th of May his GP noted that he was possibly less ataxic and referred him for a psychiatric opinion. He started to become incontinent of urine the following week. During May his family noted he had developed a childlike sense of humour and at times was probably deluded. On one occasion he told his mother not to go into the kitchen because she would be killed by the snipers that were in there. He was now needing help with washing, dressing and shaving and was having to be fed as he was very slow and his hand was 'wobbly'. Without encouragement and help he would just lay in bed and do nothing. He couldn't move his fingers as fast as previously when playing the guitar but he still played the correct notes. Mathematics become difficult, a subject he had previously studied at University.

In mid-May he was assessed by a psychiatrist. It was noted that the patient was unable to give a clear account of himself, but did report suffering from a low mood with anxiety, disturbed sleep with early morning wakening and impaired concentration, although the latter had possibly recently improved a little. His appetite and weight were said to be unaffected. He felt rather pessimistic about his future but denied any feelings of guilt, worthlessness or suicidal thoughts. Psychotic symptoms, such as delusions and hallucinations, were denied. On examination he looked low and almost preoccupied, but did smile appropriately. He was rather tremulous and agitated, constantly wringing his hands. He had no spontaneous speech and gave brief answers to concrete

questions but no answers to any open ended questions. When asked why he doesn't answer he replied 'not much sleep... can't concentrate very well'. He was orientated for date, time and place and recalled three of five items after five minutes. However, he couldn't remember the psychiatrists name or profession. Tests for concentration were performed slowly but were accurate. The psychiatrist felt the most likely diagnosis was a major depressive illness, but also raised the possibility of an adverse reaction to fluoxetine. An admission was arranged to a local psychiatric hospital a few days later for assessment.

Physical examination on admission revealed a rather clumsy, jerky gait, jerky eye movements and a tremor of his outstretched hands. He had difficulty performing the heel-shin test and his finger-nose test was jerky. Memory was by now very poor (recall after three minutes 0/5). He was unable to repeat a short shopping list. During the admission he was getting easily lost around the ward, he became more unsteady and developed frank incontinence. He was very slow when eating meals and unable to hold a cup of tea because of tremor. When his father asked him about eating only small amounts he gave an odd reply: 'the wind will blow it off'. He had lost half a stone in weight over the previous few months. Although said he felt all right, he appeared quite agitated and 'jerky', continually wringing his hands, closing his eyes and hugging himself with his arms around his chest. Further examination during this admission revealed that he was unable to give the day, date or year, he had a digit span of six and could remember his O' level but not his A' level results. An EEG at the end of May was noted to be a little polyrythmical, but showed no clear abnormality. The only medication prescribed was multivitamins. His mood was considered to have possibly lifted a little during the admission. The psychiatrists felt that his condition was not typical of depression and he was transferred to a neurology ward for further evaluation at the beginning of June.

He was assessed for three days in the neurology department. On examination he appeared generally restless, had a degree of facial hypomimia and had involuntary movements that appeared choreic. Cognitive testing yielded a Mayo short mental test score of 23/38 and he had particular deficits on orientation, digit recall and verbal fluency. Limb tone, power and sensation were normal but coordination was mildly impaired in all limbs. His gait was ataxic and he was unable to walk heel-toe. Reflexes were generally brisk, including jaw jerk, but plantars were bilaterally flexor. There was a positive pout but no palmo-mental reflexes. All routine haematological, immunological, microbiological and biochemical tests were unremarkable. CSF cell count, lactate, protein and immunoglobulins were normal. Brain MRI showed bilateral thalamic hyperintensity predominantly in the pulvinar region on PD- and T2-weighted images. He was considered to have either a degenerative or metabolic encephalopathy and as discharged home.

His family noted in June that he looked very frightened at times and hallucinations became apparent, e.g. holding a pretend gun and shooting imaginary people in the garden. By the end of June he was saying very little and was unable to stand.

A further admission for assessment took place at the end of July. On examination he was described as having generalised choreo-dystonia. His Mayo mental test score had declined to 17 and he had a mixed ataxic and extrapyramidal speech disorder. Further new features noted were reduced upgaze and an inability to perform rapid alternating tongue movements. He was now unable to wheel himself around in his wheelchair. Repeat CSF examination was again unremarkable. A second EEG showed no significant abnormality. In addition to his chorea he developed akathesia both of which were helped by clonazepam.

He was subsequently transferred at the beginning of August for continuing care to a respite ward. At this time he was still able to recognise faces from his past. His condition subsequently deteriorated further with the development of dysphagia, which led to episodes of aspiration pneumonia. He became mute later in August. A one-week course of oral steroids was given without effect. It was noted that his level of arousal varied between a restless agitated state to drowsiness depending on his dose of clonazepam. His emotional lability had persisted and be would become upset by sad music. His family noted that his best motor response was to bang balloons in front of his bed. He deteriorated further in the beginning of December with a fever and symptoms of a chest infection, which responded to antibiotics.

In mid-January 1996 he was transferred for a further EEG. At this time it was noted that over the previous 10 days his involuntary movements had stopped and he was lying immobile all the time except intermittently grimacing/risus sardonicus. The EEG was reported to show diffuse slowing. Toward the end of January he was groaning and appeared distressed and was treated with thioridazine and diamorphine. His family noted that his eyes would still open spontaneously, he would turn his head toward noise and he would laugh in response to a joke. He still had some spontaneous movement at this time but the facial twitching and severe involuntary movements had ceased over the previous few weeks. He died of bronchopneumonia in early February 1996. An autopsy was performed.

His paternal grandmother had a three-year history of 'Alzheimer's disease' and was still alive aged 83.

## Case 502

In January 1994 the family of 48-year-old telecommunications manager noticed that he had become subdued, 'keeping himself to himself', and had memory difficulties, e.g. he would forget to go to work in the morning. He was turning up for meetings that had not been organised and on occasion would arrange to pick up his wife and then forget to turn up. He was described as having 'let himself go', e.g. not getting his hair cut. Holding a cup with his right arm became difficult and he would need to steady this arm with his left hand. His work record had been excellent but he was now starting to have difficulty producing work on time and was forgetting which projects he had to do. He was described as having 'no common sense', he had difficulty sorting out minor problems with his staff and uncharacteristically was asking his wife how to sort out managerial problems.

In June 1994 he started experiencing mood swings and said he felt depressed. Previously described as 'laid back' person he was becoming occasionally bad tempered and started 'answering his wife back'. He lost interest in socialising and wanted to be by himself a lot of the time. Although not actually tearful, he was felt by his wife to be 'very close to it'. He did not consult his GP about his low mood and was not treated with antidepressants.

His minor personality change and forgetfulness persisted throughout 1994 and became slightly more marked as the year progressed. In the autumn his wife asked him to call an ambulance after someone collapse outside their house. Instead of dialling 999 he looked in the Yellow Pages for the phone number.

Early in September he consulted his GP because of a heavy feeling in the left side of his chest and retrosternal area. He also stated that he was experiencing pins and needles in both arms and pains in both upper arms. Pain on rotation of left shoulder was noted. Chest and shoulder radiographs were normal. He was reviewed two weeks later and was considered to be no better, with persistence of pain in both upper arms, particularly on the left. Limitation of

abduction left shoulder was found on examination. At a further review six days later the GP noted a history of 'shooting pain in shoulder and arms if he reaches out to do anything. Unable to lift. Finding it difficult to drive'. A referral was made for an orthopaedic opinion and for physiotherapy for his 'left frozen shoulder'.

He had been given multiple warnings by his superiors because of his inability to cope at work, and was dismissed in mid-December. Prior to losing his job he started to lose weight and was not sleeping well.

His pain had not been helped by physiotherapy, and at the beginning of January 1995 he saw an orthopaedic surgeon. It was noted that although he had initially complained of pain in his chest and left shoulder he was more concerned at that time about pain and paraesthesia in his right arm and hand. Examination showed only slight and painless restriction of neck movement, but tenderness 'over the brachial plexus' on the right. Combined abduction of both shoulders was limited to about 120 degrees, movements beyond this being painful. There was no gross neurological abnormality of the upper limbs. The diagnosis was considered to be a nerve root entrapment following a prolapsed cervical disc. Cervical spine radiographs were normal and on review two months later the patient's symptoms had become more widespread, with pins and needles now also involving the right side of his face. There were again no objective neurological signs and the patient was referred to a neurologist. At the beginning of April he saw his GP who noted that he was experiencing difficulty holding objects with his right hand because of 'pins and needles and hypersensitivity.'

At the beginning of May he saw the neurologist who noted that the paraesthesia was now additionally affecting the whole of the right side of the patient's head as well as his right chest. Examination revealed a sensitivity of the skin over the right head, chest and arm. There was no motor or reflex deficit in the limbs. The patient asked if the symptoms could be stress induced and the neurologist agreed, but arranged an MRI of the cervical cord because of the possibility of syringomyelia.

His family stated that his sensory symptoms subsequently deteriorated further, spreading to his right leg and then two weeks later to involve the whole body. He described the sensation 'as if nerve endings on my body are on fire – burning'. He didn't like to be touched and his ears were so painful that he was unable to hold the earpiece of a phone to either ear. The sensory disturbance troubled him constantly and would keep him awake at night. These symptoms persisted at least until February 1996, at which time they became obscured by his cognitive decline. Throughout the whole of 1995 he was becoming more withdrawn, depressed and stubborn. His wife found that in August he would hold books but not actually read them.

In early October his GP noted that he was walking with a limp and at the beginning of November he stopped driving because of apathy and burning pains and pins and needles in his hands when holding the steering wheel. The MRI of his cervical spine was normal and his GP reported in mid-November that he was now also complaining of an action tremor of his right hand. On examination he appeared anxious but had no clearly abnormal signs. An admission was arranged for further neurological investigation in December but the patient did not attend, despite the medical staff leaving a message on his answer machine. His wife noted that he was becoming agitated at night.

He decided not to spend Christmas with his wife because he felt depressed and thought he wouldn't be good company. His family considered this decision to be very out of character. He didn't send Christmas cards to family or friends and didn't open cards that were sent to him. His wife, a nurse, noted that he was becoming

unsteady and was 'walking like someone who has had a stroke... leaning towards one side'. After this he deteriorated quite rapidly, having several falls and on one occasion he fell through a glass door. His voice was slow and slurred and he became increasingly withdrawn, failing to answer the phone. When his wife called on him it took him a long time to answer the door and he was living 'in a mess'.

In January 1996 he still appeared very depressed, his sleep was poor (difficulties getting off to sleep and early morning waking), and he had lost two stone in weight ('he cannot concentrate on preparing food') and was living almost entirely on tinned pears. He was not suicidal although he said he was very low. Most of his day was spent watching TV, he rarely went out and he left the curtains constantly drawn. He was generally saying very little but when he returned to live with his wife on the 20th of January he became aggressive, shouting and hitting out. Jerking movements were first notice in January.

At the end of January he saw his GP again who noted that he was unshaven and had loose clothes due to weight loss. He gave little eye contact and had a low affect. His cognition, memory and concentration were poor. The GP found no objective neurological deficit, noting that his hand was strong with a good grip, but did report abnormal movements that 'defied any classical description'. A referral was made to a psychiatrist: 'Do you agree he is depressed and needs some in-patient treatment/supervision? He has a great deal of difficulty in expressing emotion and I wonder whether his hand problem may have been a somatisation phenomenon.'

The following day he saw the psychiatrist. He said that during the last few years his performance had deteriorated at his work and he was therefore made redundant. He also stated that he had recently had pins and needles in his right arm. However the psychiatrist noted that the main problems as loss of appetite and weight, various aches and pains and increasing difficulty in coping. It was reported that 'his mental state reveals no evidence of depression or other psychiatric disorder. His cognition appeared good... the main priority is full medical investigation...'.

Five days later his GP was called to see him again. He was complaining of weakness and an unsteady gait. On examination he was sluggish with slurred speech but was well orientated. He was reported to have some dystonic movements of the left hand and foot and possibly increased tone in the right arm and leg, with possible cogwheeling at the wrist. There was no obvious spasticity or sustained clonus. He was obviously ataxic with a broad-based gait.

The patient was admitted that day to a medical ward. CSF protein was 0.53 g/L, but glucose and other constituents were normal. An MRI brain scan early in February was reported to be normal. His condition deteriorated, he became disorientated in place and was requiring help to walk. A startle reaction was first noted.

In view of the lack of diagnosis he was transferred to a neurological centre for further evaluation on the 21st of February. He denied any problems and appeared to have no insight in to his illness. On examination he had poor concentration and was disorientated for day, date, month and year. At times he was also disorientated for place and person. Language assessment was normal. He registered six points of a six point address but recalled none at one minute. His semantic and autobiographical memories appeared normal. Frontal testing showed prominent deficits in that he was only able to name five animals a minute and generate three words beginning with 'f' in a minute. He performed quite well on a test similarities and gave some rather bizarre answers for his cognitive estimates such as the speed of a train being 86,000 miles per hour. He occasionally had perseveration of speech.

Cranial nerves were normal, including eye movements. Multifocal spontaneous, action and stimulus sensitive myoclonus was present and he had a prominent startle reflex. Symmetrical ataxia of all limbs was noted but tone, power, reflexes and sensation were all normal. He had a drift of the right arm which looked parietal in nature. CJD was considered a likely diagnosis. All routine haematological, microbiological, immunological and biochemical investigations were normal or unremarkable. Three EEGs were recorded during this admission. The first, on the 21st of February, was clearly abnormal, showing a generalised excess of slower activity of theta and delta frequency and a moderate amount of faster activity just reaching the alpha range. The subsequent two recordings, six and nine days after the first, showed a progressive improvement with an increasing amount of alpha activity. He was given a course of steroids and vitamins without benefit.

On the 9th of March 1996 he was transferred back to the medical ward and continued to deteriorate. He became confused at times, e.g. thinking he had been on a rocket and that his fifteen year old son was getting married. He was also misidentifying people and was unable to recall who had just visited. He didn't like being left alone and would become agitated when his wife left. He could just about scribble a birthday card. Further EEG recordings on the 11th of March and the 11th of April showed similar widespread slow activity, an appearance that was considered a deterioration compared to the previous recording at the beginning of March. His ataxia was noted to be worse when reviewed by a neurologist on the 22nd of April.

At the beginning of May he became incontinent and was needing to be fed. At the end of the month he rapidly deteriorated, becoming bedbound, restless and unable to hold a cup. Over the next few days he became rigid and akinetic mute, developing pneumonia and dying on the 6th of June. An autopsy was performed.

## Case 517

At the beginning of 1995 a 28-year-old forester was noted by his family to be less talkative. He enjoyed gardening but stopped doing this for no obvious reason. In the summer of that year he complained of feeling tired, and although previously keen on motor mechanics he lost interest in this and was uncharacteristically complaining about having to work on his car. He was troubled by headaches for the next six months.

At the start of October he experienced cold feet and took to wearing an extra pair of socks. This symptom persisted. He said that he had lost his sense of smell and that some foods had an altered taste, with sugar and salt tasting stronger. Later that month he decided to stop driving because of difficulty concentrating – he was not dipping his headlights for oncoming traffic. He became obsessed with keeping his hands clean and stopped showering because he said the water felt cold to him.

In November memory problems were first noted, e.g. he was forgetting which pieces of wood he had been told to cut. He consulted his GP twice in early December because of the headaches and loss of taste and around this time was also losing weight.

Over Christmas he became withdrawn and more forgetful. He was abnormally sleepy during the day but slept poorly at night and would wander around the house. Incontinence of urine first occurred and he became unsteady on his feet. He stopped reading and would just sit with a book open at the same page. Just after Christmas his speech started to become slurred and his welding less accurate.

By mid-January 1996 he was unable to boil a kettle and was forgetting to put a tea-bag into the tea pot. One evening in he told his family that he thought the pub was busy because it was New Year's Eve.

In February he started becoming more confused and disturbed. He was seen trying to eat a steak with two spoons and became violent, hitting his daughter for sneezing and pulling his one-year-old daughter across the floor and hitting her. His family reported that he had been visually hallucinating and when he saw his GP in mid-February he admitted that he had been hearing voices when working out in the forest. He was referred to a psychiatrist who saw him on the 23rd of February. When asked what his problem was the patient replied 'I was having treatment for something I didn't need.' He said that for the past six months he had been feeling 'just not with it' and 'rough and confused'. He reported that his mood was fairly miserable in response to recent events and that he had lost one-and-a-half to two stones in weight, but denied any suicidal ideation. It was noted that he had experienced both auditory hallucinations, hearing voices talking to and about him, occasionally in a derogatory way, and sometimes just ordinary comments. He admitted feeling a little more paranoid on occasions recently and also described very clearly that thoughts had been taken away from his mind and replaced with something else, 'it feels like a whole thought is drained from me, it's there but it's not mine'. He also reported that 'sometimes the description just gets taken over from me, as if someone is taking over the sentence'. In the past he had suffered from seasonal affective disorder but had no other relevant problems. On examination he appeared confused and perplexed. On assessing his thought he reported 'I feel that something is trying to communicate but I haven't been able to concentrate to bring it in'. He was orientated in place, person and time but gave a clearly wrong date. His concentration was very poor but there was no obvious deficit of short or long-term memory. The psychiatrist concluded, 'I feel it is essential to exclude an organic cause for the relatively late onset of what appears to be schizophreniform symptoms,' and prescribed sulpiride.

He continued to deteriorate, and by the end of the month his concentration had got worse and he was becoming increasingly confused, cutting off half his beard without knowing why, putting his boots on the wrong feet and being unable to tie a knot correctly. He was getting lost around his home and was considered a danger at work. His confusion was initially just in the morning but later occurred the whole day. A startle reaction to touch was noted by his family and he was seen to be having increasing abnormal movements of his shoulders and head.

At the beginning of March he was admitted to a psychiatric ward for assessment. He was unable to give his correct address or age and said the year was 1992 and that he had been in hospital for a year. He believed that the patient's were running the ward and he tried to leave. Medication was refused as he thought it was offered to him in an odd way. The admitting psychiatrist was concerned that he had cerebellar and long tract signs and referred him to a neurologist. The neurologist noted mild dysarthria but no other focal neurological signs. Routine haematological, immunological and biochemical tests were unremarkable and a CT brain scan was normal. CSF protein was slightly raised at 0.52 g/L but there were no leucocytes.

On the 6th of March he became more disturbed and tried to strangle a member of the staff. His medication was changed to haloperidol. An EEG was abnormal with a background consisting mainly of theta activity at 5-7Hz seen over both hemispheres and slower waves down to 3Hz were also present. Alpha activity at 8Hz was seen bilaterally and attenuated poorly on eye opening. A good deal of fast activity was seen mainly over the frontotemporal areas. Over the two weeks he failed to improve, and choreoathetosis, mainly of the shoulders, and primitive reflexes were noted. He remained confused, and said that he had two rather than three children and that there was a dog under his bed.

A transfer for further neurological investigations took place on the 19th of March. Occasionally he admitted to hearing voices, e.g. telling him that his body temperature was 176 degrees. He had mild nominal aphasia, constructional and dressing apraxia and dysgraphia with perseveration. His concentration, short and long-term memory were all severely impaired but his comprehension was relatively intact. Upgaze was limited, his gait was unsteady, narrow-based and possibly apraxic, with dystonia of his toes. He had marked increased tone in all limbs, brisk deep tendon reflexes and ankle clonus. A brain MRI was degraded by movement and was reported to show minimally prominent ventricles and one or two minute signal abnormalities in the left frontal white matter. The diagnosis of CJD was raised at this time. A repeat EEG showed frequent bilateral and independent bursts of irregular slow wave activity on a slightly slow background of 7-8Hz. A further CSF examination revealed persistence of a mildly elevated protein (0.6g/L) but no oligoclonal bands or white cells.

His condition fluctuated throughout the day and at times he was paranoid and withdrawn. Sedation was increased further due to his aggression. By the beginning of April he was requiring assistance to walk and encouragement to eat and drink. When examined on the 1st of April his MMSE was 10/30. He appeared anxious and had speech that was slurred, unintelligible and perseverative. Poverty of rapid tongue movement was apparent and paratonic rigidity was noted. Power and coordination appeared normal and he was able to hold a cup and drink from it. He had very occasion myoclonic jerks of the fingers.

A right frontal brain biopsy was performed a week later and the diagnosis of vCJD confirmed. His deterioration continued, myoclonus became more prominent and he was transferred for terminal care. Toward the end of May he was unable to stand or sit. He died on the 2$^{nd}$ of June 1996. An autopsy was performed.

## Case 571

In July 1995 the family of a 34-year-old women noted a gradual change in her personality. Uncharacteristically she was 'talking back' to her mother and being 'cheeky'. 'One minute she was placid and the next minute was in a rage, yelling, over little things.' By October she was not coping with everyday chores, which she described as being 'all too much', and she was shouting at her children. She was living with her mother at this time and in November she moved to a house near-by. Previously described as a very independent person, she became very 'clingy', wanting to be with her mother and her boyfriend constantly.

She was losing weight and at times was tearful. In addition she was feeling anxious, slightly paranoid and had difficulty concentrating. She blamed her depression on the contraceptive pill. Her GP prescribed dothiepin, and although her compliance may have been poor, her sleep pattern and panic attacks were reported to have improved.

Just before Christmas the patient developed a 'flu-like illness, took to her bed and uncharacteristically took two weeks off from her work. She subsequently developed a persistent loss of energy, and was feeling exhausted after minimal work or a good night's sleep. Her mood was now swinging from times of being normal to episodes of being low and very anxious.

The patient worked as a waitress and after Christmas she started to have difficulty with her memory, making mistakes at the till and forgetting orders. In February she consulted her GP and was prescribed diazepam for 'panic attacks'. Later that month she had to stop working because of increasing forgetfulness.

In early April she moved in with her fiancé as she was unable to look after her children. She had been leaving the cooker on, was unable to cook and was refusing to let the children out of the house. She lost interest in reading books or watching TV and became unusually passive, doing whatever she was told, e.g. when asked to peel potatoes she would just keep on until told to stop, and she would eat anything that was put in front of her. At this time she was needing help to get in and out of a bath and was occasionally falling. Her GP prescribed paroxetine.

On the 11th of April she saw a psychiatrist and stated that her mood was very low and that she was weepy, but not suicidal. She described recent episodes where anxiety 'descends down' without cause most days, being particularly bad in the morning. The psychiatrist noted a history of 'tingling in the extremities, chest pain, light-headedness and blurred vision secondary to hyperventilation.' On examination she had a flattened affect and was anxious and hyperventilating. She was fully orientated and had no abnormal neurological signs. It was felt had she possibly had an underlying depressive illness and she was also referred for anxiety management and commenced on thioridazine.

However over the next week she became paranoid, thinking her ex-husband was coming to take the children away, and disorientated, thinking she was living in a caravan. On the 22nd of April the paroxetine was increased but she started to deteriorate further. At the beginning of May she was visually hallucinating, e.g. seeing children in the room, and became unsteady, with a gait that her family described as 'like the Joe 90 puppet'. She lacked motivation and would take to her bed during the day. In the middle of May the psychiatrist decreased the dose of thioridazine because she was falling.

On the 11th of June she was reviewed in the psychiatric clinic. She had physically declined, being just able to walk unaided and was requiring help with washing and dressing. Confusion and anxiety were apparent and she stated incorrectly that she had been pregnant six weeks previously but had lost the child. She was very distressed and crying at this thought, although did accept with persuasion that this was not true and apologised for her error. Incorrectly, she stated that she only had a single son and was unable to give his year of birth. She was also upset at the sudden death of her father (correctly) three weeks previously although at times she was forgetting that he had died. A diagnosis of psychotic depression was made and she was admitted. The paroxetine dose was increased and trifluoperazine started. When examined after admission she was unkempt, seemed to have a low mood but was fully orientated. Eye movements were normal, her gait was unsteady, she was generally hyperreflexic with bilateral ankle clonus and extensor plantar responses.

In view of the abnormal neurological signs she was assessed by a physician who corroborated the findings. He discussed the case with a neurologist who suggested that as she had no evidence of physical weakness, the signs were all compatible with an arousal state and that she should be observed and referred to a neurologist if her walking got worse.

Over the following four weeks she deteriorated, becoming disorientated and very emotionally labile. She had what were described as 'depressive delusions' saying that before coming into hospital her two children had been killed in the war. She also incorrectly believed that the psychiatrist was the doctor who treated her father when he died. Her family noted that she had developed a startle response. When examined at this time her short-term memory was described as very poor and in addition to the previously noted long tract signs and unsteadiness she now had incoordination of the arms and was unable to perform tandem gait. MRI and CT brain scans were

reported to be normal but an EEG performed on the 9th of July showed mild to moderated diffuse slowing. She was assessed by a neurologist following the EEG who concluded that her abnormal neurological signs and EEG could be explained by anxiety and her major tranquillisers.

She continued to deteriorate throughout July, saying increasingly less, falling, becoming incontinent and having difficulty swallowing. A SPECT scan on the 23rd of July was abnormal, showing a generalised reduction of cortical perfusion compared to a relatively normal pattern in the thalamus, basal ganglia, anterior para-medial frontal lobes and calcarine cortex. The cerebellar hemisphere and brain stem were also normal. All routine haematological, biochemical, immunological and microbiological blood tests had been unremarkable except for a transient and minimal elevation of AST and bilirubin. The psychiatrists considered that her illness probably had an organic basis and she was transferred for further neurological investigations at the end of July. She was now virtually mute and had a severe receptive deficit. Her eye movement were normal and grasp reflexes were first noted. A second EEG showed a deterioration of slow-wave activity. CSF showed a normal protein, no leucocytes but a positive 14-3-3 test.

Over the next two weeks there was a mild improvement when anti-psychotic drugs were withdrawn. She became more alert, ate and drank more, and said occasional appropriate statements. Additional EEGs were performed on the 9th and 14th of August. These showed further slowing of background rhythms with focal slow, and occasional sharper, waves also seen independently over both temple areas, mainly on the left. From mid-August she again deteriorated, becoming bedbound and then developing myoclonus, leg rigidity and akinetic mutism. She died on the 13th of September 1996. An autopsy was performed. Her maternal grandmother was said to have a five-year history of dementia without myoclonus prior to death aged 80.

## Case 582

In January 1996 a 33-year-old housewife started to develop difficulty sleeping, became agitated and suffered from poor concentration. Her appetite had diminished and she was losing weight. She was reported by her family to have an unsteady gait. Her GP referred her to a psychiatrist whom she saw at the end of the month. The psychiatrist found her to be quite tense and restless but otherwise appeared well. He considered that she was suffering from a situational reaction related to family dynamics and arranged for her to be reviewed by a colleague from the community mental health team.

Around this time her family became concerned by her odd behaviour, such as not trying to find her young daughter when she was several hours late returning home one evening. She became tearful at times and seemed low, not smiling or laughing, and appeared to have no enthusiasm. Her family described her as uncharacteristically 'clingy', and said that she wanted to be with them more than usual. She was also not looking after her children properly, neglecting her pets and was not keeping her house clean.

By the beginning of March she started having memory difficulties, e.g. being unable to recall what she'd done the previous night, losing things and forgetting to feed the children. She was described as becoming anxious and panicky at the slightest of things. Her unsteadiness was more apparent, her speech was slurred and she had a generalised tremor that stopped her from cooking. Her GP considered that she was becoming more depressed and anxious and referred her to a psychiatric day hospital. She was taking thioridazine and paroxetine at this

time. A community psychiatric nurse visited her at home a few days later, but she said everything was fine and that she did not want to attend the day hospital.

On the 10th of March she was admitted acutely to a psychiatric ward. She had been yelling at her children who had become very frightened of her. They reported that she had been hitting herself and banging her head against a wall. However, she denied any self harm, or having thoughts of doing this and said her family were lying about her behaviour. She did, however, admit to being depressed. When examined she was agitated, extremely tearful and was pulling at her hair. She appeared depressed, said very little, made no eye contact and only occasionally smiled appropriately. There was no evidence of abnormal perception and she was fully orientated. She was diagnosed as having a severe depressive episode with marked agitation but without psychotic features and the dose of the thioridazine was increased and the paroxetine continued. A CT brain scan was normal.

When re-examined during her second week at the psychiatric hospital she was unable to give the date, recite the months of the year backward or perform serial sevens. Her memory of recent events was poor and although her registration was good she was unable to recall items after a delay. She managed to obey written commands, copy intersecting pentagons and write a sentence, although her writing was rather large, shaky and child-like. On neurological examination she was fidgety and had a staggering gait, but no other clear cerebellar signs. A formal psychology assessment showed evidence of global cognitive impairment with an overall IQ of 70, with a verbal IQ of 70 and a performance IQ of 70.

In mid-April she was discharged to live with her mother for periods of home leave. At this time the diagnosis was a stress-related depression with functional difficulties. She was requiring help to walk, wash, clean her teeth and feed. Her family reported that she didn't know the time or day and was unable to handle money. She was still confused and the family noted that at the beginning of May she asked her daughter 'Where's my daughter?'. On the 8th of May she attended hospital following a fall. When examined she was described as restless, with choreic movements, but was orientated to place and person. She returned home and her thioridazine was stopped.

On the 22nd of May she was assessed by a physiotherapist who found that she had deteriorated markedly. She was reported to be spending most of the day lying on the sofa watching TV. Conversation was minimal and she required encouragement to respond to questions. She needed help to sit up, stand and dress and she could only walk with the assistance of two people or a frame. She was able to use standard cutlery, but only with difficulty.

At the end of May she was referred for a neurological opinion. In the referral letter the psychiatrist stated that it was suspected that she was suffering from a conversion disorder. She was reviewed in the psychiatric clinic two weeks later and it was noted that her level of energy was low, she was getting tired very easily, her concentration was impaired and her appetite was still poor. Although she denied being depressed her mother said she had been crying all the time. She shook her legs continuously during the interview, but on the whole she was considered calm and did not appear depressed. Her speech was coherent and spontaneous, with no evidence of thought disorder, she was fully orientated and could accurately count one to ten in reverse order. It was considered that she may be experiencing side-effects from paroxetine and it was recommended that this be withdrawn and substituted by a different antidepressant medication.

At the end of June she was seen by the neurologist. He noted that in addition to her other symptoms she had been getting lost at home. She was able to name several simple objects and it was clear that she was dysarthric. Her intellectual function seemed very poor and she had little memory of recent events. Continuous choreic movements of the limbs and trunk were apparent. There was no evidence of abnormal eye movements or pyramidal signs. It was considered that she had an organic cerebral disease.

She was admitted for further investigation two weeks later. By now she required all assistance with washing, dressing and feeding. She had difficulty swallowing and had lost three stone in weight since the start of her illness. Although she could recognise and name her family she was disoriented in time or place. Routine blood and CSF tests were unremarkable. Brain MRI was reported to be normal and an EEG showed a diffusely slow background with no epileptiform activity.

She was discharged home after one week but because of a continued falling and progressive swallowing difficulties she was readmitted at the end of July. At this time she was virtually mute, required nasogastric feeding and was bedbound. Myoclonus was first observed early in August, and she was noted to have developed an extensor plantar response. The possibility of vCJD was raised and she was transferred back to the neurological centre for further investigation in mid-August.

Over the next three weeks she became incontinent of urine and aggressive. Her condition clearly fluctuated. At best she would make eye contact, repeats words, grunt in response to questions and following verbal commands. At other times she was drowsy and was unresponsive. She resisted forced movements of limbs, had limited upgaze and dystonia. A repeat EEG showed diffused background slowing, but with possibly left sided prominence and a suggestion of some deterioration since the previous recording. EMG and nerve conduction studies were normal. A right frontal brain biopsy was performed and confirmed the diagnosis of vCJD.

A gastrostomy feeding tube was inserted and at the end of September she was discharged home. She was examined early the next month at a time when she had a chest infection. Only minimal and non-purposeful spontaneous movements was seen. She was unresponsive to command, did not look around the room or at people and was mute. Her head was held in a twisted posture. Generalised spontaneous myoclonus was observed and she did not respond to visual menace. A pout reflex was present but other primitive responses were not. She was generally hyperreflexic with a brisk jaw jerk but no other clear pyramidal signs.

When reassessed in February 1997 the following year she could move her limbs and occasionally grasp at things and push people away. She was still mute. Her deterioration thereafter continued and she died on New Year's Eve 1997. An autopsy was not performed.

Eight of the 14 cases were female. The mean age at onset was 29 years (range 16–48 years). The median duration of illness was long compared with sporadic CJD[70] (15.5 and 4·5 months respectively). The mean length of the clinical course was 17.5 months and the oldest seven patients had the same mean duration as the seven youngest. Figures 27 and 28 show a comparison between the survival curves and age-specific incidence of sporadic and variant CJD respectively.

Two striking early features were psychiatric symptoms and sensory disturbance which are both unusual in sporadic CJD.

## Psychiatric features

All but one patient (case 433) saw a psychiatrist during their illness, six as the initial referral (see Table 25). All cases had psychiatric symptoms early in their illness, and in nine the presenting symptoms were psychiatric (see Tables 25-26). Most patients had depression, personality change, or withdrawal but one had depression and anxiety with florid paranoid delusions (case 467). Nine cases suffered from insomnia, usually at an early stage of their illness, but in none was this a severe and persistent feature. Most patients had excessive daytime sleepiness early in their illness, often before starting any psychiatric medication. Early weight loss, usually with anorexia, was experienced by all but one of the cases who had depression (see Tables 26 and 27). In all cases, psychiatric symptoms persisted until they were obscured by dementia.

Thirteen cases were seen by a psychiatrist, a median of 6·5 (range 0·5–25) months after onset of illness. Most cases were diagnosed as having depression or depression secondary to organic disease. In seven cases, symptoms of depression appeared when there was normal cognitive function, and in one case there was no evidence of organic disease nine months after the onset of depression. Suicidal ideation was described on a single occasion only in two cases. In seven cases there was evidence of organic cognitive impairment - disorientation or mild memory impairment - within six months of illness onset.

Drug treatment was used in most cases (Table 25) with little benefit, although three patients had a transient improvement, and one (case 467) appeared to achieve almost complete, if short-lived, recovery. Some fluctuation was noted in the degree of cognitive or neurological dysfunction in ten cases, with periods of minor deterioration or improvement ranging from hours to weeks, often, but not exclusively, in the setting of either acute infection or altered medication.

Although psychiatric symptoms throughout the illness in the 14 cases were heterogeneous, all patients had either depression or delusions at some stage of their illness. Two patients had Schneiderian first-rank symptoms: one believed he was being controlled by others (case 417) and another that thoughts were being taken away from his mind and replaced (case 517). Three others had symptoms suggestive of schizophrenia: one claimed that there were messages over the tannoy about her (case 467); another heard the devil talking to other people (case 474); and a third believed someone was trying to push her down the stairs and touch and wake her up at night (case 433).

A striking psychiatric symptom was unsustained delusions in 12 cases, including that there were snipers in the kitchen (case 499); that the patient had recently had a baby that died (case 571); that microscopic people were inside a patient's body (case 417); and that the patient had murdered someone (case 474). These beliefs usually, but not exclusively, occurred within a few months of onset of illness (median 5·5, range 0-26·5 months), around the time cognitive impairment and neurological deficits were first noted. The delusional beliefs were usually fleeting and did not seem to be held for more than hours to days except in one case where they persisted (case 467). Eight patients described visual hallucinations and five auditory hallucinations. There was a tendency for patients with delusions also to have hallucinations. The onset of hallucinations occurred, in most cases, at the same time as onset of delusions, and the development of neurological signs (usually ataxia or involuntary limb movements).

In most patients there was mild forgetfulness or unsteadiness of gait before psychiatric consultation. These early neurological symptoms influenced the pattern of referral for specialist investigation. Although six cases were initially referred to a psychiatrist and nine were admitted to a psychiatric hospital, three patients were initially referred to a neurologist and four to a physician. The remaining patient was first referred to an orthopaedic surgeon. All cases were referred for a specialist neurological opinion as the illness evolved and neurological signs developed. Seven patients saw a psychiatrist before a neurologist with a median time of about two months between consultations.

Results of cerebral imaging (four CT and four MRI scans) were available in six cases at the time of initial psychiatric assessment and none were reported to show any relevant abnormality. Only two patients had undergone EEG at this stage - one study was reported as normal and the other had non-specific slow wave abnormalities.

## Sensory disturbance

Four cases initially complained of sensory symptoms. One developed foot pain and was referred to a rheumatologist (case 466); another had foot pain then, within a fortnight, had dysaesthesia of hands and face (case 433); and two further patients initially complained of sensory changes (dysaesthesia, paraesthesia) in the legs (cases 480 and 485). Four of the other ten patients, although not initially complaining of sensory symptoms, developed these early in their illness - two had persistently cold feet (cases 497 and 517), one hemi-dysaesthesia (case 502), and another pain below both knees (case 467). Sensory symptoms persisted throughout in all cases. Another patient (case 417), without overt sensory symptoms, was found to have hyperaesthesia. Five patients underwent electromyography (EMG) or nerve conduction studies or both. These were normal in four cases and abnormal in one, in which there were minimal changes, including mild denervation in tibialis anterior and absent left peroneal F waves (case 485).

## Clinical course

Although a minority of cases suffered from forgetfulness or mild unsteadiness of gait from an early stage, clear neurological signs were not apparent for many months, median 6·25 (4–24·5) after disease onset (see Table 28). During this time the most prominent clinical features were psychiatric disturbance or sensory symptoms or both.

After the onset of overt neurological dysfunction, mainly ataxia, the illness rapidly progressed with global cognitive impairment, involuntary movements, incontinence of urine, and progressive immobility leading to increasing dependency, unresponsiveness and mutism (see Tables 27 and 28). Just before death, patients were usually akinetic mute and at least three developed cortical blindness. Mean delay from developing unsteadiness to becoming bedbound was six (2·5–12·5) months, and the median delay from becoming bedbound to death, 1·5 months (one week–27 months). Terminal stages, after the development of progressive cognitive impairment and involuntary movements, were similar to the late stages of sporadic CJD.

Three cases (417, 474 and 476) were noted to have transient seizure-like episodes during their clinical course (three, 7.5 and 10.5 months from onset). All three patients were in hospital, cognitively impaired and were taking chlorpromazine at the time of the seizures. The episodes took the form of 'absence attacks' in one case (who had been commenced on sodium valproate a month previously in view of cognitive impairment, an abnormal EEG and a past history of primary generalised epilepsy); hypertonia and unresponsiveness in another patient; and loss of consciousness, twitching and cyanosis in a third.

## Clinical signs

Initial neurological signs were cerebellar limb or gait ataxia in nine cases (see Table 27). These occurred in isolation in three, or in combination with involuntary movements, pyramidal signs, primitive reflexes, or sensory signs, in others. The remaining cases first developed either pyramidal signs (with or without dysphasia), dysarthria or involuntary movements, although unsteadiness of gait was noted within weeks even in these cases. Those with the longest delays to the development of neurological signs had a long prodrome with personality change or forgetfulness followed by sensory disturbance. Most cases developed primitive reflexes, cerebellar, and pyramidal signs; all had persistent involuntary movements, initially chorea (seven cases) or myoclonus (seven cases), and five of the seven patients who initially had chorea were later noted also to have myoclonus.

Although seven cases were not formally noted to have chorea, four of these were described as 'fidgety'. Seven patients were found to have upgaze paresis, an uncommon feature of CJD, after the development of other neurological signs.

## Investigations

### EEG

Each case had several EEGs (two to five). The characteristic periodic EEG pattern of sporadic CJD was not seen, even though four patients had recordings in the final month of illness (see Figure 29). Initial tracings were normal in four cases and in three of these, subsequent recordings were also normal (see Table 28). These three patients had a normal EEG even though they had cognitive impairment, cerebellar signs and involuntary movements. Abnormal recordings were noted in 12 patients, and all showed slow-wave activity which deteriorated as the illness progressed. The majority of cases thought to have a functional psychiatric illness developed an abnormal EEG within three months of their psychiatric diagnosis. However, one patient had a normal recording 7·5 months after the diagnosis of a schizophreniform psychosis.

### CSF analysis

All patients had a sample of CSF taken. No leucocyte response was seen. Four patients had slightly raised CSF protein (0·53–0·94 g/L). Results of CSF electrophoresis is available for 11 cases and in none were oligoclonal bands detected. Five cases had CSF analysed for the presence of the 14-3-3 protein - two tested positive and three negative (Table 29). One of the positive samples was taken late in the clinical course, six weeks after brain biopsy. The other was taken from a patient six weeks prior to death, before becoming bedbound and developing involuntary movements. Samples from six suspect cases of vCJD, subsequently judged not to have CJD, were negative.

### Imaging

Ten cases had CT brain scans (Table 29); eight patients had normal scans (Figure 30A) and two had non-specific abnormalities; dural calcification in one case and a slightly enlarged lateral ventricle and cerebellar interhemispheric cistern in another. Cranial MRI was reported to show no abnormalities in eight cases (see Figure 30B), three of whom had normal repeat studies. Four patients were reported to have mild generalised atrophy, and one as having slightly prominent ventricles with one or two small areas of high signal in the left frontal white matter. Two others were reported to have posterior thalamic high signal on T2-weighted (and one case also PD-weighted) images (see Figure 30C). Abnormal areas of cerebral perfusion were detected in both patients (cases 480 and 571) who had SPECT studies (Figure 31). One patient (case 474) had PET which was normal (Figure 32).

### Genetic analysis

Sequencing of the PrP gene open reading frame identified no mutation in any of the 14 cases. All had the MM genotype at codon 129.

### Blood and other tests

The results of haematological, biochemical, immunological, genetic and microbiological testing is presented in Tables 30-34. The results of other miscellaneous tests are given in Table 34.

**Figure 27: Survival curves for sporadic and variant CJD**

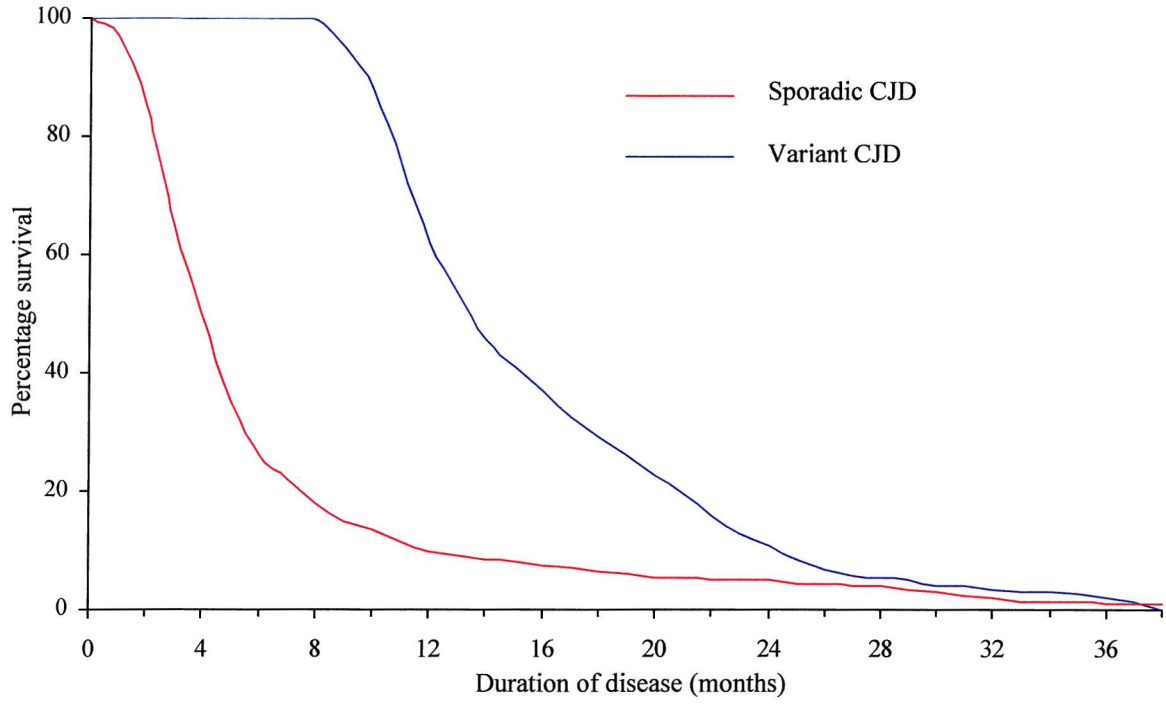



**Figure 28: Age-specific incidence of death from sporadic and variant CJD (1995-6) in the UK**

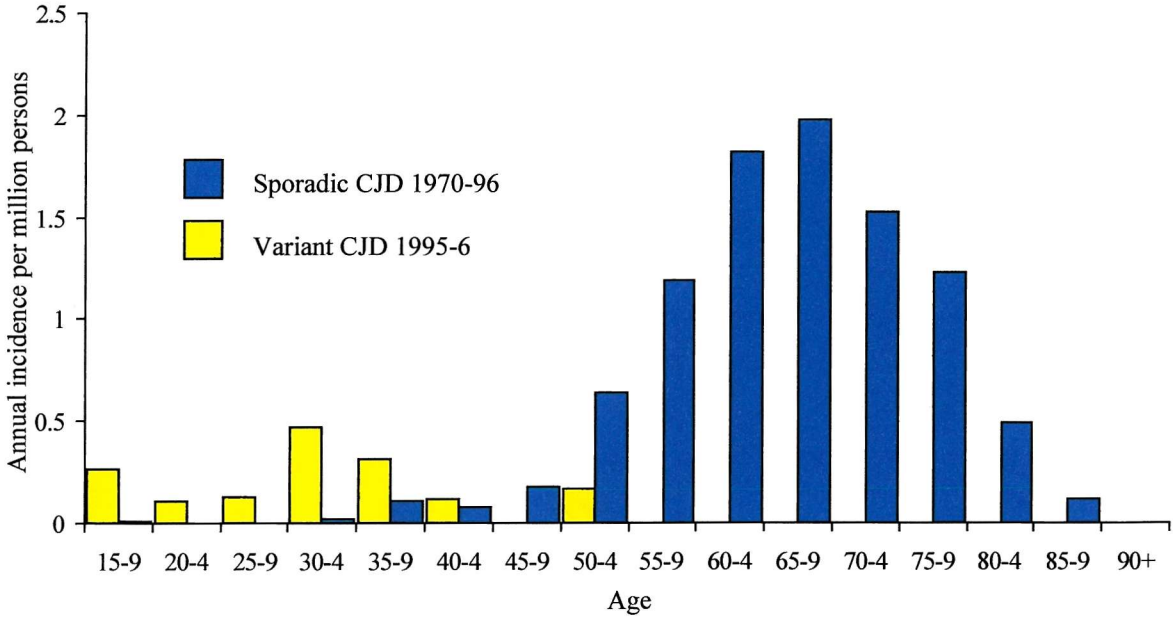
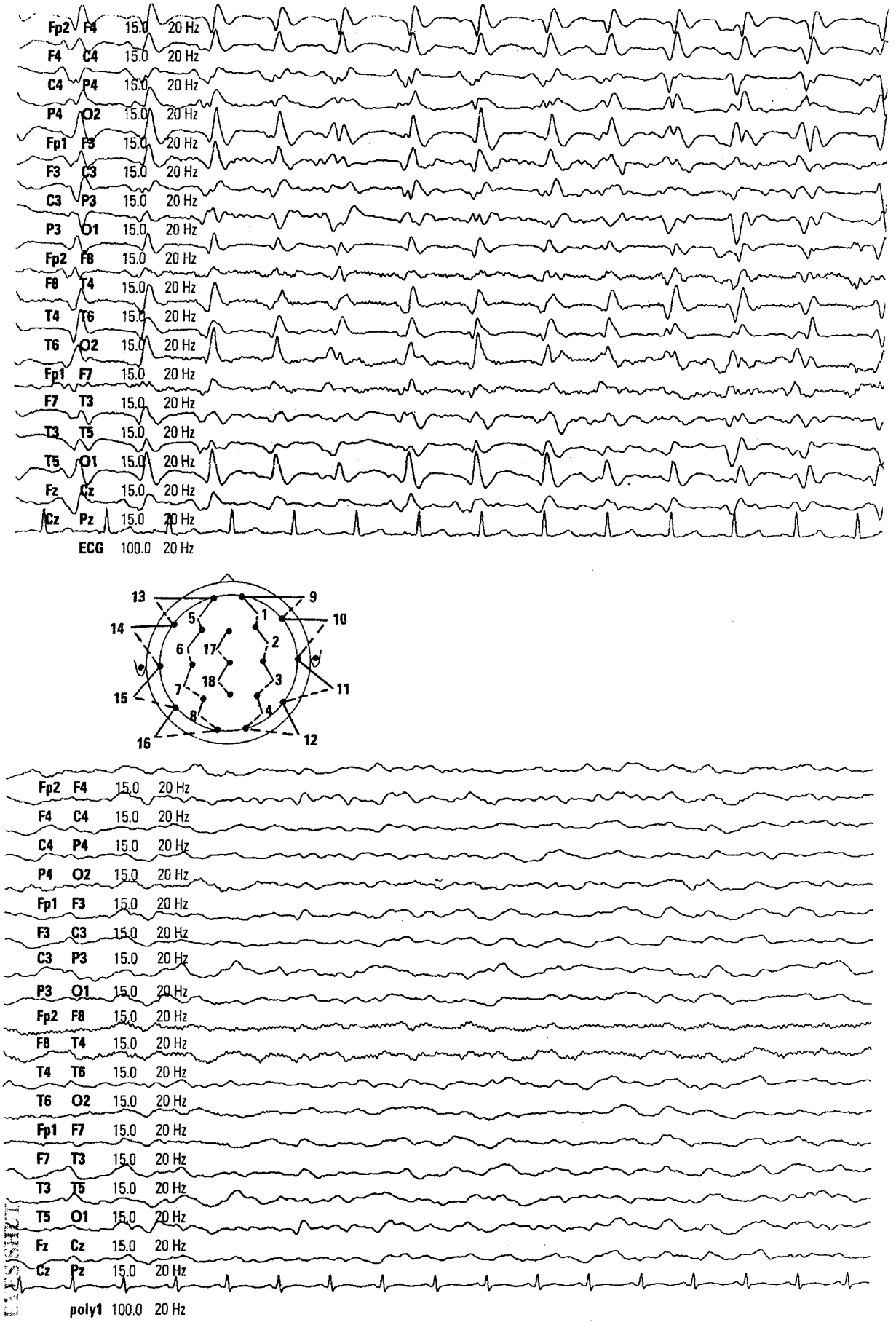
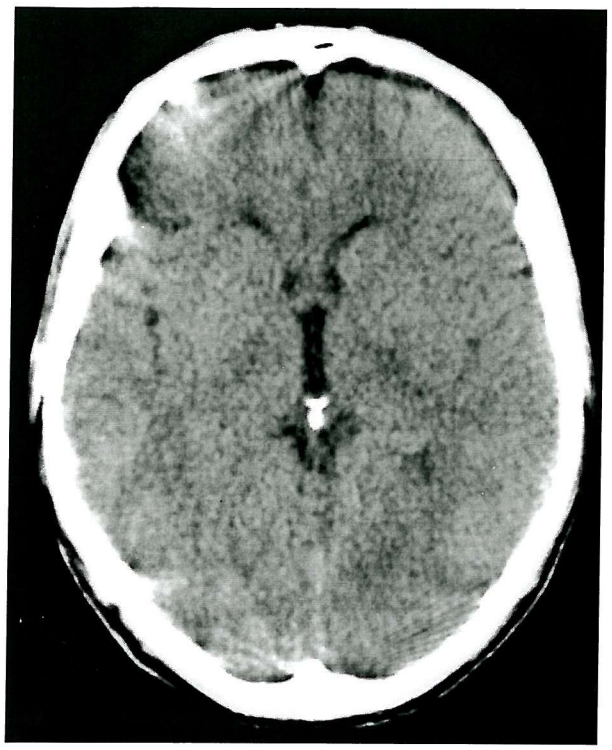


**Figure 29: EEG in sporadic and variant CJD**

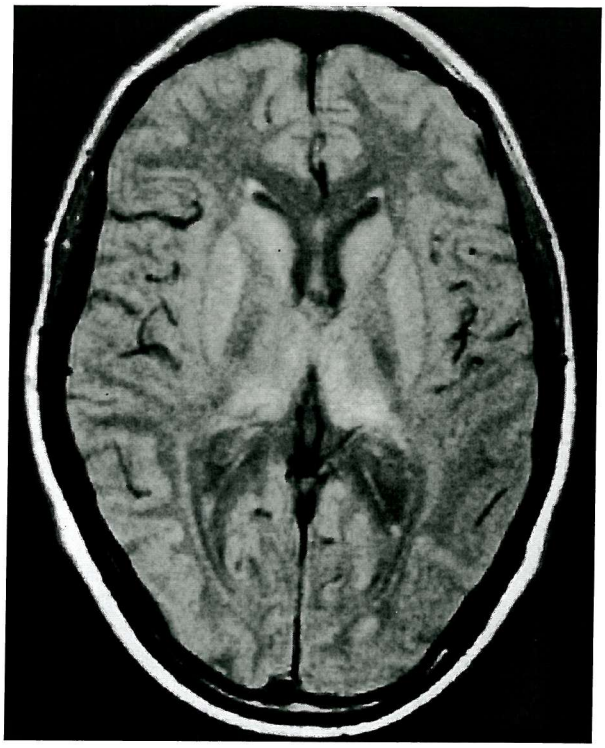



Top - Typical periodic EEG in sporadic CJD. Bottom - Non-specific slow-wave changes in vCJD

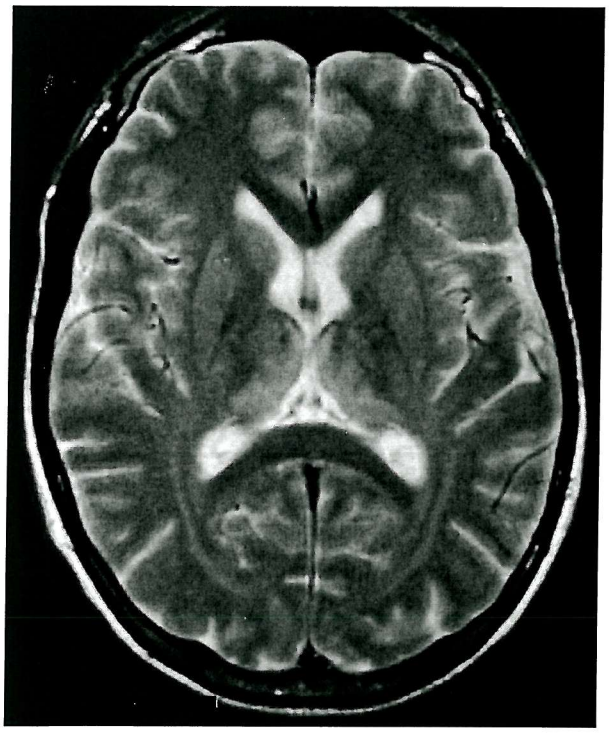
**Figure 30: Cerebral imaging in vCJD**

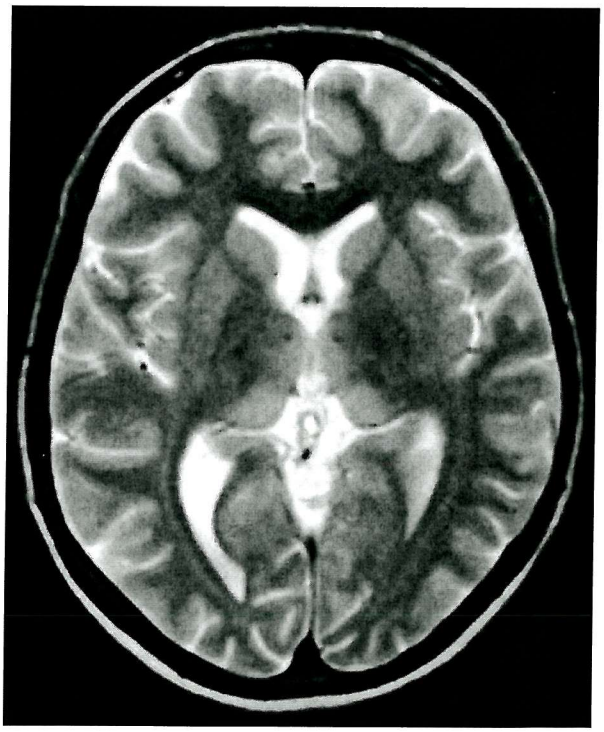


A. Normal axial CT in case 466



B. PD-weighted axial MRI reported as normal in case 502. In retrospect the signal from the caudate nuclei and the posterior and medial thalami is increased





C. T2-weighted axial MRI reported as showing posterior thalamic high signal in cases 474 (left) and 499 (right)

**Figure 31: Axial SPECT scanning in a patient with vCJD (A) and a control (B)**

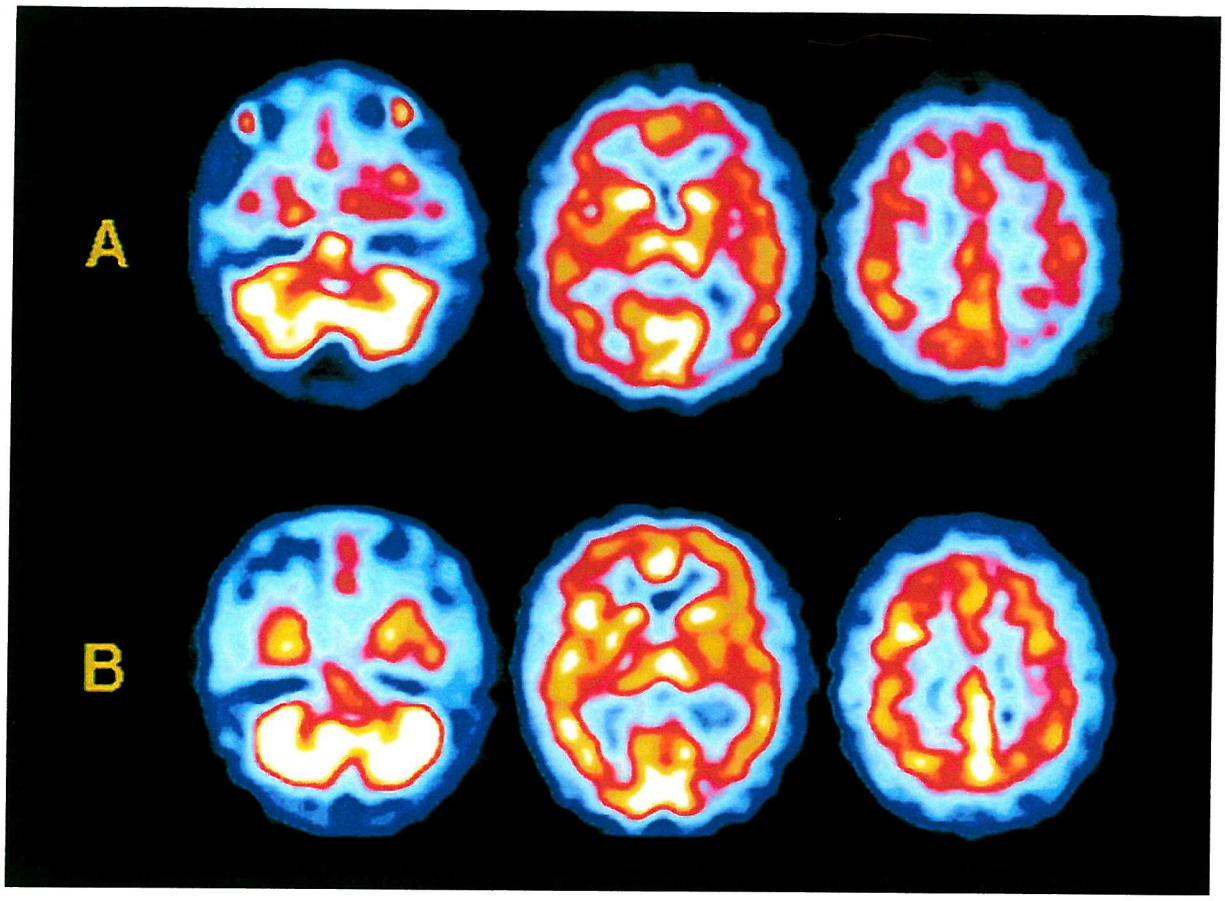



**Figure 32: Normal axial PET scan in a patient with vCJD**

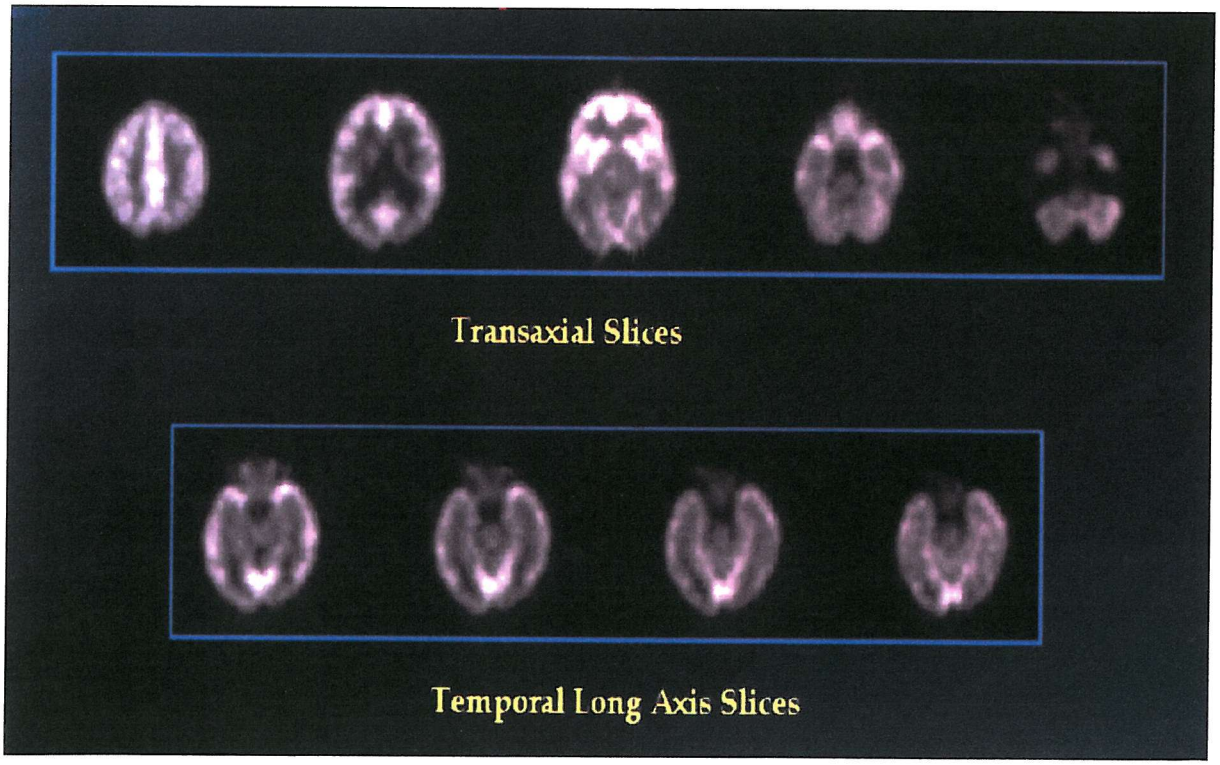


**Table 25: Presenting and psychiatric features**

| Case No. | Initial psychiatric symptoms | Presenting features* | Sensory symptoms | Initially assessed by | Psychiatric diagnosis | Psychiatric medication |
|---|---|---|---|---|---|---|
| **417** | Withdrawn | - | No | Psychiatrist | Major depressive illness | Sertraline, chlorpromazine, dothiepin, sulpiride, zuclopenthixol |
| **433** | Believed someone was trying to touch her at night | Dysaesthesia | Yes | Neurologist | Nil | Nil |
| **466** | Difficulty concentrating, anxious & agitated | Foot pain | Yes | Rheumatologist | Organic rather than functional† | Nil |
| **467** | Depressed, anxious, agitated & paranoid delusions | - | Yes | Psychiatrist | Paranoid illness with possible first rank symptoms | Trifluoperazine, diazepam |
| **474** | Emotional lability | Forgetful | No | Physician | Thought that amnesia may well be hysterical rather than organic† | Chlorpromazine, diazepam, risperidone, haloperidol, moclobemide |
| **476** | Aggression & apathy | - | No | Psychiatrist | Severe agitated depression | Amitriptyline, thioridazine, chlorpromazine, diazepam |
| **480** | Withdrawn with intermittent low mood | Paraesthesia | Yes | Neurologist | Neuro-degenerative disorder† | Amitriptyline |
| **485** | Depression | Dysaesthesia | Yes | Physician | Depressive illness with significant anxiety symptoms† | Fluvoxamine, fluoxetine, lofepramine |
| **497** | Recklessness & mild aggression | - | Yes | Physician | Both organic and functional symptoms - thought to be developing psychosis† | Diazepam |
| **499** | Depressed, quiet & withdrawn | - | No | Neurologist | Major depressive illness | Lofepramine, clomipramine, fluoxetine |
| **502** | Withdrawn | - | Yes | Orthopaedic surgeon | No evidence of depression or any other psychiatric disorder | Nil |
| **517** | Withdrawn | - | Yes | Psychiatrist | Schizophreniform psychosis† | Sulpiride, haloperidol, lorazepam , chlorpromazine |
| **571** | Emotionally lability | - | No | Psychiatrist | Anxiety, hyperventilation - possible underlying depression | Dothiepin, diazepam, paroxetine, trifluoperazine, thioridazine |
| **582** | Agitation, poor concentration, & insomnia | - | No | Psychiatrist | Severe depression without psychotic features. Marked agitation | Paroxetine, thioridazine, lorazepam |

*If not psychiatric †Possibility of organic cause raised.

**Table 26: Psychiatric features during clinical course**

| Case number | Psychiatric admission | Suicidal ideation | Emotional lability | Anxiety | Apathetic/ withdrawn | Aggression | Insomnia | Depression | Delusions | 1st rank symptoms† | Auditory hallucinations | Visual hallucinations | Fluctuating course |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 417 | + | - | - | + | + | + | + | + | + | + | - | + | + |
| 433 | - | - | + | + | + | - | + | - | + | - | - | - | - |
| 466 | - | - | + | + | + | + | + | - | + | - | - | - | - |
| 467 | + | - | + | + | + | + | - | + | + | - | + | - | + |
| 474 | + | + | + | + | + | + | - | - | + | - | + | + | + |
| 476 | - | - | - | + | + | + | - | + | + | - | + | + | + |
| 480 | - | - | + | + | + | - | + | + | - | - | - | - | + |
| 485 | + | - | - | + | + | + | + | + | + | - | + | + | + |
| 497 | + | - | + | + | + | + | - | - | + | - | - | + | - |
| 499 | + | + | + | + | + | - | + | + | + | - | - | + | + |
| 502 | - | - | - | - | + | + | + | + | + | - | - | - | - |
| 517 | + | - | - | - | + | + | + | - | + | + | + | + | + |
| 571 | + | - | + | + | - | + | - | + | + | - | - | + | + |
| 582 | + | - | + | + | + | + | + | + | - | - | - | - | + |
| **Total** | **9** | **2** | **9** | **12** | **13** | **11** | **9** | **9** | **12** | **2** | **5** | **8** | **10** |

**Table 27: Symptoms and neurological signs noted during clinical course**

| Case number | First neuro sign* | Early Weight loss | Seizures | Early tremor | Myoclonus | Chorea | Dystonia | Pyramidal signs | Cerebellar signs | Rigidity | Primitive reflexes | Upgaze paresis | Akinetic mutism | Dysphagia | Cortical blindness |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **417** | Cer+Pyr | + | + | - | + | -† | - | + | + | + | - | + | + | - | - |
| **433** | Cer+Inv | - | - | + | + | + | + | + | + | + | + | - | + | + | - |
| **466** | Pyr+Dysp | - | - | - | + | -† | - | + | + | + | + | - | + | - | - |
| **467** | Cer | + | - | - | - | + | - | + | + | + | - | - | - | + | + |
| **474** | Pyr | - | + | - | + | - | + | + | - | + | + | + | + | + | + |
| **476** | Cer+Inv | - | + | - | + | -† | - | + | + | + | + | - | + | - | - |
| **480** | Cer+Sen | + | - | - | + | + | + | + | + | + | + | - | + | + | - |
| **485** | Cer | + | - | - | + | -† | - | + | + | + | + | + | + | - | - |
| **497** | Cer+Prim | + | - | - | + | + | - | + | + | - | + | + | + | + | - |
| **499** | Cer | + | - | + | - | + | - | + | + | - | + | + | + | + | - |
| **502** | Cer+Inv | + | - | + | + | - | - | - | + | + | - | - | + | - | - |
| **517** | Dysa | + | - | - | + | + | + | + | - | + | + | + | - | - | - |
| **571** | Pyr | + | - | - | + | - | - | + | + | + | + | - | + | + | - |
| **582** | Inv | + | - | + | + | + | + | + | - | + | + | + | - | + | + |
| **Total** | | **11** | **3** | **4** | **12** | **7** | **5** | **13** | **11** | **12** | **11** | **7** | **11** | **8** | **3** |

*Cer, cerebellar; Pyr, pyramidal; Inv, involuntary movements; Dysp, dysphasia; Dysa, dysarthria, Sen, sensory; Prim, primitive reflexes.

† But described as 'fidgety'

**Table 28: Months to clinical milestones of vCJD**

| Case Number | Forgetful | Delusions | Unsteadiness | Neuro signs | Psychiatric assessment | Neurological assessment | Objective cognitive impairment | Involuntary movements† | Urinary incontinence | Mute | Bedbound | Abnormal EEG | Normal EEG | Duration |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **417** | 5 | 5.5 | 6 | 6.5 | 5.5 | 7.5 | 5.5 | $8^{M}$ | 8 | 10 | 11 | 6 | - | 11 |
| **433** | 5.5 | 0 | 4.5 | 5.5 | - | 5.5 | 5.5 | $5.5^{C}$ | 17 | 13 | 17 | - | 5.5 & 8.5 | 38 |
| **466** | 4 | 5 | 4 | 6 | 6.5 | 5.5 | 5.5 | $8.5^{M}$ | 8 | 95 | 8.5 | 6 | - | 10 |
| **467** | 6.5 | 0 | 6.5 | 8.5 | 1 | 9 | 8.5 | $9^{C}$ | 11 | 15 | 16 | 9.5 | 8.5 | 18 |
| **474** | 0 | 2.5 | 2.5 | 4.5 | 1.5 | 2.5 | 2.5 | $7^{M}$ | 4.5 | 6 | 5 | 1.5 | - | 11 |
| **476** | 1 | 6.5 | 5 | 9 | 8 | 10 | 8 | $9^{M}$ | 9.5 | 10.5 | 10 | 10 | - | 11 |
| **480** | 9 | - | 10 | 3.5 | 13 | 3.5 | 9 | $11^{C}$ | 11 | 20 | 20 | - | 11.5 & 14.5 | 22.5 |
| **485** | 5 | 13 | 11 | 11 | 11.5 | 11 | 15.5 | $16.5^{M}$ | 16 | 17 | 17 | 16 | - | 17.5 |
| **497** | 1 | 3.5 | 4.5 | 5.5 | 4 | 5.5 | 3.5 | $5.5^{C}$ | 7 | 8 | 8 | 5 | - | 9.5 |
| **499** | 3.5 | 4.5 | 3.5 | 4 | 4.5 | 4 | 4 | $5^{C}$ | 4 | 7.5 | 11 | 12.5 | 4.5 & 6.5 | 13 |
| **502** | 0 | 26.5 | 23.5 | 24.5 | 25 | 15.5 | 24.5 | $23^{M}$ | 27.5 | 28.5 | 28.5 | 25 | - | 28.5 |
| **517** | 10.5 | 14 | 12 | 14 | 14 | 14 | 14 | $14^{C}$ | 12 | 17 | 16.5 | 14 | - | 17 |
| **571** | 5.5 | 9 | 9.5 | 11 | 9 | 12 | 11 | $13.5^{M}$ | 12 | 13.5 | 13 | 12 | - | 14 |
| **582** | 1 | - | 0 | 4 | 1 | 6 | 2.5 | $4^{C}$ | 7.5 | 8.5 | 7 | 6.5 | - | 23.5 |
| **Median** | **4.5** | **5.25** | **5.5** | **6.25** | **6.5** | **6.75** | **6.75** | **8.75** | **10.25** | **11.75** | **12** | **9.75** | | **15.5** |

† First abnormal movements , C = chorea, M = myoclonus

**Table 29: Other investigations**

| Case number | CT | MRI* | Other imaging | NCS | 14-3-3 protein | Codon 129 |
|---|---|---|---|---|---|---|
| **417** | Normal | N | - | Normal | - | MM |
| **433** | Normal | N [1,2,3] | - | Normal | - | MM |
| **466** | Normal | N | - | - | - | MM |
| **467** | Normal | A | - | - | - | MM |
| **474** | Normal | N[1], A [2], A & ↑T [3] | PET normal | - | neg† | MM |
| **476** | - | N [1,2] | - | - | - | MM |
| **480** | - | N[1], A[2] | SPECT abnormal | Normal | - | MM |
| **485** | - | N [1,2] | - | Abnormal | - | MM |
| **497** | Slightly enlarged lateral ventricle | A | - | - | POS | MM |
| **499** | Dural calcification | ↑T | - | - | - | MM |
| **502** | - | N | - | - | - | MM |
| **517** | Normal | N‡ | - | - | neg† | MM |
| **571** | Normal | N | SPECT abnormal | - | POS | MM |
| **582** | Normal | N | - | Normal | neg† | MM |

* N - normal; A - atrophy; ↑T - thalamic high signal; 1, 2, 3 - 1st, 2nd, 3rd scan

† Suboptimal sample

‡ But had minor signal abnormalities in frontal white matter and minimally prominent ventricles

NCS, nerve conduction studies.

**Table 30: Investigations: routine haematology and biochemistry**

| Test | Number | Comments |
|---|---|---|
| **Haematology** | | |
| Full blood count | 14 | All had normal counts at least once. Eight had minor, usually transient, abnormalities, e.g. increased WCC with infection, post operative anaemia |
| Blood film | 8 | Five normal, one changes of thalassaemia trait, one reactive lymphocytes, one polychromasia and on a single occasion some atypical lymphocytes |
| ESR | 13 | 11 cases values always ≤15. One case range 1-38 and another 42-94 (but pregnant and infection) |
| Viscosity | 2 | Both normal |
| Clotting | 7 | All normal |
| Bone marrow | 4 | Two normal, one active marrow probably reactive (pregnant and infection), one changes of thalassaemia trait |
| **Blood biochemistry** | | |
| Urea and electrolytes | 14 | All had normal values at least once. Six cases showed changes that were minor or transient or both. |
| Osmolality | 1 | Slightly low serum value with sodium of 127 mmol/l and normal urine osmolality |
| Glucose | 13 | All normal, but three cases with had values 6.3-9.5 mmol/l at least once |
| Liver function tests | 14 | All had normal values at least once, seven cases showed abnormalities that were minor or transient or both. |
| Thyroid function tests | 14 | All normal except one patient with thyrotoxicosis |
| Calcium | 11 | All normal |
| Phosphate | 10 | All normal except one case with a transiently low value |
| CPK | 8 | All had normal values at least once. Five cases showed changes that were minor or transient or both |
| Cholesterol | 4 | All in range 3.3–4.0 mmol/l |
| Triglycerides | 4 | All in range 0.5-1.1 mmol/l |
| Ammonia | 4 | All normal except one case with a transiently raised value |
| Lactate | 8 | All normal except two cases with slightly low values |
| Pyruvate | 5 | Three normal. Two cases had slightly increased values |
| Urate | 3 | All normal |
| Androstendione | 1 | Decreased |
| Prolactin | 1 | Normal |
| Synacthen test | 1 | Normal |
| Cortisol | 2 | One normal. One case uncertain, as time of sample not stated |

**Table 31: Investigations: blood and CSF biochemistry**

| Test | Number | Comments |
|---|---|---|
| **Blood biochemistry (continued)** | | |
| Porphyrins | 2 | All normal except one case with slightly positive blood values followed by urine screening which was normal |
| Urinary porphyrins | 2 | Both normal |
| Vitamin A | 1 | Normal |
| Vitamin $B_1$ | 1 | Normal |
| Vitamin $B_{12}$ | 12 | All had normal values. One case had a raised level on repeat testing. |
| Vitamin E | 5 | All normal except one case with a slightly low value |
| Folate | 12 | All normal except two cases with raised values |
| Ferritin | 1 | Normal |
| Phytanic acid | 1 | Normal |
| ACE | 3 | All normal |
| Arsenic | 1 | Normal |
| Cadmium | 1 | Normal |
| Copper studies | 12 | All normal except one case with raised blood caeruloplasmin and copper (pregnant) and another case had a slightly low caeruloplasmin (history of weight loss) |
| Lead | 3 | All normal |
| Magnesium | 4 | All normal at least once. One very slightly low repeated value |
| Mercury | 1 | Normal |
| Zinc | 1 | Slightly low (but weight loss) |
| CRP | 8 | All normal except two cases, one had a normal repeated value |
| CEA | 1 | Normal |
| CA 125 | 1 | Normal |
| AFP | 2 | Normal |
| Ca 19-9 | 1 | Normal |
| Biotin | 1 | Results not available |
| Homocysteine | 1 | Results not available |
| Methylmalonic acid | 1 | Results not available |
| Apo A1, A2 and B | 1 | Normal |
| **CSF Biochemistry** | | |
| Pyruvate | 2 | Both normal |
| Lactate | 6 | All normal except one slightly raised |
| ACE | 2 | Normal |

**Table 32: Investigations: congenital metabolic defect screening and genetic analysis**

| Test | Number* | Comments |
|---|---|---|
| **Congenital metabolic defect screening** | | |
| α-N-acetylgalactosaminidase (Schindler's) | 1 | Normal |
| α-N-acetylneuraminidase (Sialidosis) | 1 | Normal |
| Acid sphingomyelinase (Niemann-Pick, types A and B) | 1 | Low |
| Arylsulphatase A (Metachromic leucodystrophy) | 3 | Two normal and one low |
| α-fucosidase (alpha-fucosidosis) | 2 | Both normal |
| α-galactosidase(Fabry's disease) | 1 | Normal |
| β-galactosidase (GM 1 gangliosidosis) | 2 | Both normal |
| Glucocerebrosidase (Gaucher's disease) | 2 | Both normal |
| β-glucuronidase (Sly's disease) | 2 | One normal and one slightly low |
| Hexosaminidase A (Tay Sachs) | 4 | Three normal and one slightly low |
| Hexosaminidase (A and B ) (Sandhoff's) | 4 | All normal |
| α-mannosidase (alpha-mannosidosis) | 2 | Both normal |
| β-mannosidase (Beta-mannosidosis) | 2 | Both normal |
| Nac-α-glucosaminidase (Sanfilippo B) | 1 | Normal |
| N-acetylglucosamine phosphotransferase (I cell disease) | 1 | Normal |
| N-aspartyl-β-glucosaminidase (Aspartylglycosaminuria) | 1 | Normal |
| Orotate (orotic aciduria) | 1 | Normal |
| Very long chain fatty acids | 2 | Normal |
| Urine amino acid analysis | 4 | All normal |
| Urine organic acids | 2 | Both normal |
| Fibroblast enzyme activity† | | |
| **Genetic analysis** | | |
| Huntington's disease | 5 | All negative |
| DRPLA | 4 | All negative |
| Mitochondrial screen | 1 | Negative |

* In two additional cases white cell enzymes were normal but the specific enzymes tested were not detailed.

†β-glucosidase, acid esterase, β-glucuronidase, neuraminidase, α-mannosidase, Lowry protein, B-Galactosidase, $GM^2$ type 1, arylsulphatase A and hexosaminidase A were all tested once only and were all normal. B-Galactosidase was tested in two cases - both normal. Sphingomyelinase and α-fucosidase tested in a single case and both found to be low, although result not considered significant.

**Table 33: Investigations: blood and CSF microbiology**

| Test | Number | Comments |
|---|---|---|
| **Microbiology (Blood)** | | |
| Borrelia | 2 | Negative |
| L. Pneumophilia | 1 | Negative |
| Mycoplasma | 2 | One negative. One persisting high titre predating onset of symptoms |
| Psittacosis | 1 | Negative |
| Q fever | 1 | Negative |
| Syphilis | 12 | All negative |
| Toxoplasma | 1 | Negative |
| Adenovirus | 1 | Negative |
| CMV | 3 | Two negative. One positive IgG and negative IgM |
| Coxsackie B | 2 | Negative |
| Epstein–Barr virus | 1 | IgG positive but IgM negative |
| Paul Burnell | 2 | Negative |
| Herpes simplex | 4 | Three negative. One positive with low titre |
| Herpes zoster | 3 | All negative |
| HIV | 6 | All negative. Two cases had normal CD subtyping (one HIV tested) |
| Hepatitis A | 1 | Negative |
| Hepatitis B | 3 | All negative |
| Hepatitis C | 2 | Both negative |
| Influenza A and B | 1 | Negative |
| Measles | 4 | No evidence of recent infection in any case |
| Mumps | 2 | Both negative |
| Rubella IgG antibodies | 5 | One negative and four positive |
| **Microbiology (CSF)** | | |
| Measles | 6 | All negative |
| Mumps | 1 | Negative |
| HSV | 2 | Normal |
| CMV | 1 | Normal |
| Zoster | 2 | Normal |
| Enterovirus | 1 | Normal |
| Rubella | 2 | No evidence of acute infection. In both cases serum IgG was positive |
| TPHA | 1 | Negative |
| Faecal culture | 1 | Negative |

**Table 34: Investigations: immunology, tissue biopsy and miscellaneous neurophysiology and imaging**

| Test | Number | Comments |
|---|---|---|
| **Immunology** | | |
| Auto-immune profile* | 14 | All negative, except one cases with positive thyroid antibodies and a transient positive ANA |
| Ganglioside antibodies | 1 | Negative |
| ENA | 1 | Negative |
| ANCA | 6 | All negative |
| Lupus anticoagulant | 1 | Normal |
| Purkinje cell antibodies | 3 | All negative |
| Neuronal antibodies | 4 | All had negative Hu and Ri. One also negative Yo antibodies. |
| Endomysial antibodies | 1 | Normal |
| Anti-cardiolipin antibodies | 4 | All normal |
| Electrophoresis | 7 | All normal |
| IgG, IgA and IgM | 8 | All normal except a single case with a slightly low IgA |
| C3 and C4 | 2 | Both values slightly low in one case, but normal on repeat |
| **Tissue biopsy** | | |
| Skin biopsy | 2 | One normal the other result not available |
| Muscle biopsy | 4 | One normal, one marked type 2 muscle fibre atrophy, one occasional fibre degeneration and another one suggestion of regeneration on EM. |
| Rectal biopsy | 1 | Normal |
| Duodenal biopsy | 2 | Normal |
| Liver biopsy | 1 | Normal, including copper level |
| **Neurophysiology** | | |
| Visual EPs | 1 | One normal, one marginally abnormal configuration without any delay |
| Somatosensory EPs | 1 | Abnormal bilaterally, considered probably central conduction problem |
| Thermal thresholds | 1 | Grossly diminished sensation of hot & cold, but inconsistent responses. |
| **Miscellaneous** | | |
| Endoscopy | 5 | Results available for three cases – all normal |
| Chest X-ray | 8 | All normal except one case showed changes compatible with infection |
| Abdominal ultrasound | 5 | Four normal. One other case result not available |
| ECG | 1 | Normal |
| Bone scan | 1 | Normal |

*All cases had ANA tested, in nine the auto-immune profile was normal but full details of tests performed were not stated. The following normal tests were specifically noted: rheumatoid factor (two cases), dsDNA (two cases) and thyroid antibodies in one case.

## VARIANT CJD: PATHOLOGY

### General observations

The diagnosis of CJD was established pathologically in all 14 cases. Twelve underwent necropsy and six frontal cerebral biopsy (cases 417, 433, 474, 497, 517 and 582). Biopsy was diagnostic in all but case 417. Although the tissue from this patient showed a strongly positive reaction with two of three PrP monoclonal antibodies there was no spongiform change. In contrast to the pathological variations that occur between cases of sporadic, iatrogenic and familial TSE, the neuropathology of the 14 cases was relatively stereotyped, although variation in the severity of the pathology was seen from case to case.

The pathological characteristics of vCJD have been summarised by WHO and are presented in Annex 7.[919]

### Macroscopic findings

The brain weight after fixation was within the normal range for age. Accordingly, no evidence of cerebral cortical atrophy, ventricular dilatation or white matter loss was identified, but in cases with a lengthy clinical history (>18 months) there was evidence of cerebellar atrophy (particularly involving the vermis), with a corresponding reduction in myelinated axons in the white matter.[920]

### Florid plaques

A striking abnormality was the presence of large amyloid plaques, which were most numerous in the occipital and cerebellar cortex, but were identified in all cortical areas, particularly at the bases of the sulci. These plaques were large, fibrillary and surrounded by a rim or corona of spongiform change (Figure 33A). The plaques resembled the florid plaques first described in experimental transmission of Icelandic scrapie into mice,[921] and which also occur in several other unrelated TSEs, including chronic wasting disease in white-tailed deer.[922] In vCJD the florid plaques were identified on H&E stains, and were particularly well visualised using the periodic acid-Schiff and alcian blue stains and the Gallyas silver impregnation technique.[920]

### Spongiform change

Spongiform change in the cases was variable in distribution and frequently occurred as a patchy abnormality in the cerebral cortex (most evident in the occipital and inferior frontal regions), often at the base of gyri, and in a random distribution within the cerebellar cortex. Extensive confluent spongiform change was not a prominent feature in the cerebral cortex (in comparison to cases of sporadic CJD), but was more often observed in the molecular layer of the cerebellum. Spongiform change was most conspicuous in the basal ganglia, particularly the caudate nucleus and putamen. Similar changes were present in the anterior thalamus, with focal spongiform change involving many of the nuclei, often in the absence of plaques. Spongiform change was detected in the

midbrain (particularly in the periaqueductal grey matter) and in the pontine nuclei, but was not present in either the medulla or spinal cord.[920]

## Neuronal loss and astrocytosis

Cerebral cortical neuronal loss was most evident in the primary visual cortex, with accompanying moderate astrocytosis. The neuronal populations in the hippocampus were relatively well-preserved. All layers of the cerebellar cortex suffered neuronal loss, particularly the granular cell layer. In cases with a lengthy clinical course, there was accompanying severe astrocytosis and mild to moderate cerebellar cortical atrophy. In the dorsomedial and posterior regions of the thalamus there was severe and extensive neuronal loss with marked astrocytosis (see Figure 33B), which was most apparent in a symmetrical distribution in the pulvinar. In the midbrain, severe neuronal loss and astrocytosis occurred in the superior and inferior colliculi and the periaqueductal grey matter.[920]

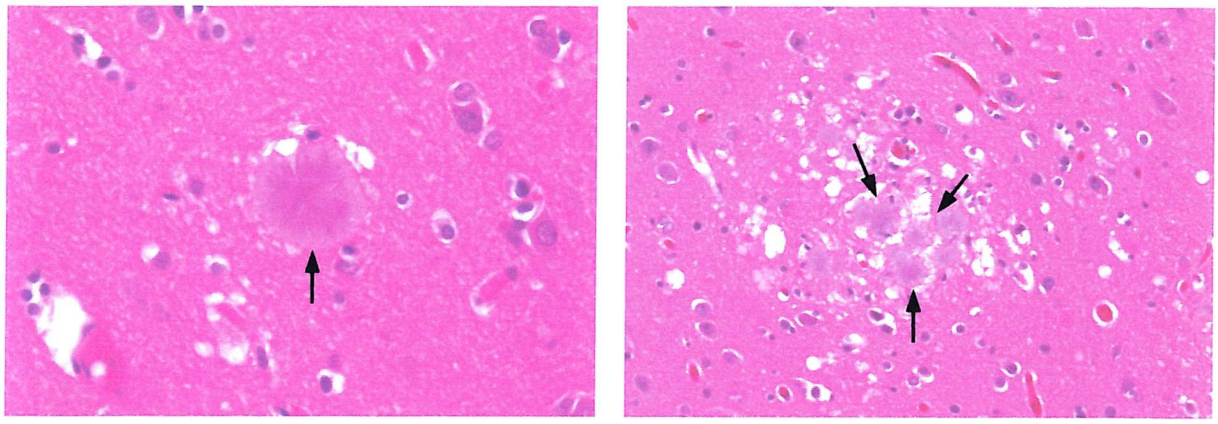
## Immunocytochemistry

A striking pathological abnormality was the massive accumulation of disease-associated PrP in a distribution unlike any other human TSEs (see Figure 33C). This not only involved the large florid plaques, but also occurred as multiple small plaques which were frequently clustered together in the neuropil ('cluster plaques'). In addition, amorphous pericellular and perivascular deposits of PrP were seen throughout the cerebellar cortex and in the cerebral cortex. In the basal ganglia and thalamus a different pattern of PrP accumulation occurred; PrP was present in neurons and around axons in the basal ganglia and a synaptic pattern of staining was seen in the thalamus, with only a few plaques identified.[897] PrP also accumulated in the brain stem and in the grey matter of the spinal cord in a perineuronal and synaptic distribution. Quantitative assessment found that PrP accumulation in the occipital cortex and the cerebellar cortex exceeded that in other cortical regions, the basal ganglia and thalamus.[897]

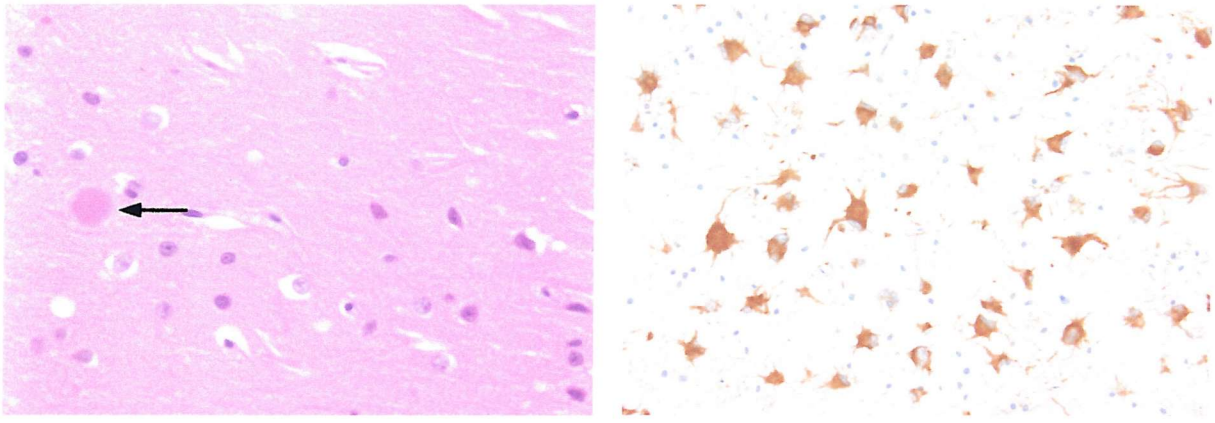
## Non-CNS tissues

The commonest immediate cause of death was bronchopneumonia. Very few significant pathological features were detected in histology of non-CNS tissues, although some cases exhibited mild steatosis in the liver, probably as a consequence of terminal inanition. Routine morphological analysis of the peripheral nerves revealed no evidence of a neuropathy. Positive staining for PrP was identified in follicular dendritic cells within germinal centres in palatine tonsillar tissue (see published report of case 571[923] and Figure 33C) and Peyer's patches in the ileum. PrP-positivity was also found in the appendix, spleen and lymph nodes from the cervical, mediastinal, para-aortic and mesenteric regions. PrP immunocytochemistry in the heart, lung, skeletal muscle, salivary gland, oesophagus, stomach, liver, gall bladder, pancreas, kidney, adrenal gland, thyroid gland, parathyroid gland, bladder, testes, placenta, pelvic organs (vagina, cervix, uterus, Fallopian tubes and ovaries) and skin was negative.[920]

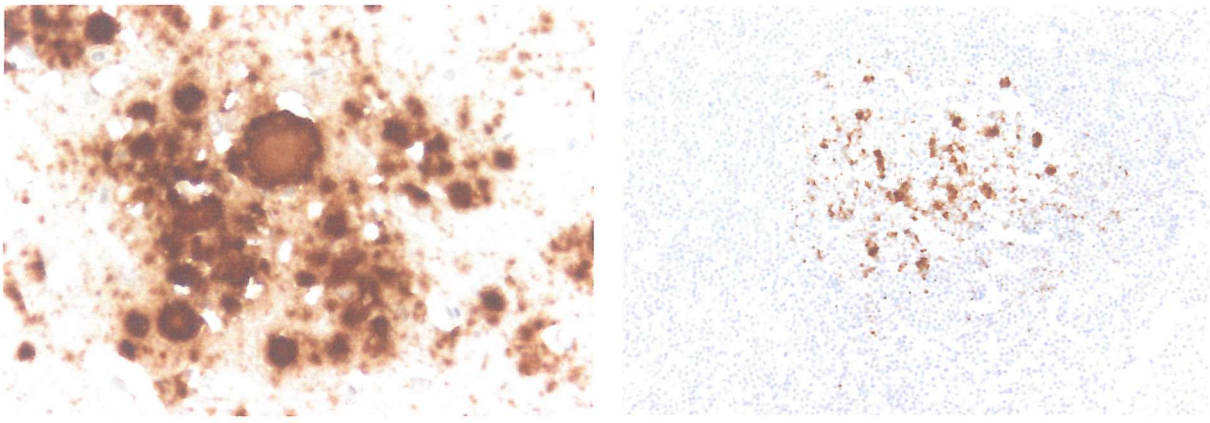
**Figure 33: Pathological features of vCJD**



A. Cerebral cortex in vCJD H&E: Left - florid plaque (arrowed - magnification x400) and right multiple florid plaques (arrowed - magnification x200)



B. In contrast to vCJD, a PrP amyloid plaque in the cerebral cortex of a patient with sporadic CJD (arrowed - left, H&E x200) does not show a halo of spongiform change. Promient thalamic astrocytosis in vCJD (right - GFAP x200)



C. PrP immunocytochemistry with KG9: Left - cerebral cortex x400 showing abundant PrP-positive plaques. Right - palantine tonsil x200 showing PrP-positive straining associated with follicular dendritic cells in germinal centres

## SUSPECT VCJD CASES WITH ALTERNATIVE FINAL DIAGNOSES

Up to the end of 1996 27 cases were identified as suspected vCJD who were later classified as non-cases. The characteristics of these patients are presented in Table 35. Table 36 shows a comparison of the features of the non-cases and the vCJD patients. The history of one patient with a clinical phenotype similar to vCJD is presented below.

### Case 527

In September 1995 the wife of a 47-year-old man noticed that he had become quiet, withdrawn and slightly depressed. His mood remained low over the next few months and he was having difficulty sleeping. From December onward he became disinhibited and developed progressive unsteadiness. He complained of pains in his limbs, which were initially intermittent but later moved to his back and become persistent. He used bath salts to try to ease the pains which in January 1996 were severe enough for him to attend A&E and to be referred to a rheumatologist.

At the beginning of February he was complaining of double vision and when assessed had an inaccurate finger-nose test. By the end of the month he was unable to walk unaided.

In March he was admitted to a neurology ward. Around this time he was first noticed to be forgetful and had developed difficulty with arithmetic. Routine investigations including cranial CT and MRI, EEG and CSF analysis were reported to be normal or unremarkable. CSF 14-3-3 test was positive.

At the end of April he returned home. His wife reported that he was visually hallucinating, became preoccupied with violent thoughts and said odd things: 'a fellow has been murdered you know... it wasn't me... he's outside you know'.

In May there was an episode when both the patient and his wife had to pull at his right hand which was trying to strangle his neck. By the end of month he was unable to recognise his daughter, he required help feeding and had become incontinent. His MMSE score was 12, he had decreased vertical gaze, paratonic rigidity, primitive reflexes and myoclonus. He later became akinetic mute and died in mid-August 1996, 11 months after the onset of symptoms. Neuropathology showed typical changes of sporadic CJD and genetic analysis did not identify any of the mutations associated with familial TSEs. He was homozygous for valine at codon 129 of the PrP gene.

## THE 'NON-VARIANT' CJD CASES

During the period August 1994 to March 1997 I assessed 92 cases of 'non-variant' CJD that were referred to the NCJDSU. Eight-eight were sporadic (76 definite and 12 probable), four were familial and none were known to be iatrogenic. Fifty (54%) of the patients were visited during life and had a physical examination performed by a neurologist from the NCJDSU (myself for all but one). The features of the 92 cases and the vCJD patients are compared in Table 37

**Table 35: The 'non-cases': suspected vCJD cases with alternative final diagnoses**

| Case no. | Age at onset | Presenting features | Duration (months) | Final diagnosis |
|---|---|---|---|---|
| **269** | 15 | Apathy, tearful, withdrawn, tired, weight loss and chorea | 54 | Sporadic CJD (codon 129 MV) |
| **398** | 29 | Memory problems | 139 | Alzheimer's disease |
| **429** | 41 | Apathetic and forgetful | 12.5 | Sporadic CJD (codon 129 VV) |
| **488** | 35 | Tremor of hands | Alive | Encephalopathy of unknown cause - improved |
| **496** | 59 | Quiet and depressed | 47 | Sporadic CJD (codon 129 MM) |
| **514** | 14 | Depression and mood swings | Alive | Got completely better |
| **515** | 39 | Memory loss | 33 | Sporadic CJD (codon 129 VV) |
| **518** | 42 | Dry mouth, odd taste, tired, legs 'wobbly', poor sleep and depressed | 19 | Autoimmune limbic and brainstem encephalitis |
| **520** | 42 | Slurred speech and decreased balanced | 11.5 | Multiple system atrophy |
| **521** | 21 | Indecisive, withdrawn and agitated after childbirth | Alive | Uncertain |
| **527** | 46 | Quiet and depressed | 11 | Sporadic CJD (codon 129 VV) |
| **531** | 25 | Odd behaviour and fugue-like state | Alive | Possible encephalitis - improved |
| **532** | 17 | Repeating words and aggressive | Alive | Encephalitis - improved |
| **534** | 43 | Numb fingers and forgetfulness | Alive | Painful neuropathy |
| **537** | 29 | Ataxia and agitation | Alive | Familial spinocerebellar ataxia |
| **542** | 58 | Aggressive and disinhibited | 15 | Possible metabolic disorder |
| **560** | 48 | Ataxia | Alive | Progressive ataxia and dementia of uncertain cause |
| **561** | 43 | Poor personal hygiene | 34 | Uncertain - no specific abnormalities at autopsy |
| **562** | 50 | Withdrawal, tearful and weight loss | 22 | Alzheimer's disease |
| **565** | 42 | Odd behaviour, refusing access to house | 9 | Cerebrovascular disease |
| **572** | 55 | Emotional lability | Alive | Uncertain - stabilised |
| **575** | 50 | Confusion, ataxia and myoclonus | Alive | Uncertain – improved to normal |
| **576** | 43 | Severe depression | Alive | Uncertain – improved to normal |
| **579** | 55 | Emotionally labile, aggressive, mania and hallucinations | Alive | Possible metabolic disorder |
| **592** | 48 | Personality change and apathy | Alive | Cerebral biopsy showed no diagnostic features |
| **594** | 11 | Tired, depressed and mood swings | Alive | Uncertain |
| **600** | 15 | Personality change and focal shaking | 4.5 | Encephalitis |

**Table 36: Clinical features of non-cases vs. variant CJD**

| Feature | Variant (n=14) | Non-cases (n=27) |
|---|---|---|
| **General** | | |
| Mean age at onset (years) | 29 | 38 |
| Median duration of illness (months) | 15.5 | 19* |
| Dementia | 100 | 81 |
| Ataxia | 100 | 67 |
| Early weight loss | 79 | 19 |
| Fluctuating course | 71 | 26 |
| Sensory disturbance | 57 | 19 |
| Dysphagia | 57 | 15 |
| Seizures | 21 | 30 |
| **Psychiatric** | | |
| Early psychiatric symptoms | 100 | 89 |
| Saw psychiatrist during illness | 93 | 48 |
| Apathy/withdrawn | 93 | 41 |
| Delusions | 86 | 22 |
| Anxiety | 86 | 19 |
| Aggression | 79 | 26 |
| Emotional lability | 64 | 44 |
| Depressed | 64 | 44 |
| Insomnia | 64 | 22 |
| Admitted to psychiatric unit | 64 | 22 |
| Visual hallucinations/misinterpretations | 57 | 30 |
| Antidepressants | 50 | 37 |
| Auditory hallucinations | 36 | 11 |
| Suicidal ideation | 14 | 7 |
| 1st rank | 14 | 4 |
| **Involuntary movements** | | |
| Myoclonus | 86 | 63 |
| Chorea or 'fidgety' | 79 | 26 |
| Dystonia | 36 | 19 |
| Early tremor | 29 | 11 |
| **Signs** | | |
| Pyramidal | 93 | 37 |
| Rigidity | 86 | 44 |
| Cerebellar | 79 | 52 |
| Primitive reflexes | 79 | 48 |
| Akinetic mutism | 79 | 30 |
| Upgaze paresis | 50 | 7 |
| Cortical blindness | 21 | 4 |

Values are percentages unless otherwise stated. *(n=13)

**Table 37: Clinical features of non-variant CJD vs. variant CJD**

| Feature | Non-variant (n= 92)* | Variant (n=14) |
|---|---|---|
| Mean age at death | 66 | 30 |
| Median duration of illness (months) | 4.5 | 15.5 |
| Saw psychiatrist as initial referral | 16% | 43% |
| Saw psychiatrist during illness | 36% | 93% |
| Admitted to psychiatric unit | 17% | 64% |
| Depressed | 25% | 64% |
| Antidepressants | 22% | 50% |
| Delusions | 24% | 86% |
| Visual hallucinations/misinterpretations | 41% | 57% |
| Auditory hallucinations | 2% | 36% |
| Sensory disturbance | 18% | 57% |
| Chorea† | 12 (+ 5%) | 50 (+ 29%) |
| Upgaze paresis | 13% | 50% |
| Duration >6 months | 33% | 100% |
| Duration >9 months | 25% | 100% |
| Duration >12 months | 20% | 57% |
| Duration >24 months | 8% | 14% |
| Cerebellar signs | 70% | 79% |
| Pyramidal signs | 53% | 93% |
| Primitive reflexes | 65% | 79% |
| Myoclonus | 97% | 86% |
| Cortical blindness | 35% | 21% |
| Akinetic mutism | 86% | 79% |
| Typical EEG | 50% | 0% |

All non-variant CJD cases were sporadic except four: case 282 (possible GSS), case 328 (96bp insert), case 431 (144bp insert) and case 509 (insert)

* Some cases had insufficient details to allow assessment of one or more of the features and were excluded from the analysis for those features

† Figures in brackets indicate cases not formally noted to have chorea, but who were described as 'fidgety'

## Residence

The place of residence of the vCJD cases at onset of symptoms is shown in Figure 34. None had ever lived outside of the UK. Only two moved a distance of more than 15 km over the period 1980 until the onset of their illness.

## Racial background

One patient was of Turkish Cypriot parentage, but was born in England. All the other cases were Caucasian and were born in the UK (one in Wales, one in Northern Ireland, three in Scotland and nine in England).

## Control patients

Paired case-control data is available for all 14 cases. The list of diagnoses of the controls is given in Table 38. Data on the controls was obtained solely from interview of a relative for three cases, solely from interview of the patient for seven cases and from interview of both the control and their relative for two cases. For these latter two patients the data used is that given by the relative. The reason for hospital admission was a neurological illness for nine of the controls.

## Occupation

Table 39 lists the lifetime occupations of the cases and controls. Case 476 case was a shop assistant in a butcher's shop in 1985 for 8-9 months and in the same shop in 1987 for eight months. As part of her job she would make burgers (moulding and placing into a pressing machine) and would clean the shop, including the slicing machines, cutting boards and refrigerators. The shop sold liver, kidneys, heart, tripe and cow heels, but not brain or sheep's eyes. Sheep heads had been sold in the shop, but not during the period that she worked there. Animals were not slaughter on the premises. She did not wear gloves at work and is not known to have eaten raw meat. No other case or control was employed as a butcher, but one control helped a butcher mince beef and deliver meat approximately once a week when aged 5-8 in 1967-70.

Case 417 worked part-time in a hardware shop on saturdays and during school holidays from 1993-5. The shop had a garden centre and part of his job involved bagging fertiliser. Case 466 studied food engineering as part of a mechanical engineering degree, and for 1-2 days in 1987 visited an abattoir and probably saw cows being slaughtered. It is not known if he had contact with any of the animals at this time. Case 467 worked for the Ministry of Agriculture, Fisheries and Food for three months in 1988. Her job was purely clerical. The father of case 480 worked in an animal feed factory up to 1989, but the patient never visited the factory. One control worked in a chicken factory in 1977. He did not kill the animals but part of his job included emptying bones onto a bucket. Another control helped out on a farm in 1960-1 when age 17-18.

Case 485 worked as a nurse in nursing homes or psychogeriatric hospitals between 1972 and 1995. It is not known if she had any contact with a patient suffering from CJD. One control worked as a porter and driver for the NHS in 1990. There was no other relevant occupational history for any of the cases or controls.

## Past medical and surgical history

No case has a history of neurosurgery or exposure to human or animal pituitary-derived hormones. Seven cases have a history of surgery (excluding childbirth) after 1980 in comparison with six controls. Between January 1985 and January 1990 four cases and three controls had procedures which required sutures. Table 40 lists the surgical histories of the cases and controls.

Six cases had suffered from a previous medical condition that led them to consult a doctor on a regular basis. Case 417 had asthma since 1989 and used salbutamol and becotide inhalers once or twice a year since this time. Case 467 had a 'nervous breakdown' in 1992 and was treated with an antidepressant (type unknown). Case 474 had epilepsy since 1979 and took sodium valproate and another anti-epileptic medication for a few years. No other case or control had a previous history of epilepsy. Case 476 had suffered from migraine since before 1980 and used coproxamol or codeine for this. Case 485 was asthmatic dating to before 1984 and used a salbutamol inhaler. She also had familial polyposis coli. Case 497 was diagnosed as having a hypertensive cardiomyopathy in 1992 and had been treated with various antihypertensive medication since. Four controls had suffered from a previous medical condition (unrelated to the illness for which they were admitted) that led them to consult a doctor on a regular basis. One had had Raynaud's syndrome since 1979 and suffered from menorrhagia in 1992. Another control had suffered from back pain between 1991-5 and took coproxamol. A further control was diagnosed as having diabetes mellitus in 1993 and since this time had taken metformin. Another control experienced recurrent tonsillitis throughout his life and had received penicillin courses about once a year.

All but one case and all controls were known or presumed by the interviewee to have had the usual childhood vaccinations. One case was reported to have had no previous vaccinations because of a fear of needles and concern regarding the risk of brain damage. Five cases and five controls had not had any vaccination since 1986.

Tables 41 and 42 list the lifetime medication and vaccination histories respectively of the cases and controls. Case 517 had used 'bodybuilding supplements' when aged 18-19 in 1984-5 that had been bought from a health food shop. He had not injected any medication.

One case probably received blood following a road traffic accident in 1977 and two had been blood donors. Six cases and four controls had had minor head injuries. No case or control had a history of jaundice. Seven cases and three controls had a history of herpes simplex infection (cold sores). One case and three controls had had shingles. No cases but two controls were reported to have had glandular fever. No case or control had a history of polio. Two cases and one control were known to have had acupuncture. Both the cases only had acupuncture during their illness. One case and one control were reported to have been tested for glaucoma.

**Table 38: Characteristics of controls**

| Case Number | Interviewee | Age at interview* | | Diagnosis |
|---|---|---|---|---|
| | | Case | Control | |
| **417** | Both | 19 | 14 | Chest infection and cystic fibrosis |
| **433** | Relative | 17 | 14 | Wilson's disease post-liver transplantation |
| **466** | Both | 29 | 28 | Fractured ankle |
| **467** | Patient | 30 | 30 | Multiple sclerosis |
| **474** | Patient | 29 | 28 | Multiple sclerosis |
| **476** | Patient | 29 | 27 | Possible multiple sclerosis |
| **480** | Patient | 29 | 31 | Pneumothorax |
| **485** | Patient | 41 | 40 | Possible epilepsy |
| **497** | Patient | 31 | 34 | Cerebral arterio-venous malformation |
| **499** | Patient | 20 | 18 | Cystic fibrosis |
| **502** | Relative | 50 | 53 | Possible post-infectious transverse myelitis |
| **517** | Patient | 29 | 31 | Chronic inflammatory demyelinating polyneuropathy |
| **571** | Patient | 35 | 30 | Possible idiopathic intracranial hypertension |
| **582** | Patient | 33 | 33 | Multiple sclerosis |

*Age at death is taken if interview conducted after death

**Table 39: Lifetime occupations of vCJD cases and controls**

| No | YOB | Cases | Dates | Control | Dates |
|---|---|---|---|---|---|
| **417** | 1976 | Schoolboy | | Schoolboy | |
| | | Hardware shop assistant | 1993-5 | | |
| **433** | 1978 | Schoolgirl | | Schoolgirl | |
| **466** | 1965 | University student | 1983-8 | Electronics engineer | 1983-95 |
| | | Power station engineer | 1988-91 | | |
| | | Mechanical engineer | 1991-91/2 | | |
| | | Manager in estates office | 1991/2-5 | | |
| **467** | 1965 | Technical college student | 1983-5 | Catering assistant | 1982-7 |
| | | Teacher training college | 1985-7 | | |
| | | Clerical assistant | 1987-9, '94 | | |
| **474** | 1966 | Weekend job at stables | 1976-84 | Legal secretary | 1985-94 |
| | | University student* | 1984-7 | | |
| | | Solicitor | 1990-5 | | |
| **476** | 1966 | Shop assistant | 1982-3 | Dish washer | 1982-84 |
| | | Butcher-shop assistant | 1985 and '87 | Yeast packer | 1984 |
| | | | | Office worker/ shop assistant | 1984-95 |
| **480** | 1966 | Civil servant | 1984- 94 | Office work | 1981-2 |
| | | | | Waitress | 1982 |
| | | | | Fruit shop assistant | 1983-6 |
| | | | | Cleaner | 1986-92† |
| **485** | 1954 | Electrical factory worker | 1970-1 | Hairdresser | 1970-5 |
| | | Nursing | 1971-2, '84-7 | Dancer, barmaid, magician's assistant | 1976-89 |
| | | Nursing | 1972–95† | | |
| | | Laundry worker | 1979-82 | Hairdresser | 1990-96 |
| **497** | 1964 | Electrical engineer | 1980-95 | Chicken factory worker | 1977 |
| | | | | Miner | 1978-83, '85-9 |
| | | | | Electrical factory worker | 1984 |
| | | | | NHS porter/driver | 1990 |
| | | | | Tyre factory worker | 1991-6 |
| | | | | Fireman | 1994-6 |
| **499** | 1975 | University Student | 1993-5 | Hairdresser's assistant | 1995-6 |
| **502** | 1945 | British Telecom engineer | 1963-95 | Toolmaker | 1960-96 |
| **517** | 1966 | Forester | 1985-96 | Mechanical engineer | 1982-88 |
| | | | | Car quality inspector | 1989-96 |
| **571** | 1961 | Hospital cleaner | 1977 (One month) | Fairground ride worker | 'All life' |
| | | Clerical | 1977-85 | Clothes shop assistant | 1987- 91† |
| | | Waitress/barmaid | 1989-96 | | |
| **582** | 1963 | Cook/waitress | 1979 | Clerical worker | 1981-96 |

YOB, year of birth of case

* Clerical job during University holidays. Unclear what occupation 1987-90 but probably training in law

† Intermittently

**Table 40: Past surgical history of vCJD cases and controls**

| No. | Cases | Dates | Controls | Dates |
|---|---|---|---|---|
| **417** | Soluble sutures for cut lip | 1982 | Skin cyst resected | 1995 |
| | Toes straightened | 1984 | | |
| | Corn removed | 1989 | | |
| **433** | Tonsillectomy | 1991 | No surgery | |
| **466** | No surgery | | Appendectomy | 1979 |
| **467** | Dental extractions | 'Not recent' | Childbirth | 1990 |
| | | | Sterilisation | 1994 |
| **474** | Tonsillectomy | 1974 | No surgery | |
| **476** | Childbirth | 1986 & '90 | Nodule removed from ankle | 1981 |
| | Miscarriage and D&C | 1989 | Laparoscopy | 1994 |
| | Possible miscarriage | 1993 | | |
| **480** | Childbirth | 1991 | Squint operation | 1972 |
| | | | Childbirth no sutures (all?) | 1987, '90 & '91 |
| **485** | Hand operation | 1971 | Tonsillectomy | 1961 |
| | Caesarean section | 1974 | | |
| | Laparoscopy | 1986/7 | | |
| | Colonoscopy | 1992 & '94 | | |
| **497** | No surgery | | FB resected from thumb | 1976 |
| | | | Vasectomy | 1986 |
| | | | Sutures in A&E | 1995 |
| **499** | Nasal FB removed | 1979 | Sutures to hand | 1990 |
| | Teeth extracted | 1985 | Sutures to arm | 1991/2 |
| | Sutures to eyebrow | 1993 | | |
| **502** | No surgery | | Tonsillectomy | 1948 |
| | | | Cholecystectomy | 1973 |
| **517** | Dental extraction | 1978/9 | Sutures to cut hand (+ op. '71) | 1970 & '71 |
| | | | Sutures to laceration on foot | 1985 |
| **571** | Foot sutures | 1977 | Sutures to cut on ankle | 1984 |
| | Tonsillectomy | 1979 | Appendectomy | 1986 |
| | Childbirth* | 1987 & '89 | Dental extraction | 1986 |
| | Bunion operation | 1992 | Childbirth | 1994 |
| **582** | Caesarean sections | 1982 & '88 | Dental extractions | 1972 & '73 |
| | Miscarriage | 1985 | | |
| | Salpingectomy (ectopic) | 1986 | | |
| | Laparoscopic sterilisation | 1988 | | |
| | Laparoscopy | 1992 | | |
| | Hysterectomy | 1994 | | |

FB – foreign body

* Sutures '87, unknown'89

**Table 41: Oral medication histories of vCJD cases and controls**

| Case | Cases | Dates | Controls | Dates |
|---|---|---|---|---|
| **417** | Ventolin & becotide inhalers | 1989-95 | Nil | |
| | Alpha Keri bath-oil | 1981-95 | | |
| **433** | Ferrous sulphate | 1991-5 | Oral contraceptive pill | 1994-95 |
| | | | Eye drops and eye cream | 1995 |
| | | | Antibiotics for various reasons | All life |
| | | | Occasional cough mixture | |
| **466** | Nil | | Ear drops for wax | 1996 |
| | | | Occasional cough mixture, night nurse | |
| **467** | Unknown antidepressant | 1992 | Prozac | 1996 |
| | Occasional cough medication | | Oral contraceptive pill | 1986-90 |
| | and paracetamol | | Conjunctivitis – course of eye gel | 1994 |
| | | | Occasional paracetamol | |
| **474** | Epilim & other AED | 1979- few years | Occasional antibiotics | 1992 |
| | Oral contraceptive pill | 1986-94 | | |
| | Temazepam and aspirin | 1994 | | |
| | Occasional Hedex tablets | | | |
| **476** | Oral contraceptive pill | 1982 & 1994 | Penicillin | 1979-84 |
| | Coproxamol or codeine | Pre-1980-1995 | | |
| **480** | Oral contraceptive pill | 1987-90 | Coproxamol | 1992-6 |
| | | | Oral contraceptive pill | 1982 |
| | | | Eye drops ? type | 1971 |
| | | | Occasional cough medication/Lemsip | |
| **485** | Ventolin | Pre-1984 | Eye drops ? type | 1994-6 |
| | Coproxamol | 1985-95 | Occasional aspirin and cough medication | |
| | Metatone tonic | 1983-7 | | |
| | Herbal medication ?what | 1993 | | |
| | Eye dew eye drops | 'Years' -1993 | | |
| **497** | Hypertension tablets ?name | 1992-1996 | Eye drops for dry eyes | 1994 |
| | Occasional Benilyn or Venos | | Protein supplements for weight lifting | |
| **499** | Multivitamins | 1992-5 | Drug for dysentery | 1995 |
| | | | Nightol & herbal sleeping tablets | 1996 |
| **502** | Paracetamol and codeine | 1984-96 | Metformin | 1993-6 |
| | | | 'Bowel medication' | 1996 |
| | | | 'Heart tablets' | 1993-6 |
| | | | Occasional eye drops for conjunctivitis | 1993 |
| | | | Occasional neurofen for headaches | |
| **517** | Courses of antibiotics | 1975, 86 & '93 | Eye drops for a few days | ~1988 |
| | 'Bodybuilding supplements' | 1984/5 | Occasional Lemsip or paracetamol | |
| **571** | Oral contraceptive pill | 1994-6 | Penicillin courses | All life |
| | Ampicillin | 1975-9 on/off | Oral contraceptive pill | 1992-6 |
| | Occasional paracetamol | | Anadin and paracetamol | 'few years' |
| **582** | HRT | 1995 | Oral contraceptive pill | 1986-96 |
| | Occasional paracetamol or cough medication | | Oral steroids | 1996 |

**Table 42: Vaccinations and injection histories of vCJD cases and controls**

| Case | YOB | Cases | YOB | Controls |
|---|---|---|---|---|
| **417** | 1976 | Triple vaccine 1978<br>Polio in early childhood<br>BCG unknown<br>Anti-tetanus up-to-date | 1981 | All school vaccinations<br>Tetanus since 1995 |
| **433** | 1978 | All school vaccinations including rubella, pertusis & measles. No tetanus known | 1981 | All school vaccinations<br>Tetanus 1992 |
| **466** | 1965 | All childhood vaccinations.<br>1987 travel vaccination. ?Tetanus and others ?type | 1967 | All school<br>Tetanus 1992 |
| **467** | 1965 | All childhood vaccinations including BCG.<br>No injections since 1986 | 1966 | All childhood vaccinations including rubella and BCG<br>Nil past since 1986 |
| **474** | 1966 | All school vaccinations<br>1993 Travel vaccinations ?which | 1967 | Usual school<br>BCG 1979 |
| **476** | 1966 | Nil | 1968 | All school vacs<br>Tetanus 1985 and 1990 |
| **480** | 1966 | Probably childhood vaccinations<br>Painkillers during childbirth 1991 | 1965 | All childhood<br>1991-95 depot contraception |
| **485** | 1954 | All school and probably up-to-date with tetanus<br>1989-1995 Flu vaccination (x 4) | 1955 | All childhood vaccinations<br>Nil since 1986 |
| **497** | 1964 | Usual childhood<br>Possible tetanus 1968 | 1962 | All school vaccinations<br>1990 Hepatitis B<br>1994 Tetanus |
| **499** | 1975 | Usual childhood including triple vaccination but not polio<br>BCG 1986 | 1978 | All school |
| **502** | 1945 | All childhood vaccinations<br>1986 Tetanus and polio | 1943 | Polio and others as child<br>1993-6 Flu vaccination (x 2-3)<br>1993 Tetanus |
| **517** | 1966 | All school vaccinations<br>1976 tetanus<br>1994 Heaf test – not vaccinated | 1965 | Usual childhood vaccinations (but not TB)<br>Nil since 1986 |
| **571** | 1961 | All usual childhood vaccinations<br>No travel/tetanus since 1986 | 1965 | All school<br>1984 tetanus |
| **582** | 1963 | All usual childhood vaccinations<br>Nil since 1986 | 1962 | All usual school vacs<br>Tetanus 1996 |

## Diet

The reported consumption of various different meats and meat products by cases and controls is shown in Tables 43-46. All cases for whom data was available had eaten beef, beefburgers, sausages, meat pies, poultry and lamb since 1985. Most, but no all, were reported to have consumed pork, fish, liver and shell fish since 1985. Only a small number of cases and controls were reported to have eaten haggis, heart, faggots, tongue, trotters or tripe since 1985. Three cases and three controls were reported to have eaten venison since 1985, while one case and six controls had eaten veal. About half of the cases and controls had eaten kidneys. None were reported to have ever eaten sweetbreads or eyes, while only one control was reported to have tried 'a spoonful' of brains on one occasion, in Italy in the mid 1980s. Cases appeared to have consumed beef, beefburgers, pork and poultry more frequently than the controls. There were no striking differences in the frequency of consumption of any of the other items. A statistical analysis based on a lager number of cases is discussed later.

History of consumption of pet food is available for four cases and four controls. Case 476 ate dog biscuits aged two in 1968. None of the others cases or control were reported to have eaten pet food.

Case 499 had been a 'strict' vegetarian (not vegan) since 1991. Two controls were reported to be vegetarians, one during 1988-93, and another since 1985 (but ate occasional sausages). All cases and controls consumed dairy products.

## Contact with animals and animal products

Two cases and three controls reported a previous history of living or working on a farm. Case 485 worked for a cat rescue organisation once a week for a four-month period in 1991. This was situated on a farm which kept horses but not cattle or sheep. Case 517 lived on a farm from 1966-76. Cattle were not kept on the farm but were present on near-by farms. Aged three (1969) he had 'pottered-about' in cow sheds and age 10-11 he had helped to round-up sheep. One control worked on a farm picking potatoes and bailing hay in 1980 for two weeks, another control helped out on a farm by delivering milk in 1960-1 and a further control spent two weeks on a farm working with sheep and cows in 1975.

Case 417 used to stay on his aunt's farm in Kent each year for a week during 1976-86. The farm had a small milk herd of up to 150 cows in addition to a few pigs and an arable crop. He would play with the cows and drink unpasteurised milk. No case of BSE (confirmed by MAFF) or definite 'odd' cows has been reported on the farm and the cattle were said to have been fed fish meal.

Seven cases and seven controls were known or considered to have a possible history of contact with cattle and six cases and five controls had a history of contact with sheep. Case 417 had contact with cattle as described above, case 485 would occasionally stroke cows in a field or sheep in a zoo from childhood to 1994 and 1993 respectively. Case 474 was in the Young Farmers from 1984 to 1986 and it was thought that she may have possibly touched a cow or sheep during this period 'if their head was sticking over a fence'. Case 497 touched sheep once in 1973 and possibly touched cattle in 1974. Case 502 would occasional touch cattle during the

period 1975-91 and sheep during the period 1975-94. Case 517 was reported to have had contact with cattle from 1969 to possibly 1991 and sheep from 1977 to 1991. Case 571 would have patted cows on two or three occasions during the period 1994-6 and would have picked up one-month old lambs on two or three occasions in 1994.

No case or control had a history of contact with live mink or working with fur or leather. Case 502 had contact with deer during the period 1975-1994. No other case and only one control had a history of deer contact. No case and only one control had a history of contact with ferrets.

Details of household pets is given for cases and controls in Table 47. Ten cases and 11 controls kept pets during the period 1985-90. Four cases had never kept any kind of pet.

Four cases and one control had a history of contact with fertiliser or bone meal. Case 417 worked in a shop during 1993-5 and would bag fertiliser (bone meal, hoof and horn and dried blood). Case 485 would use bone meal on her garden and case 517 would use horse mature as fertiliser and fed bone meal (he would have touched this) to his dog in 1992. Case 502 used fertiliser once a year in his garden. This was not thought to be bone meal, hoof and horn or dried blood.

Information on history of bull's eye dissection at school is available for nine cases and four controls. Case 497 did this in 1978 or 1979 as did one control (1977). Case 480 was thought to have possibly dissected a bull's eye and for two cases the history was unknown. Five other cases and the four controls were reported to have never dissected a bull's eye.

## Family history of dementia

Eight cases had possible or probable family history of dementia, whereas only two of the controls reported a possible history of dementia: one had a paternal grandfather who was alive in his 80s and was said to be 'a little confused recently', and the other had a paternal grandfather who was alive aged 80 and was said to have been increasingly confused for the previous two years.

## Miscellaneous factors

Six cases and five controls were current smokers, six cases and six controls had never smoked and two cases and three controls were ex-smokers. All except one case and one control were current drinkers of alcohol. One case had never drank alcohol and one control had not drank in the previous year. Five controls but no case had had a tattoo. Eight cases and 13 controls had had ear-piercing.

**Figure 34: Residence at onset of symptoms for the 14 cases of vCJD**

**Table 43: Consumption of meat ever and since 1985 for cases and controls**

| Type of meat | Frequency of consumption | Cases | | Controls | |
|---|---|---|---|---|---|
| | | Ever | Post-'85 | Ever | Post-'85 |
| **Lamb** | Never | 0 | 0 | 1 | 3 |
| | Less than yearly but not never | 0 | 2 | 3 | 1 |
| | At least yearly but less than monthly | 4 | 5 | 3 | 5 |
| | At least monthly but less than weekly | 6 | 5 | 6 | 3 |
| | Weekly or more | 4 | 2 | 1 | 2 |
| **Pork** | Never | 1 | 1 | 0 | 1 |
| | Less than yearly but not never | 0 | 0 | 0 | 0 |
| | At least yearly but less than monthly | 0 | 0 | 1 | 1 |
| | At least monthly but less than weekly | 2 | 1 | 5 | 4 |
| | Weekly or more | 11 | 12 | 8 | 8 |
| **Poultry** | Never | 0 | 0 | 0 | 1 |
| | Less than yearly but not never | 0 | 0 | 0 | 0 |
| | At least yearly but less than monthly | 0 | 0 | 2 | 2 |
| | At least monthly but less than weekly | 4 | 3 | 7 | 5 |
| | Weekly or more | 10 | 11 | 5 | 6 |
| **Venison** | Never | 8 | 11 | 11 | 11 |
| | Less than yearly but not never | 6 | 1 | 1 | 2 |
| | At least yearly but less than monthly | 0 | 1 | 2 | 0 |
| | At least monthly but less than weekly | 0 | 1 | 0 | 1 |
| | Weekly or more | 0 | 0 | 0 | 0 |
| **Fish** | Never | 0 | 1 | 0 | 1 |
| | Less than yearly but not never | 0 | 0 | 2 | 0 |
| | At least yearly but less than monthly | 3 | 3 | 4 | 3 |
| | At least monthly but less than weekly | 7 | 6 | 2 | 4 |
| | Weekly or more | 4 | 4 | 6 | 6 |
| **Raw fish** | Never | 14 | 14 | 11 | 11 |
| | Less than yearly but not never | 0 | 0 | 1 | 2 |
| | At least yearly but less than monthly | 0 | 0 | 1 | 0 |
| | At least monthly but less than weekly | 0 | 0 | 1 | 1 |
| | Weekly or more | 0 | 0 | 0 | 0 |

**Table 44: Consumption of beef and meat products ever and since 1985 for cases and controls**

| Type of meat or product | Frequency of consumption | Cases | | Controls | |
|---|---|---|---|---|---|
| | | Ever | Post-'85 | Ever | Post-'85 |
| **Beef** | Never | 0 | 0 | 0 | 1 |
| | Less than yearly but not never | 0 | 0 | 1 | 2 |
| | At least yearly but less than monthly | 2 | 1 | 2 | 3 |
| | At least monthly but less than weekly | 2 | 3 | 4 | 4 |
| | Weekly or more | 10 | 10 | 7 | 4 |
| **Veal** | Never | 10 | 13 | 8 | 8 |
| | Less than yearly but not never | 3 | 1 | 3 | 3 |
| | At least yearly but less than monthly | 0 | 0 | 0 | 1 |
| | At least monthly but less than weekly | 0 | 0 | 1 | 1 |
| | Weekly or more | 0 | 0 | 2 | 1 |
| **Beefburgers*** | Never | 0 | 0 | 0 | 2 |
| | Less than yearly but not never | 1 | 1 | 1 | 0 |
| | At least yearly but less than monthly | 1 | 2 | 2 | 4 |
| | At least monthly but less than weekly | 2 | 3 | 6 | 3 |
| | Weekly or more | 7 | 6 | 3 | 3 |
| **Sausages** | Never | 0 | 0 | 0 | 0 |
| | Less than yearly but not never | 0 | 1 | 0 | 0 |
| | At least yearly but less than monthly | 2 | 1 | 2 | 2 |
| | At least monthly but less than weekly | 5 | 7 | 7 | 9 |
| | Weekly or more | 6 | 5 | 5 | 3 |
| **Meat pies**† | Never | 0 | 0 | 1 | 1 |
| | Less than yearly but not never | 0 | 0 | 0 | 0 |
| | At least yearly but less than monthly | 1 | 1 | 2 | 2 |
| | At least monthly but less than weekly | 0 | 1 | 3 | 2 |
| | Weekly or more | 4 | 3 | 3 | 4 |
| **Faggots**‡ | Never | 5 | 6 | 4 | 6 |
| | Less than yearly but not never | 2 | 1 | 4 | 2 |
| | At least yearly but less than monthly | 1 | 1 | 1 | 1 |
| | At least monthly but less than weekly | 0 | 0 | 0 | 0 |
| | Weekly or more | 0 | 0 | 0 | 0 |

* Cases n=12, controls n=12. † Cases n=5, controls n=9. ‡ Cases n=8, controls n=9. For one case the frequency of veal, sausage and beefburger consumption is known since 1985, but not for the category 'ever'

**Table 45: Consumption of various meats or meat products ever and since 1985 for cases and controls**

| Type of meat | Frequency of consumption | Cases | | Controls | |
|---|---|---|---|---|---|
| | | Ever | Post-'85 | Ever | Post-'85 |
| **Puddings*** | Never | 4 | 5 | 4 | 7 |
| | Less than yearly but not never | 3 | 4 | 4 | 3 |
| | At least yearly but less than monthly | 4 | 4 | 3 | 1 |
| | At least monthly but less than weekly | 1 | 0 | 2 | 2 |
| | Weekly or more | 1 | 1 | 1 | 1 |
| **Brains*** | Never | 13 | 14 | 13 | 14 |
| | Less than yearly but not never | 0 | 0 | 1 | 0 |
| | At least yearly but less than monthly | 0 | 0 | 0 | 0 |
| | At least monthly but less than weekly | 0 | 0 | 0 | 0 |
| | Weekly or more | 0 | 0 | 0 | 0 |
| **Tripe*** | Never | 11 | 14 | 12 | 13 |
| | Less than yearly but not never | 2 | 0 | 2 | 1 |
| | At least yearly but less than monthly | 0 | 0 | 0 | 0 |
| | At least monthly but less than weekly | 0 | 0 | 0 | 0 |
| | Weekly or more | 0 | 0 | 0 | 0 |
| **Liver*** | Never | 4 | 5 | 0 | 1 |
| | Less than yearly but not never | 1 | 2 | 6 | 3 |
| | At least yearly but less than monthly | 7 | 7 | 4 | 5 |
| | At least monthly but less than weekly | 1 | 0 | 3 | 3 |
| | Weekly or more | 0 | 0 | 1 | 2 |
| **Kidneys†** | Never | 7 | 8 | 5 | 6 |
| | Less than yearly but not never | 1 | 1 | 2 | 1 |
| | At least yearly but less than monthly | 2 | 3 | 4 | 4 |
| | At least monthly but less than weekly | 1 | 1 | 1 | 1 |
| | Weekly or more | 1 | 1 | 2 | 2 |
| **Sweetbreads*** | Never | 13 | 14 | 14 | 14 |
| | Less than yearly but not never | 0 | 0 | 0 | 0 |
| | At least yearly but less than monthly | 0 | 0 | 0 | 0 |
| | At least monthly but less than weekly | 0 | 0 | 0 | 0 |
| | Weekly or more | 0 | 0 | 0 | 0 |

* For one case the frequency of consumption is known since 1985 but not for the category 'ever'

† For two cases the frequency of consumption is known since 1985 but not for the category 'ever'

**Table 46: Consumption of various meats or meat products ever and since 1985 for cases and controls**

| Type of meat | Frequency of consumption | Cases | | Controls | |
|---|---|---|---|---|---|
| | | Ever | Post-'85 | Ever | Post-'85 |
| **Tongue**† | Never | 7 | 12 | 11 | 12 |
| | Less than yearly but not never | 3 | 0 | 1 | 1 |
| | At least yearly but less than monthly | 2 | 1 | 1 | 0 |
| | At least monthly but less than weekly | 0 | 1 | 0 | 0 |
| | Weekly or more | 0 | 0 | 1 | 1 |
| **Trotters*** | Never | 10 | 13 | 14 | 14 |
| | Less than yearly but not never | 3 | 1 | 0 | 0 |
| | At least yearly but less than monthly | 0 | 0 | 0 | 0 |
| | At least monthly but less than weekly | 0 | 0 | 0 | 0 |
| | Weekly or more | 0 | 0 | 0 | 0 |
| **Eyes*** | Never | 13 | 14 | 14 | 14 |
| | Less than yearly but not never | 0 | 0 | 0 | 0 |
| | At least yearly but less than monthly | 0 | 0 | 0 | 0 |
| | At least monthly but less than weekly | 0 | 0 | 0 | 0 |
| | Weekly or more | 0 | 0 | 0 | 0 |
| **Haggis*** | Never | 6 | 8 | 7 | 8 |
| | Less than yearly but not never | 5 | 4 | 6 | 5 |
| | At least yearly but less than monthly | 2 | 2 | 1 | 1 |
| | At least monthly but less than weekly | 0 | 0 | 0 | 0 |
| | Weekly or more | 0 | 0 | 0 | 0 |
| **Heart*** | Never | 10 | 12 | 13 | 13 |
| | Less than yearly but not never | 0 | 1 | 0 | 0 |
| | At least yearly but less than monthly | 3 | 1 | 0 | 0 |
| | At least monthly but less than weekly | 0 | 0 | 1 | 1 |
| | Weekly or more | 0 | 0 | 0 | 0 |
| **Shell fish** | Never | 6 | 6 | 5 | 6 |
| | Less than yearly but not never | 1 | 1 | 2 | 0 |
| | At least yearly but less than monthly | 1 | 3 | 4 | 2 |
| | At least monthly but less than weekly | 5 | 4 | 2 | 5 |
| | Weekly or more | 1 | 0 | 1 | 1 |

* For one case the frequency of consumption is known since 1985 but not for the category 'ever'

† For two cases the frequency of consumption is known since 1985 but not for the category 'ever'

**Table 47: Pets**

| No | Case | Dates | Control | Dates |
|---|---|---|---|---|
| **417** | Hamster | 1979-81 | Dogs | 1984-94 |
| | Dog | 1980-7 | Cat | 1993-5 |
| | Bullfinch | Up to 1995 | | |
| **433** | Nil | | Dogs | 1983-5, 1989-95 |
| | | | Fish | 1992-3 |
| **466** | Nil | | Dogs | 1972-88 |
| **467** | Dogs | All life | Dog | 1987-91 |
| | Cat | 1969 | Cat | 1991-4 |
| | | | Fish | 1993-4 |
| | | | Guinea pig | 1994-6 |
| | | | Rabbit | 1996 |
| **474** | Guinea pig | 1975-6 | Rabbits | 1976-7 |
| | Dog | 1984-90 | Cats | 1980-86 |
| | Hamsters | 1994-5 | | |
| **476** | Dogs | 1980-95 | Rabbit | 1979-80 |
| | | | Dog | 1982-90 |
| **480** | Nil | | Cat | 1981 |
| | | | Dogs | 1981-2, 1988 |
| | | | Hamster | 1993 |
| **485** | Cats | All life | Nil | |
| | Parrots | 1991-5 | | |
| **497** | Rabbit | 1975-6 | Dogs | 1978-86, 1990-6 |
| | Budgies | 1981-5 | Goldfish | 1995-6 |
| | Cat | 1986-9 | | |
| **499** | Hamster | 1989-91 | Dogs | All life |
| **502** | Tropical fish | 1976-81 | Rabbit | 1993 |
| | Cat | 1978-91 | Goldfish | 1994-6 |
| | | | Budgie | 1996 |
| **517** | Dogs | All life | Cat | 1970-81 |
| | Cats and rabbits | Up to 1980 | Guinea pigs | 1980-1 |
| | Bird | 1989-96 | | |
| | Cat | 1994-5 | | |
| **571** | Nil | | Dogs | 1965-96 |
| **582** | Chinchilla | 1994 | Nil | |
| | Cats | 1995-6 | | |
| | Dogs | 1995-6 | | |

## MRI STUDY

MRI scans were obtained for 93 patients (see Table 48); 36 had histologically confirmed vCJD. The control group consisted of 57 individuals with a diagnosis other than vCJD. The mean age of the 36 pathologically-confirmed vCJD cases was 30 years (range 15-52) and of the 57 controls 52 years (range 14-79). Twenty-seven controls were under the age of 52. The age distribution of the 14 controls who were initially suspected of having vCJD but were later classified as non-cases was similar to that of the pathologically-confirmed vCJD cases (mean age 33, range 14-58).

All film sets contained T1-weighted and T2-weighted images. In 21 (58%) of the histologically confirmed vCJD cases PD-weighted images were also available. Any abnormal thalamic signal was seen on T2- or PD-weighted images (or both), but no changes were seen on T1-weighted images.

### Results of initial blinded assessments

For the 36 pathologically-confirmed cases of vCJD, radiological diagnoses of vCJD were reported by the first and second radiologist for 29 and 32 individuals respectively at the first reading, and for 32 and 31 at the second reading. Twenty-eight individuals (78%) were considered positive on all four assessments, while one, four, one and two individuals were scored positively on three, two, one and no assessments respectively. Of the 124 positive scan assessments the scans were classified as showing marked changes on 89 assessments, moderate changes on 26 assessments and minimal/equivocal changes on nine assessments. Of the 20 negative MRI assessments in the confirmed vCJD cases, the radiological diagnosis was normal on 12 occasions, sporadic CJD on three, and on five occasions non-specific white matter cerebrovascular disease.

A false positive radiological diagnosis of vCJD was made on seven out of 228 MRI assessments on the 57 controls. Observer 1 judged a 50-year-old women with familial CJD and a 51-year old man with probable sporadic CJD to have a radiological diagnosis of vCJD with minimal/equivocal changes on one and both assessments respectively. Observer 2 judged three sporadic CJD cases (age 55-63) to have a radiological diagnosis of vCJD (changes graded as minimal/equivocal in two cases and moderate in a single case). Observer 2 also judged a suspect vCJD case who has recovered (age 14) to have minimal/equivocal pulvinar signal change. The other radiological assessments of each of these individuals were considered normal.

Intra-observer agreement between the first and second scan assessments (graded as positive or negative for vCJD diagnosis) was high for both observers. Overall concordances were 96% for observer 1 and 92% for observer 2 with corresponding kappa coefficients of 0.90 (standard error 0.10) and 0.84 (standard error 0.10). Similarly high levels of concordance (>90%) were observed when assessments were graded as positive for the diagnosis of vCJD only if scans were considered to show moderate or marked changes.

Estimates of the sensitivity of radiological changes for the diagnosis of vCJD are presented in Table 49. These fell from the order of 80% or more to the order of 50-70% as stricter criteria for positivity were applied. At the same time, minimum estimates of specificity increased from 95% to 100%.

## Consensus review of MRI examinations

On consensus review of the scans of the 36 pathologically-confirmed vCJD cases the pulvinar high signal was graded as nil in two cases, + in four cases, ++ in two cases, +++ in 10 cases and ++++ in 18 cases. When present pulvinar high signal was always symmetrical (see Figures 34-36). Three of the control scans, all from middle-aged sporadic CJD cases, were considered to show pulvinar high signal (+) on consensus review. The 28 vCJD patients with the most prominent pulvinar high signal (+++ or ++++) were all diagnosed as vCJD radiologically on all 112 blinded assessments (marked changes 88 assessments, moderate changes 22 assessments and minimal/equivocal changes two assessments). The two pathologically-confirmed vCJD cases considered not to show pulvinar changes on consensus review (see Figure 36) had both been scored negative on all four of their blind assessments.

Basal ganglia (including thalamus) signal changes were more conspicuous on PD-weighted images than T2-weighted images in 12 out of 14 pathologically-confirmed vCJD cases for whom both types of imaging were available (see Figures 34 and 35). FLAIR images were available for three patients and thalamic signal changes were most conspicuous on these images in two cases (Figure 37). Positive scans were characterised by high signal changes with a well-defined anterior border in the pulvinar. Movement artefact was present in 16 of the pathologically-confirmed vCJD cases, of marked degree in three cases. However, the scans remained diagnostic (see Figure 36).

Prominent pulvinar high signal (+++ or ++++) accompanied by increased signal (+ or greater) in the dorsomedial thalamic nuclei was noted in 27 (75%) of the pathologically-confirmed vCJD cases (Table 50) giving a 'hockey-stick' appearance (see Figure 30). Cerebral atrophy was present in only 8/36 patients. These patients had scans performed a mean of 11 months into the illness (range 6-25 months) and 3.5 months (range 2-8 months) before death compared to those without atrophy whose last scans were performed a mean of 8.5 months into their illness (range 3-22 months) and six months (range 1-27 months) before death. Other features included increased signal (+ or greater) in the putamen in 12 cases (33%) and the caudate head in eight cases (22%) (see Table 50). The peri-aqueductal grey matter signal was increased in 72% of cases (+ in six, ++ in nine, +++ in 10 and ++++ in one) and 37% of controls (+ in 13, ++ in seven and +++ in one). There were no changes observed in the globus pallidus in any of the 36 pathologically-confirmed vCJD cases.

For nine of the pathologically-confirmed vCJD cases two scans performed at different times during their illness and showing adequate images of the thalamus were available. In seven of these cases there was no difference in the pulvinar appearance between the earlier and later scans (time between scans two weeks to 12 months). In two cases the scans evolved from showing very slight pulvinar signal change (+) to very prominent abnormalities (++++) (scans at six and 10 months of an 11 month-illness, and six and 18 months of a 23-month illness).

Eight of the pathologically-confirmed vCJD cases were considered negative on at least one of the four blinded radiological assessments and on consensus review all of these were judged to have pulvinar signal graded less than +++. Scans from two of these cases were considered to be normal on consensus review. One of these had only T2- weighted images available and these were of poor quality. Only coronal PD-weighted images were available in the second 'normal' case and another case with pulvinar grade +. Differences in signal changes between the thalamus and other basal ganglia are more difficult to assess in the coronal plane, and the axial or

sagittal plane are preferred. In another case the pulvinar changes were masked by high signal changes in the putamen and caudate heads and in scans from two further patients image contrast was flat resulting in poor quality images. Two pathologically-confirmed vCJD cases had good quality T2- and PD-weighted images available and had only minimal signal change (+) in the pulvinar. The mean age of these eight cases with minimal or absent pulvinar changes was 35 years compared with 29 years for the 28 cases with prominent changes (pulvinar signal +++ or ++++) ($p = 0.13$). They had their last scan at a similar stage of their illness, mean 10.5 months through an illness of mean 15 months compared with mean 8.5 months through an illness of mean 15.5 months.

Reviewing the MRI reports from the referring centre, pulvinar high signal had been noted in 12 of the 36 cases. In our study all of these cases were considered to have prominent pulvinar high signal (+++ two cases, ++++ 10 cases) on consensus review.

All 26 cases of vCJD examined pathologically for this study exhibited a consistent pattern of thalamic gliosis which was variable in severity from case to case, but was always most severe in the pulvinar. The dorsomedial nucleus was also severely involved in 16/26 cases. In the pulvinar, severe astrocytosis was accompanied by widespread neuronal loss and rarefaction of the neuropil (see Figure 33), particularly around blood vessels. Spongiform change of mild to moderate severity was present in the pulvinar; this pathological feature was most marked in the caudate nucleus and the putamen, which exhibited considerably less astrocytosis. No relationship was identified between the severity of the gliosis and either PrP accumulation or amyloid plaque formation in the pulvinar. Posterior thalamic gliosis and severe neuronal loss was not identified in any of the 20 controls with sporadic CJD.

**Table 48: MRI diagnoses by patients group**

| Patient Group | Final Diagnosis | Number of patients | Mean age (range) | Positive MRI diagnosis of vCJD* |
|---|---|---|---|---|
| **Suspected vCJD** | Pathologically-confirmed | 36 | 30 (15-52) | 124/144 |
| | Not-vCJD | 14 | 33 (14-58) | 1/56 |
| **Suspected sporadic CJD** | Pathologically-confirmed | 21 | 64 (46-79) | 1/84 |
| | Probable | 11 | 62 (49-75) | 4/44 |
| | Possible | 1 | 65 | 0/4 |
| | Non case | 2 | 58 (54, 61) | 0/8 |
| **Suspected familial CJD†** | Pathologically-confirmed | 2 | 49, 50 | 1/8 |
| **Suspected Iatrogenic CJD‡** | Pathologically-confirmed | 6 | 31 (25-36) | 0/24 |
| **Total** | | **93** | | |

* Each scan was examined on four occasions, so the denominators are equal to four times the number of patients

† Both with codon 200 mutations

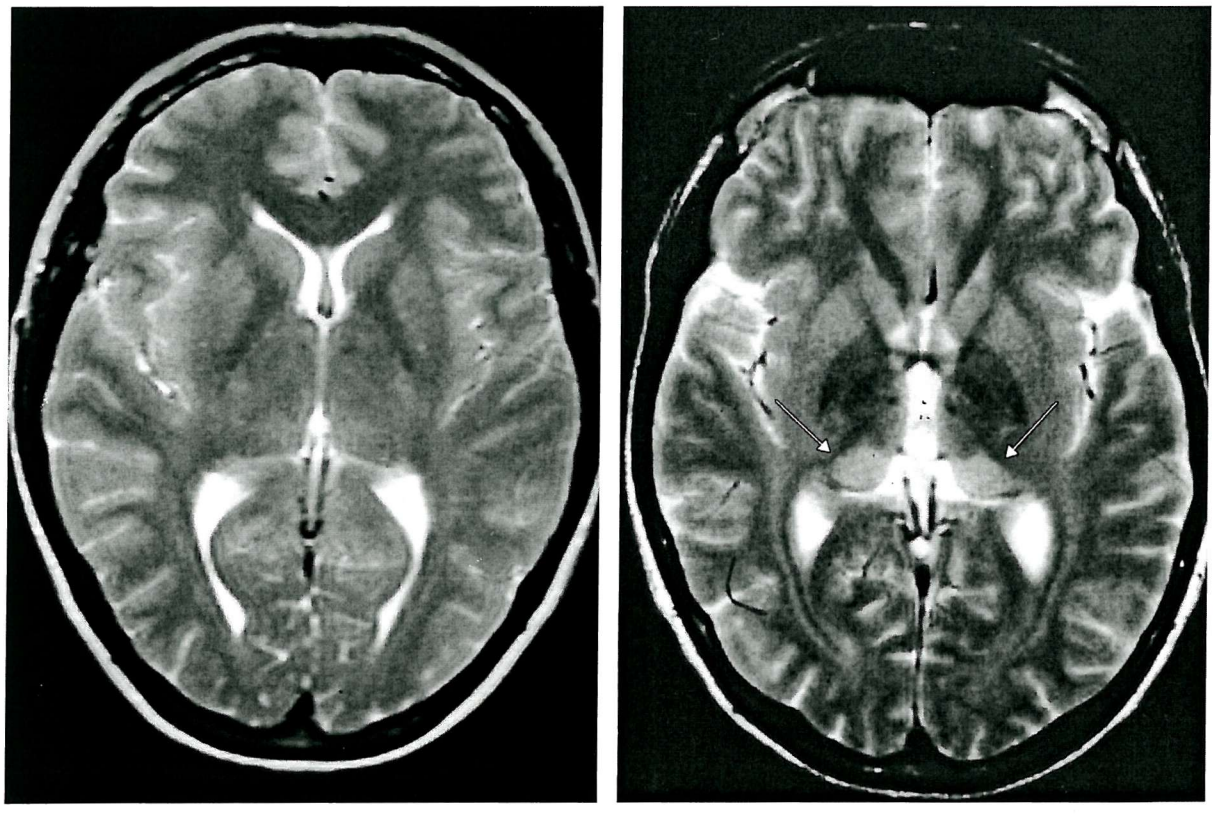
‡ All hGH cases

**Table 49: Sensitivity of radiological diagnosis of vCJD using different MRI criteria for positivity**

| Positive cut-off point | Observer 1 | | Observer 2 | |
|---|---|---|---|---|
| | Assessment 1 | Assessment 1 | Assessment 1 | Assessment 2 |
| Minimal/equivocal changes or greater | 81 (64-92) | 89 (74-97) | 89 (74-97) | 86 (71-95) |
| Moderate changes or greater | 78 (61-90) | 81 (64-92) | 81 (64-92) | 78 (61-90) |
| Marked changes | 53 (35-70) | 69 (52-84) | 69 (52-84) | 58 (41-74) |

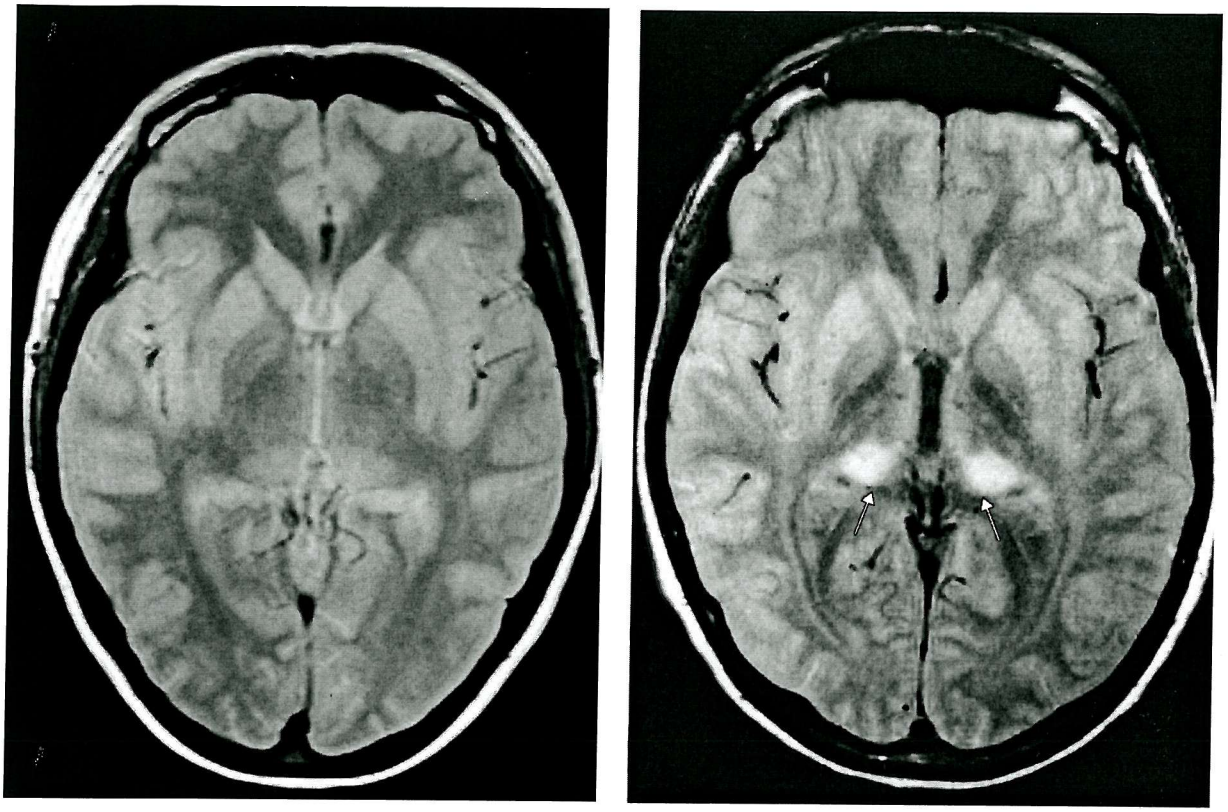
Data are percentage sensitivity (95% CI)

**Figure 35: Axial T2-weighted MRI in control and vCJD**



Normal axial T2-weighted MRI in a control (left) and showing posterior thalamic high signal in vCJD (right)

**Figure 36: Axial PD-weighted MRI in control and vCJD**



Normal axial T2-weighted MRI in a control (left) and showing posterior thalamic high signal in vCJD (right)

**Figure 37: Diagnostic MRI in vCJD despite movement artefact (left) and normal appearance (right)**

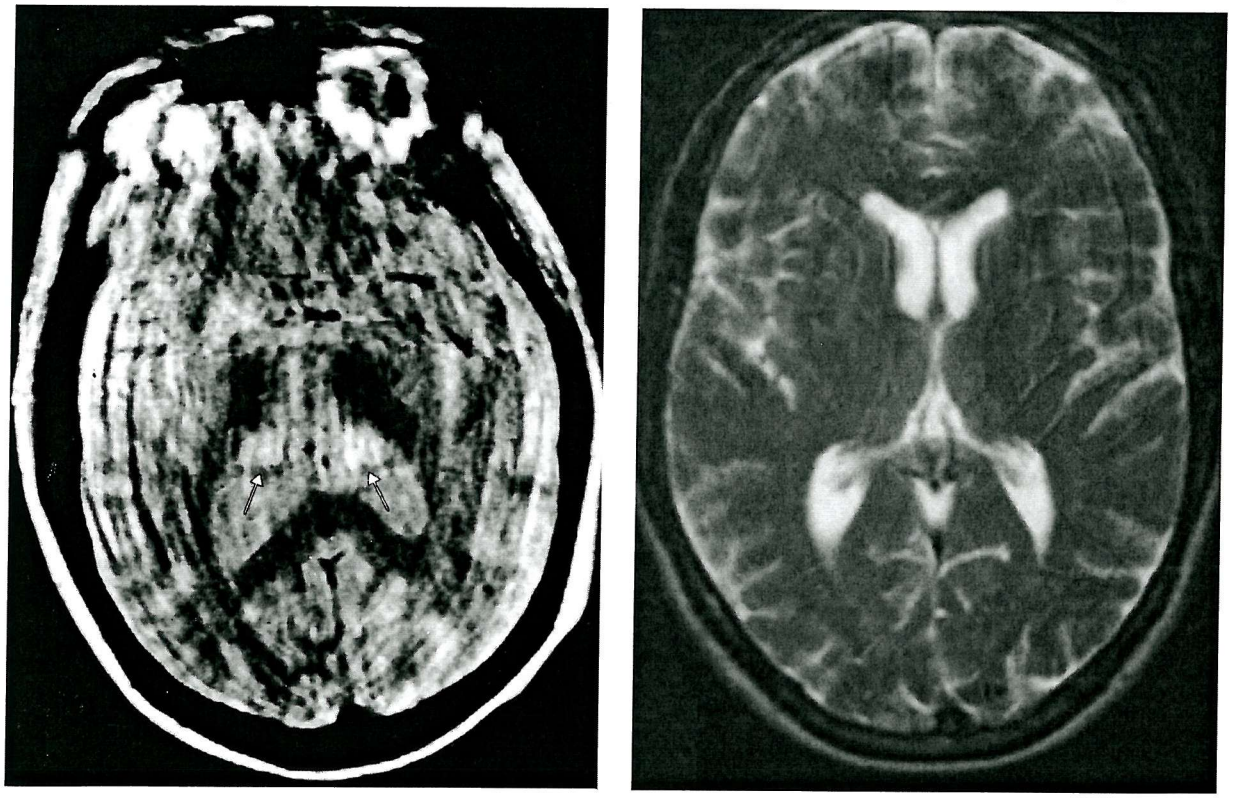


**Figure 38: Axial, coronal and sagittal FLAIR MRI in normal controls (left) and vCJD (right)**

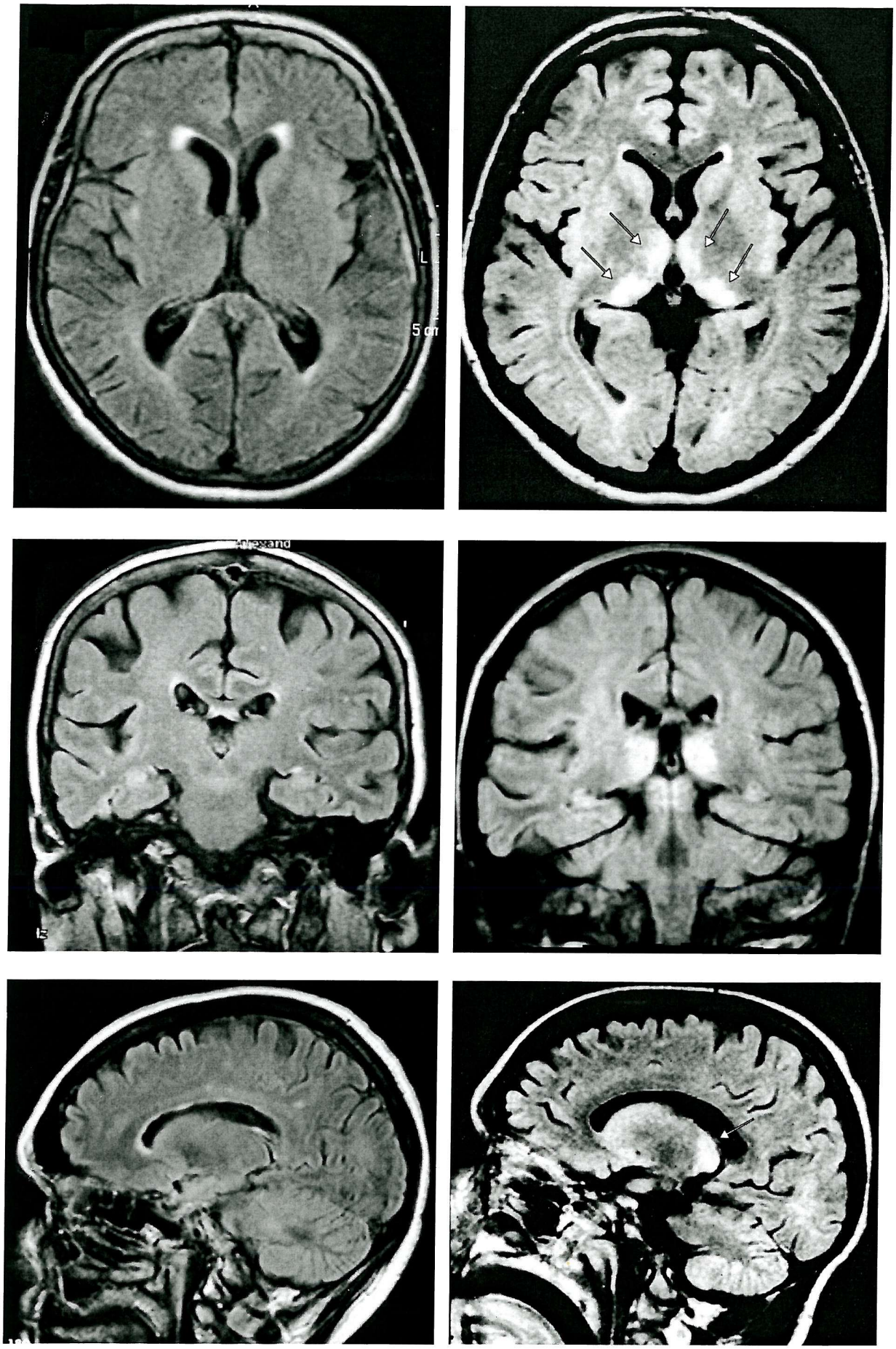


**Table 50: Neuroradiological assessment of MRI from 36 pathologically-confirmed cases of vCJD**

| Case No. | Age | Scan time* | Total illness duration* | Pulvinar signal | Medial thalamus | Peri-aqueductal grey | Putaminal change | Caudate | Atrophy | Movement artefact |
|---|---|---|---|---|---|---|---|---|---|---|
| **417** | 19 | 8 | 11 | +++ | ++ | ++ | - | - | + | + |
| **433** | 17 | 6 | 38 | + | - | - | - | - | - | |
| | | 11 | | ++++ | ++ | ++ | + | + | - | |
| **466** | 30 | 6 | 10 | +++ | + | ++ | - | - | + | ++ |
| **467** | 30 | 9 | 18 | + | ++ | + | - | - | - | + |
| **474** | 29 | 1 | 11 | N/A | N/A | +++ | N/A | N/A | N/A | |
| | | 4 | | ++++ | +++ | +++ | - | - | - | |
| **476** | 29 | 10 | 11 | +++ | ++ | + | - | - | - | + |
| | | 10 | | +++ | ++ | + | - | - | - | + |
| **480** | 29 | 5 | 23 | +++ | + | +++ | + | + | - | |
| | | 14 | | +++ | ++ | +++ | + | + | - | |
| **485** | 41 | 11 | 18 | +++ | + | - | - | - | - | |
| **497** | 31 | 5 | 9.5 | ++++ | ++ | - | - | - | - | +++ |
| **499** | 20 | 5 | 13 | ++++ | +++ | ++ | - | - | - | |
| **502** | 50 | 25 | 29 | ++++ | +++ | +++ | + | ++ | + | |
| **517** | 29 | 14 | 17 | +++ | - | - | - | - | + | ++ |
| **571** | 35 | 12 | 14 | ++++ | ++ | ++ | - | - | + | |
| **582** | 33 | 6 | 23 | ++++ | +++ | +++ | ++ | ++ | - | |
| **637** | 19 | 9 | 12 | ++++ | ++ | + | - | - | - | ++ |
| **642** | 22 | 11 | 14 | ++++ | ++ | ++ | + | - | + | + |
| **698** | 24 | 13 | 25 | ++ | + | - | ++ | + | - | |
| | | 15 | | ++ | + | - | ++ | + | - | |
| **707** | 36 | 9 | 12 | - | - | - | - | - | - | + |
| **730** | 15 | 4.5 | 33 | ++++ | +++ | +++ | - | - | - | + |
| **755** | 52 | 8 | 11 | + | + | - | - | - | - | |
| | | 10 | | + | + | - | - | - | - | |
| **759** | 18 | 4 | 13 | ++++ | +++ | +++ | - | - | - | |
| **797** | 34 | 4 | 12 | ++++ | +++ | +++ | - | - | - | |
| **827** | 45 | 8 | 24 | - | - | - | - | - | - | |
| | | 22 | 24 | - | - | - | - | - | - | |
| **834** | 20 | 6 | 11 | + | - | + | - | - | - | |
| **839** | 25 | 10 | 13 | ++++ | ++ | ++ | - | - | - | +++ |
| | | 10 | | ++++ | ++ | ++ | - | - | - | +++ |
| **845** | 36 | 11 | 15 | +++ | ++ | ++ | + | - | - | |
| **866** | 20 | 3 | 7 | ++ | ++ | - | - | - | - | ++ |
| **869** | 39 | 6 | 11 | + | - | - | - | - | - | |
| | | 10 | | ++++ | ++++ | ++++ | +++ | +++ | - | |
| **872** | 16 | 7 | 14 | ++++ | +++ | +++ | - | - | - | |
| **877** | 25 | 8 | 14 | +++ | ++ | - | - | - | - | + |
| **907** | 39 | 6 | 14 | ++++ | ++++ | ++ | + | - | + | |
| **921** | 24 | 7 | 12 | ++++ | +++ | ++ | - | + | - | ++ |
| | | 8 | | ++++ | ++++ | ++ | + | ++ | - | |
| **927** | 51 | 10 | 11 | + | + | - | + | - | - | + |
| **930** | 28 | 13 | 16 | +++ | ++ | +++ | - | - | - | |
| **964** | 41 | 2 | 7 | +++ | ++ | + | - | - | - | +++ |
| **1030** | 29 | 6 | 7 | ++++ | +++ | +++ | +++ | ++ | + | |

* Months. N/A = not applicable as images showing the relevant area were not available

# DISCUSSION

## HAS A NEW VARIANT OF CJD BEEN IDENTIFIED IN THE UK?

The postulated mechanisms by which a novel form of CJD caused by BSE might be identified are:

1. An increase in the overall incidence of CJD in the UK
2. An excess of cases in groups most likely to have high exposure to the BSE agent
3. A change in the epidemiological pattern of CJD, such as a change in the age distribution
4. A change in the clinical or neuropathological characteristics of CJD.

### Has there been an increase in the overall incidence of CJD in the UK due to BSE?

The number of sporadic cases of CJD recorded in England and Wales increased over the period 1970 to 1996 (see Figure 23). During a period of prospective surveillance before the advent of BSE (1980-4) the yearly number of deaths from sporadic CJD in England and Wales averaged 24.8. During prospective surveillance after the onset of the BSE epidemic (1990-6) the yearly number of deaths averaged 33.6. However, similar increases in the incidence of CJD have been observed in countries where BSE is rare or absent (Table 51).[7] These increases most likely reflect better case ascertainment rather than real increases in incidence. Improved case detection is probably due to a combination of improved surveillance (a change anticipated in a Lancet editorial in 1990[924]) and greater awareness of issues relating to CJD and BSE by the public, media and the neurological communities.[925]

**Table 51: Incidence of CJD in countries other than the UK over time**

| Country | Period | Incidence cases/million |
|---|---|---|
| **Austria** | 1969-1985 | 0.18 |
| | 1986-1994 | 0.67 |
| | 1995 | 1.5 |
| **Australia** | 1970-1980 | 0.66 |
| | 1987-1996 | 1.07 |
| **Chile** | 1955-1972 | 0.10 |
| | 1973-1977 | 0.31 |
| | 1978-1983 | 0.69 |
| **France** | 1968-1977 | 0.34 |
| | 1978-1982 | 0.58 |
| | 1993-1995 | 0.84 |
| **Germany** | 1979-1990 | 0.31 |
| | 1993*-1995 | 0.55 |
| **Israel** | 1963-1972 | 0.75 |
| | 1963-1987 | 0.91 |
| **Italy** | 1958-1971 | 0.05 |
| | 1993-1994 | 0.11 |
| | 1993-1995 | 0.56 |
| **Japan** | 1975-1977 | 0.45 |
| | 1985-1996 | 0.58 |
| **US** | 1973-1977 | 0.26 |
| | 1986-1988 | 0.83 |

* Extrapolated from part-year data.

Table modified from Will[2]

## Has there been an excess of CJD in groups most likely to have high exposure to the BSE agent?

Over the period 1990-6 there has been a significant excess of cases of CJD in the UK among workers on dairy farms and on farms with a confirmed case of BSE.[7,926] None of the six patients (see Table 18) who might have been occupationally exposed to BSE had the clinical or pathological phenotype of vCJD, supporting the hypothesis that they have a different aetiology. However, evidence from iatrogenic cases that route of infection may be a determinant of the clinical and pathological pattern of disease[25] supports the possibility that 'occupational' cases and vCJD arise from exposure to the same agent by different routes. One potential explanation for the apparent excess risk among farmers is that case ascertainment of CJD in the UK has been better in this group than among other groups because of awareness of a possible link between BSE and CJD. Moreover, though the incidence of CJD among dairy farmers in the UK is higher than in the general population, it does not seem remarkable when compared with the incidence among dairy farmers in other European countries where BSE is rare or absent (see Table 52).[206,927] This suggests that either there is widespread ascertainment bias in this occupational group, which would not be surprising given the pan-European concern regarding BSE, or that dairy farmers may be at increased risk of CJD for reasons other than exposure to BSE.

**Table 52: Incidence rate of CJD in dairy farmers in different European countries**

| Country | Period | Number of cases | Yearly incidence/million |
|---|---|---|---|
| France | 1992-4 | 4 | 4.8 |
| Germany | 1993-4 | 2 | 3.8 |
| Italy | 1993 | 2 | 9.4 |
| Netherlands | 1993-4 | 0 | 0 |
| United Kingdom | 1990-6 | 4 | 4.0 |

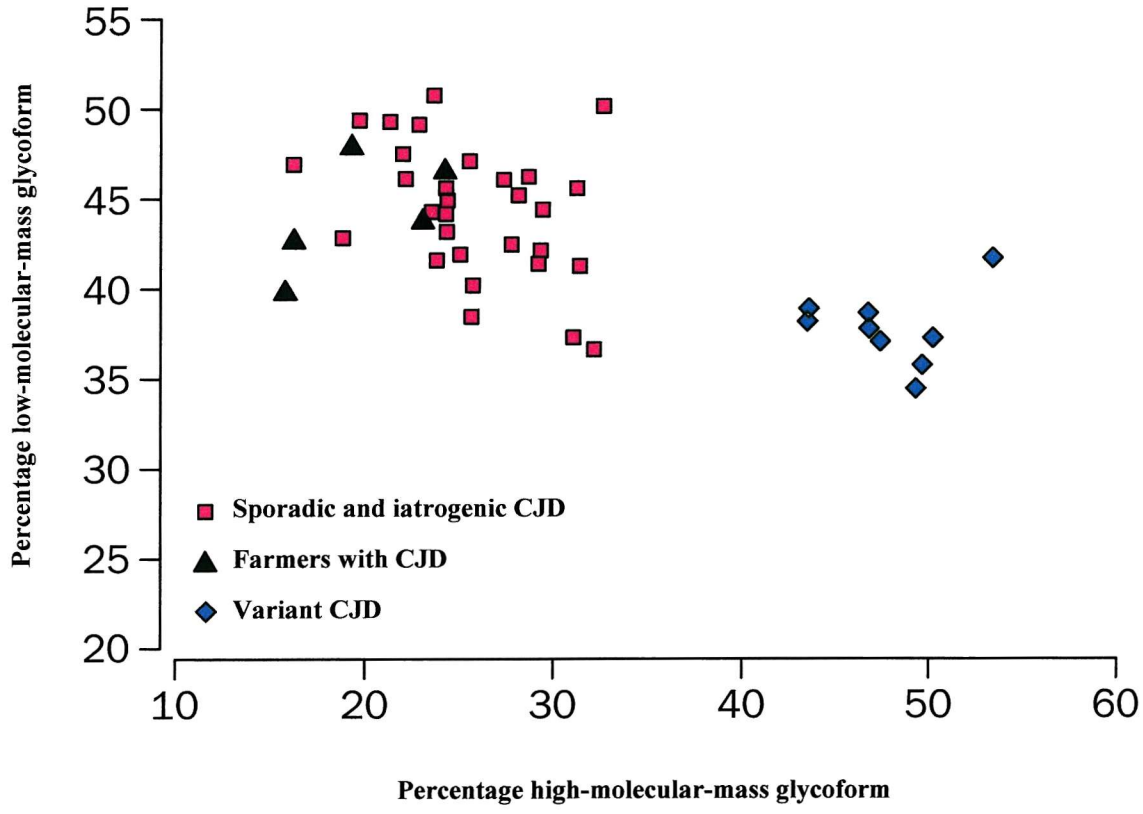
The biological plausibility of cattle farmers acquiring CJD from occupational exposure to BSE also needs to be addressed. As the BSE agent appears to be confined to the CNS of naturally-affected cattle, day-to-day handling of infected animals would seem unlikely to provide a mechanism of direct exposure to the BSE agent. Farmers may, though, have been exposed through contact with MBM, either through handling or eating feed pellets or through nasal inhalation.[928,929] Ridley argues, however, that 'if these farmers had been at risk from handling cattle feed one would expect feed producers to have been at greater risk, and if the farmers had been at risk from handling affected cattle one would expect abattoir workers to have been at greater risk.'[930]

The above analyses are based on occupation at the time of disease onset, as national data are not available on the numbers of people ever employed in particular occupational groups. Therefore excluded from the analyses was one patient (case 489) who had worked in an abattoir for 10 months, six years before disease onset in 1995; one

patient (case 597) who had worked on a farm with dairy and beef herds until retiring in 1991, four years before disease onset in 1995; and one patient (case 476) with vCJD who had worked as a butcher between 1985 and 1987. Another case (case 442) of interest that was similarly excluded was of a man who transported MBM from an animal feed compounder to farms for many years up to 1991 but who changed his job before onset of CJD in 1993.

Both molecular and biological strain-typing studies have now been performed on a number of the UK farmers with CJD. Bruce showed that the transmission characteristics in a series of inbred strains of mice using brain tissue from two of the farmers were distinct from that observed with BSE and vCJD.[12] Hill reported the findings of Western blot analysis of $PrP^{Res}$ from five of the six farmers reported above (see Figure 39).[11] On the basis of fragment size and glycoform ratio all five had PrP subtypes associated with sporadic CJD and distinct from that observed in vCJD (discussed later). These authors concluded that their results, in conjunction with the clinicopathological phenotype of the cases, do not support a link with occupational exposure to the agent of BSE.

**Figure 39: PrP glycoform analysis in farmers with CJD compared to iatrogenic, sporadic and variant CJD**



## Has there been a change in the epidemiological pattern, such as a change in the age distribution?

There has been no significant change in the sex ratio or the geographical distribution of CJD cases before and after the occurrence of BSE (unpublished data from NCJDSU).

It has long been suspected that the age distribution of CJD in the UK and elsewhere - with a decline in incidence in people over 70 - might reflect under ascertainment of cases in older age groups. In the UK the greatest increase in CJD incidence over the period 1970-96 has been in people aged over 70 (see Figure 25). For many diseases case ascertainment is likely to be poorest among old people and best among age groups in which death from any cause is unusual. Therefore a general improvement in ascertainment is most likely to be reflected in a relatively better detection of elderly cases. Another possible explanation for the greatest increase in CJD incidence being observed in the elderly is that older persons might be particularly susceptible to the BSE agent. However, a similar increase in the incidence of CJD in the elderly has been observed in countries in which BSE is rare or absent.[192] Furthermore, no other unusual clinicopathological change has recently been observed in elderly CJD cases in the UK. Therefore the relatively high increased CJD incidence in the elderly is most likely due to ascertainment bias.

Over the period 1995-96 inclusive, a total of nine cases of CJD (excluding familial and iatrogenic) under the age of 30 and 13 under the age of 40 at onset were identified in the UK. Case reports relating to the first two of these patients, both teenagers, were published in October 1995[902,903] and the following month the BMJ[902,903] produced a group of articles discussing the implication of these cases and the cases identified in UK farmers.[931] At this time a literature search[894] identified that only four sporadic CJD cases in persons under the age of 20 had been previously reported in the world[212,904-906] and only 17 cases had been documented in people under the age of 30 (see Table 21). The statistician Sheila Gore, an author of one of the BMJ articles, expressed clear concern about the two teenagers and concluded that their occurrence was 'more than happenstance'[926] (although others did not share this view[932]). She implied that the appearance of two or more additional cases under the age of 40 would be a highly improbable chance occurrence.[926] Over the following four months a further eight pathologically-confirmed cases of CJD under the age of 40 at onset were identified in the UK by the NCJDSU.[84] These and the earlier two teenager cases constituted the 10 patients reported by the UK Government on 20 March 1996.

Gore published a further statistical analysis in a BMJ editorial on 30 March 1996 titled 'Bovine Creutzfeldt-Jakob disease?'[933] As part of her calculations she estimated the number of CJD cases that would be expected to occur in persons under the age of 40 over the six-year period since surveillance began in the UK in May 1990. As the incidence of CJD in the 15-39 age group was not precisely known she used three estimates: 0.05 per million person years, (the same as for people aged 40-44 years), giving an expected number of 6.3 cases; 0.0286 per million person years, based on three sporadic cases of CJD in this age group reported in the UK from 1985 to 1989, giving an expected number of 3.6 cases; or a previous guesstimate of 0.01 per million person years, (one fifth the incidence at age 40-44), giving an expected number of 1.26 cases. Against each of these expectations, the probability of 10 or more sporadic cases of CJD occurring in people aged 15-39 in the six years from 1 May 1990 was 11 in a hundred, four in a thousand, or 0.9 in a million respectively. However, the fact that the 10 cases were identified over a period of less than one year and most were aged less than 30 would

indicate that their occurrence by chance was even less likely than Gore's calculations suggest. Indeed, Morrison calculated that the probability of the seven deaths from CJD in the age group 15-35 occurring by chance was one in a billion, although he did not described his methods.[934]

A key issue is whether the occurrence of these young CJD cases could be explained by increased ascertainment, perhaps as a result of a heightened awareness of CJD.[935] Three of the 10 cases under the age of 40 identified in the year up to 20 March 1996 were notified to the NCJDSU by unconventional means (see Table 53 – this also includes the cases 502, 517, 571 and 582 identified after 20 March 1996): one was seen by myself in a general neurology clinic (case 497), another was notified following a lecture in which young cases of CJD were discussed (case 480) and a further patient was identified through an enquiry by the NCJDSU following newspaper report (case 499). Arguably these cases were only notified because of the concern that young people in the UK might be developing CJD as a result of BSE. Furthermore, of the 13 CJD cases (excluding familial and iatrogenic) under the age of 40 at referral identified during the period 1995-6, all but one (case 580 – see Annex 8) was diagnosed pathologically and 11 patients underwent necropsy. None of these cases would have fulfilled the criteria for probable sporadic CJD used at that time and therefore would not have been classified as a CJD had they not undergone pathological examination. Aylin reports that since 1979 only about 46% of patients aged under 45 dying of dementias with which CJD might be confused have come to necropsy, with no evidence that necropsy rates have increased over that time.[936] The high incidence of pathological diagnosis in these 13 CJD cases is therefore remarkable, and probably reflects the existence of CJD surveillance (with an autopsy rate of 70%), augmented by the concern of the patients' families and clinicians that the patients' illnesses may have been related to BSE. By implication, had this concern and surveillance not existed one-half of the cases may not have been detected. However, even if only five or six cases of CJD in persons under the age of 40 had been detected over the period 1995-6 this would still have to be considered a remarkable occurrence given the rarity of the disease in this age group.

**Table 53: Variant CJD mechanism of referral**

| |
|---|
| Direct referral (cases 466, 517, 502 and 571) |
| Direct referral post cerebral biopsy (cases 433, 582 and 474) |
| Neuropathologist asked to review cerebral biopsy (case 417) |
| Neurology registrar phoned for advice (case 476) |
| Query about genetic test for CJD (case 467) |
| Query from immunology department (case 485) |
| Seen by myself in neurology clinic (case 497) |
| Following lecture in which young cases of CJD were discussed (case 480) |
| Enquiry from NCJDSU following newspaper report (case 499) |

A further issue is whether CJD in young persons had previously occurred, but been undiagnosed or unreported. In support of this hypothesis, a 16-year-old British patient was identified who had been pathologically diagnosed before 1990 as not CJD, but upon re-examination of pathological material in 1996 was found to have CJD. Furthermore, three CJD cases, aged 19, 23, and 27 years, were identified in Poland in the course of a study of subacute sclerosing panencephalitis (SSPE), and arguably these patients would not have undergone pathological examination and reporting in the literature had this study not existed. To assess the possibility of previous under-reporting of young CJD cases the NCJDSU contacted groups and individuals connected with CJD surveillance around the world. In France, between 1968 and 1982, only two patients aged less than 30 years old were identified; only one in Japan between 1975 and 1977; and none at all in Israel between 1963 and 1987. During 1993-95 the European CJD surveillance project identified two cases, aged 22 and 34, in the Netherlands; two, aged 31 and 33, in Germany; two, aged 26 and 37, in France; and one aged 37 in Italy. Only a single patient aged less than 30 (16 years-old - mentioned above) was identified following a reassessment of CJD cases in the UK during 1970-90. Furthermore, a review of the clinical details of suspect but unconfirmed cases of SSPE held by the UK SSPE register did not provided evidence that cases of CJD were misdiagnosed as SSPE in the UK. Thus, although CJD in young persons had occurred around the world, this was clearly a very rare phenomenon. Perhaps the strongest argument against widespread under-diagnosis and under-reporting, is that in the months and years following the dissemination of the clinical and pathological details of vCJD, there has not been a torrent of reports of previous young CJD cases in the literature or at meetings.

In conclusion, there is reason to believe that underascertainment of CJD has occurred previously in the UK and elsewhere, in particular in the elderly, but also in young persons. Furthermore, there is evidence that young CJD cases in the UK in the period 1995-6 were more likely to undergo pathological examination, and hence be diagnosed as having CJD, than might be expected, probably reflecting concern that their illness was BSE-related. However, all 10 CJD cases (excluding iatrogenic and familial) under the age of 30 occurring in the UK since 1990 were detected over a short period from September 1995 up to the end of 1996. Although there was extensive publicity surrounding the report of the two teenage cases in late 1995, there has been considerable publicity regarding CJD and BSE since 1990, suggesting that a reason other than ascertainment bias is required to explain the occurrence of this group of cases. This hypothesis is supported by the lack of young CJD cases identified by surveillance in other European countries despite evidence of a general increased ascertainment over time and concern in these countries regarding BSE and CJD.

## Has there been a change in the clinical characteristics of CJD?

In parallel with the appreciation in early 1996 that the young CJD cases recently identified in the UK had an unusual and common neuropathology profile (discussed below), it became apparent that their clinical features were also atypical and consistent. This recognition was hampered by the fact that four other young CJD patients (three sporadic- cases 429, 515 and 527 and one familial - case 431) were also under investigation at this time. The clinical pattern of vCJD that emerged, and was described in the April 1996 Lancet paper,[84] was of a relatively long clinical duration (median 13 months vs. 4.5 for sporadic CJD) and presentation with behavioural or painful sensory symptoms following by progressive ataxia and dementia.[84] Choreiform movements were more frequently observed than in sporadic CJD and a characteristic periodic EEG was not seen. The clinical and investigative features of the 14 vCJD described in this thesis were later published[937,938] and it was noted that the

most recent four cases had a clinical phenotype similar to the previous 10 patients.[937,938] A number of additional characteristics were described in these more recent papers, in particular the presence of fleeting delusions, visual and auditory hallucinations and upgaze paresis. The potential novelty of the vCJD clinical phenotype and specificity of the various clinical characteristics are discussed later, and to avoid repetition are not detailed here.

## Has there been a change in the neuropathological characteristics of CJD?

Both of the teenage CJD patients (cases 417 and 433) reported in the Lancet in October 1995 had PrP-positive amyloid plaques seen on neuropathology.[902,903] Plaques are a characteristic feature of GSS, kuru and hGH-related iatrogenic CJD, but are seen in only 10% of sporadic CJD cases.[939] Plaques were also noted on review of a brain biopsy in October 1995 taken from a 29 year-old patient, (case 474) whose case was later published as a detailed report in April 1996.[898] By the end of January 1996, the diagnosis of CJD had been confirmed by neuropathology on four further young patients (cases 466, 476, 480 and 485, aged 30, 29, 29 and 41 respectively at death) and again all showed plaque deposition. Over the next seven weeks similar appearances were identified on examination of neuropathology from three further young patients (cases 467, 497 and 499, aged 30, 31 and 20 respectively at death). The plaques were noted to be widespread throughout the brain and spinal cord in those cases with complete CNS tissues available. Furthermore, the plaques demonstrated an unusual flower-like ('florid') appearance (see Figure 33) and PrP deposition was found to be more extensive than usually observed in CJD. The histological profile in these cases was reported to be so consistent that neuropathological samples from the cases were virtually indistinguishable.[84] Hence by March 20 1996 a series of 10 young CJD cases had been identified with a common and unusual neuropathological appearance. PrP gene analysis was available for most of these cases at that time and all were codon 129 methionine homozygotes without a genetic mutation. Previous work by the NCJDSU and others had found that plaques were associated with the VV or MV genotypes,[465,467,468] strengthening the hypothesis that these young patient had an unusual and potentially novel neuropathological phenotype.

An important issue was whether a similar pathological appearance had previously been reported, in particular in young CJD cases. A review of published reports of young patients worldwide did not reveal any descriptions of neuropathology identical to these UK cases (see Table 21).[84] Of the 14 cases of sporadic CJD aged less than 30 years identified outside the UK before March 1996 for whom pathological information was published, plaques were described in only one, and no mention was made as to whether these were surrounded by a halo of spongiform change, as seen with florid plaques.[914] In four of these cases,[212,655,904,906] pathological reports have been reviewed and there was no evidence of PrP plaques.[84] Immunocytochemical staining performed at the NCJDSU in early 1996 on a previously reported case of CJD aged 27 years from Poland[905] (courtesy of Professor Kulczycki) and on a 16-year-old patient from the UK dying of CJD in 1980, showed no evidence of plaque formation in either case.[84] The NCJDSU performed immunocytochemical staining on 11 hGH-related CJD cases (mean age 27.5 years) and although PrP plaques were present predominantly in the cerebellum, the neuropathological features in these cases were otherwise quite distinct from vCJD.[84] Pathological material from the vCJD cases was reviewed by neuropathologists from centres other than the NCJDSU, all with experience of human TSEs, and their independent view was that 'this was a distinct entity unlike any previously seen form of CJD'.[894]

Subsequent to the 20 March 1996 announcement by the UK Government of a new variant of CJD, cases 502, 517, 571 and 582 were identified during 1996 as having identical neuropathological features to the earlier vCJD patients. Similar appearances were found on neuropathological examination of a 26-year-old Frenchman who had died in December 1995.[343,940] In 1997 Budka reported that the pathological analysis of 232 definite CJD cases from around Europe between 1970 and 1996 demonstrated that at that time there was no evidence of cases of vCJD outside of the UK and France.[941]

Case 571 had palatine tonsillar tissue analysed following necropsy and this showed $PrP^{Res}$ deposition associated with follicular dendritic cells.[923] Hill subsequently reported widespread lymphoid involved in eight vCJD cases, including tonsil, spleen and lymph nodes (cervical, mediastinal, para-aortic, and mesenteric).[942] Similar involvement of lymphoid tissues was not identified in small numbers of cases of sporadic, familial or iatrogenic CJD, strengthening the hypothesis that vCJD is pathologically distinct from other forms of CJD.[942,943] The reason for this observation is unclear, but may be a property of the strain (presumed to be BSE) of the infectious agent.

Since the original published description of vCJD in April 1996[84] a number of authors have described cases with pathological features that they have considered suggestive of vCJD, in particular the presence of florid plaques. Takashima,[944] Shimizu[731] and Kopp[945] reported dura mater-associated CJD cases with florid plaques. All four of their patients showed pathological changes similar to vCJD and codon 129 methionine homozygosity. Three had an illness duration of eight months or more and only a single case had a periodic EEG. However, all four cases presented with ataxia, had a history of a dura mater graft 9-11 years previously, were aged 47 or older at onset.[731] and did not exhibit any of the other distinguishing neuropathological features of vCJD.[920] Additional PrP size and glycotyping was performed in three of the cases[731,946] and all showed a pattern distinct from the type 4 of vCJD (see later).[947] A florid plaque was illustrated in a picture accompanying the case report of a 54-year-old neurosurgeon with CJD.[377] This patient also had some clinical features reminiscent of vCJD: long duration, paraesthesia and lack of a periodic EEG. The pathology of this case has been reviewed by the NCJDSU, and although the presence of an occasional florid plaque in the cerebral cortex was confirmed, none were present in the cerebellum and other characteristic neuropathological features of vCJD were absent.[920]

In conclusion over the period 1995-6, 14 cases of CJD in patients under the age of 50 at onset were identified in the UK with a consistent neuropathological profile which appears not to have been described previously.

## A summary of the evidence that a novel form of CJD has occurred in the UK

The overall incidence of CJD in the UK has increased over the period 1970-96, particularly in the oldest age groups. These observations probably represent improved case ascertainment rather than a real change in incidence. The high rate of CJD in dairy farmers in the UK is comparable to that observed in dairy farmers in countries where BSE is rare or absent, suggesting a cause not directly related to BSE. The clinical characteristics of the UK cattle farmers with CJD in addition to biological and molecular strain typing experiments support the hypothesis that the cases they are not due to BSE.

The detection of a group of young patients with CJD during 1995 and 1996 in the UK is very unlikely to be explained by chance alone. Increased ascertainment of CJD in young persons could be anticipated due to

improved surveillance and concern relating to CJD and BSE. However, data from the surveillance of CJD in other European countries in the 1990s and from the UK for the years 1990-94, showing a much lower incidence of CJD in young persons compared to that seen in the UK from 1995-6, argues against ascertainment bias as the sole explanation for the occurrence of CJD in this group of young patients. Furthermore an unusual and consistent pathological phenotype is seen in these cases, who also share clinical characteristics which are relative uncommon for sporadic CJD, even in young people (see later). Taken together the above data argues that a new variant of CJD had been detected in the UK in 1995-6.

## Variant CJD - nomenclature

One issue that followed the identification of this novel form of CJD was that of nomenclature. Initially the cases were referred to as 'new variant CJD' as a result of the title of the Lancet paper in which they were first described as a group – 'A new variant of Creutzfeldt-Jakob disease in the UK'. However a number of other names have been suggested or used:

- Human BSE[948]
- Narang disease[949]
- Will-Ironside syndrome[950]
- Kuru[951]
- Atypical CJD (proposed by Dr CJ Gibbs Jr.)
- Bovine Creutzfeldt-Jakob disease[933]
- Pediatric CJD[952]
- A new form of transmissible spongiform encephalopathy[953]

Concern was expressed about the appropriateness of the term 'new' variant and many journals referred to the disease as variant CJD instead. The UK SEAC discussed this issue at its meeting on 18 March 1999 and elected that the term variant CJD should be used instead of new variant CJD.[954]

## WHAT IS THE EVIDENCE THAT VARIANT CJD IS CAUSED BY THE BSE AGENT?

On 20 March 1996 the UK SEAC issued a statement regarding the 10 cases of vCJD that had recently been identified. The committee concluded: 'in the absence of any credible alternative the most likely explanation at present is that these cases are linked to exposure to BSE before the introduction of the SBO ban in 1989'. Some authors were sceptical about this proposed link. Wickham asked 'How can a committee of scientists come to a conclusion on the basis of no credible alternative? Why exposure to BSE and not to microwave ovens, high voltage power lines, or organophosphorus sheep dips?[955] Fitzpatrick states that 'there is only the weakest circumstantial backing' for the proposed connection between BSE and vCJD.[956] Foncin wrote that 'the case for a causal relation between BSE and CJD is too weak to warrant the claim even of a "possible" link', and suggested that the 10 cases might be a form of GSS.[957] In July 1996 a group of specialists were asked about their belief in the proposition that BSE causes the variant form of CJD. Their responses averaged 5.4 (SD 2.8) on a scale of 0 (no belief) to 10 (absolute certainty).'[958] The following pages discuss the evidence that vCJD is causally linked with BSE.

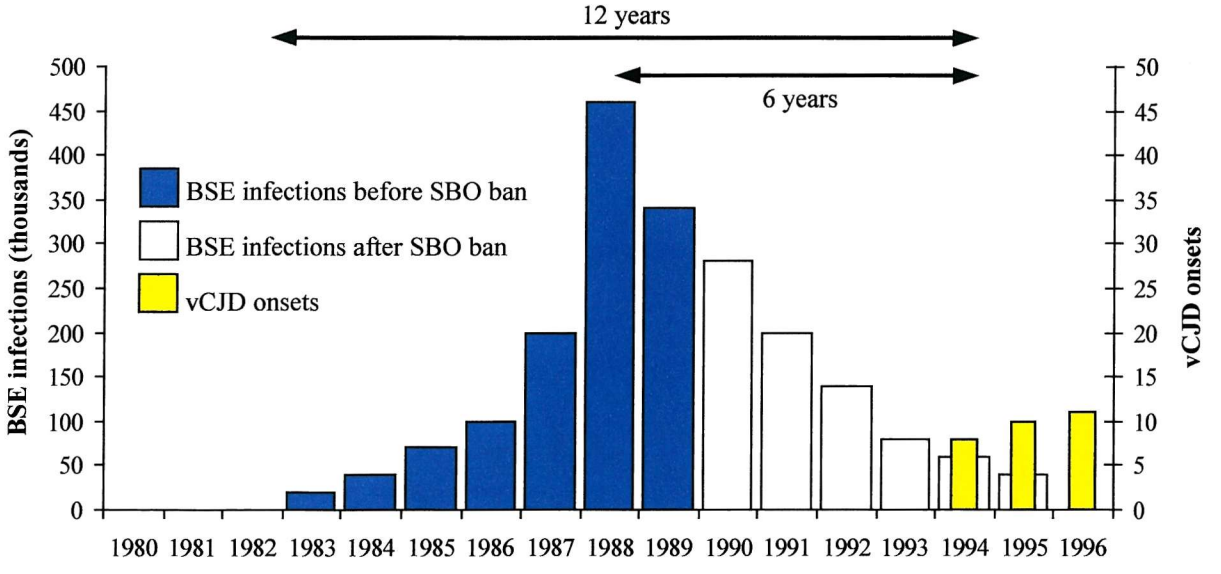
## Spatial and temporal association

Up to the end of 1996 14 definite and one 'probable' case (see Annex 8) of vCJD had been identified in the UK and a single case in France. By the end of June 2001 102 vCJD cases have been identified in the UK, three cases in France and a single case in the Republic of Ireland (who had lived in the UK for several years in the 1980s).

Over 99% of BSE cases have occurred in the UK, thus providing a strong spatial link with vCJD. Three cases of vCJD had never visited the UK and were longstanding residents of France, a country with an extremely small number of cases of BSE relative to the UK (see Table 14). However, the occurrence of these cases is not incompatible with the hypothesis that the BSE agent is the source of vCJD. France has been one of the biggest markets for exported British beef, beef products, and cattle[7,959] and prior to 1996 about 10% of beef consumed in France originated from the UK.[960] One possible explanation for the occurrence of over 90% of the vCJD cases arising in the UK is that there is a greater degree of case ascertainment in the UK compared to other countries in which CJD surveillance is minimal or absent. WHO found as part of its activities to promote global CJD surveillance[961] that most countries in Africa, Asia and, to a lesser extent, South America have an extremely low incidence of CJD or have never reported a case.[194] However, many countries have well developed surveillance and the incidence of CJD in Austria, Germany, Italy, USA and Australia is comparable to that observed in the UK, suggesting a high degree of case ascertainment. Furthermore suspect, but unconfirmed, vCJD cases have been reported in many countries (Belgium, Chile, France, Germany, Hungary, Japan, Russia, South Africa, South Korea, Spain and Thailand) arguing that clinicians are aware of vCJD outside of the UK, and that if vCJD cases had occurred in these countries they could have been identified.

Figure 40 shows the temporal association between BSE and vCJD (estimates for number of the BSE infections – used as a surrogate for exposure - is taken from Anderson[962]). Exposure of the human population to the BSE agent in the UK is likely to have been greatest in the 1980s, and especially toward the end of that decade, before the SBO ban was introduced. This would be consistent with an incubation period of 5-10 years for the first vCJD cases, a range compatible with what is known about the incubation period of TSEs. For example, the youngest age of a patient with kuru was 4.5 years[4] and the shortest incubation period of hGH-related CJD is estimated at five years.[25] Verdrager argued that the identification of the 10 cases of vCJD 10 years after the beginning of the BSE epidemic in the UK 'can be considered as epidemiological proof of a causal link…'[963]

**Figure 40: BSE 'exposure' and number of cases of vCJD by year of onset**

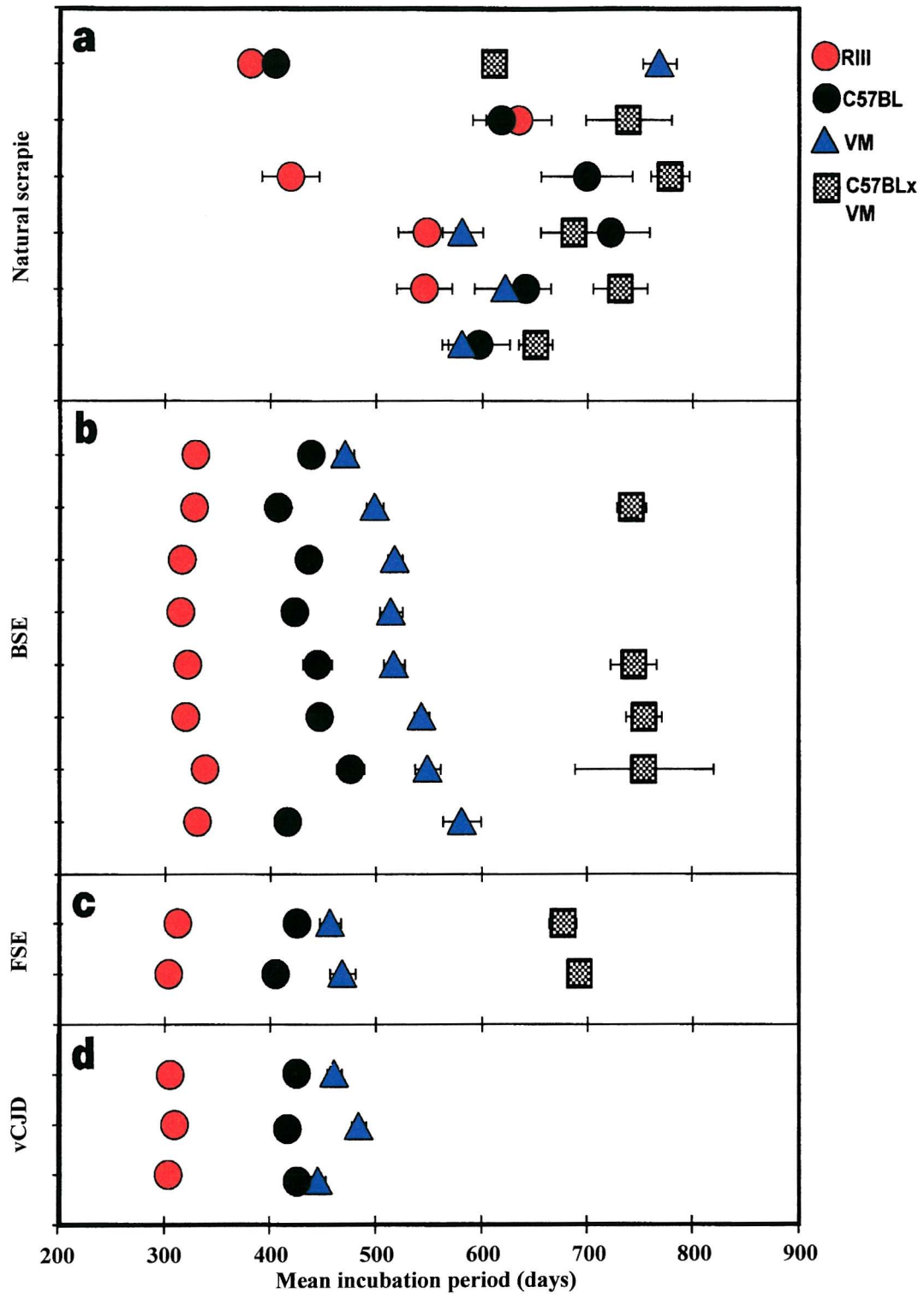


## Biological strain type: Transmission characteristics in mice

The most persuasive experimental evidence that vCJD is due to the BSE agent comes from 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 (see Figure 41-42).[964-967] Such experiments have shown that at least 20 distinct strains of scrapie exist, whereas BSE is due only to a single strain of agent, which is distinct from those of scrapie.[183,968,969] Furthermore, the transmission characteristics of FSE, the novel spongiform encephalopathies of kudu and nyala, 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.[183] Similar strain typing experiments subsequently performed for sporadic CJD and vCJD have shown that the vCJD agent has the same transmission characteristics as the BSE agent and that these characteristics differ from those of the limited number of sporadic CJD cases tested (see Figures 41-43).[12,13]

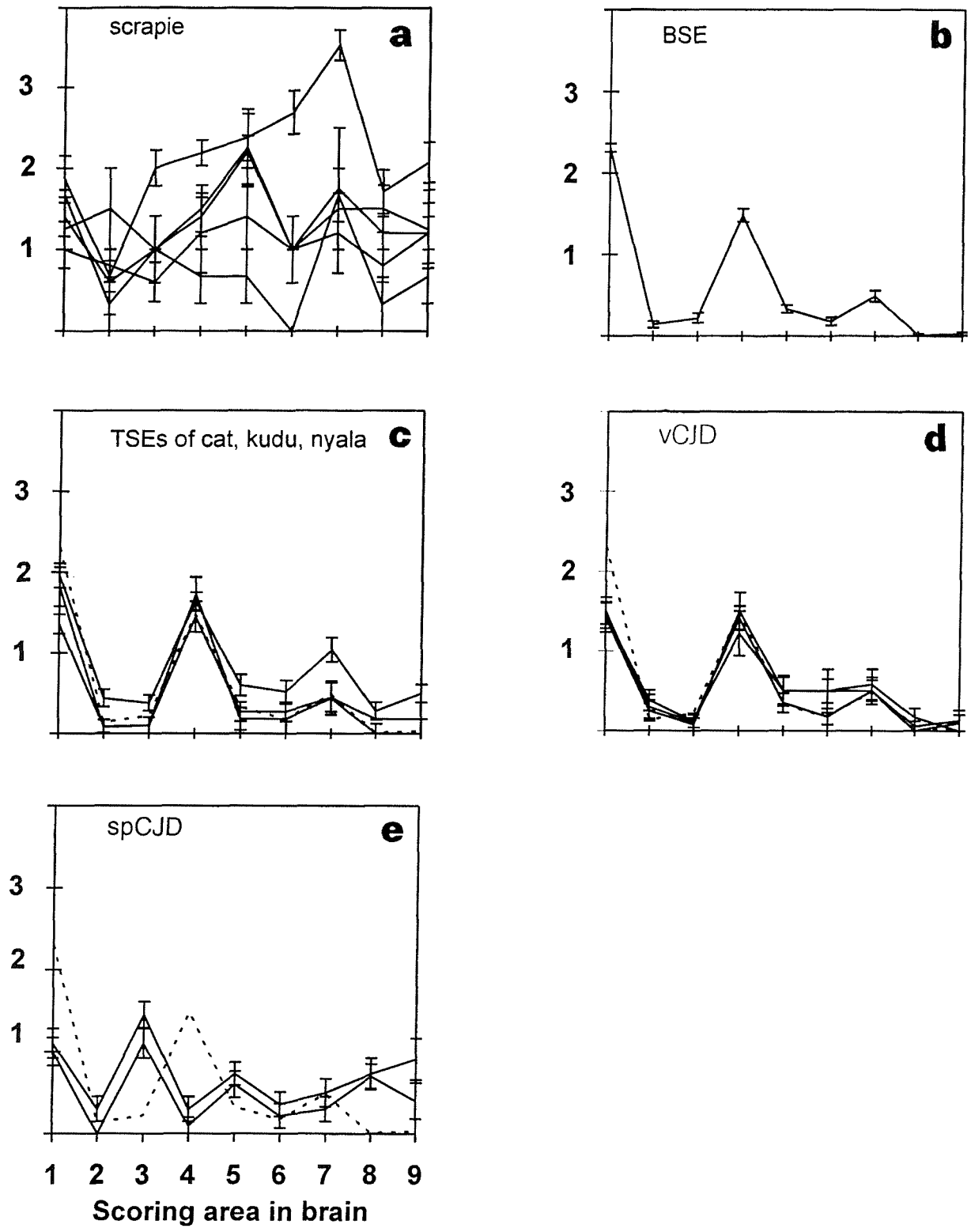
Hill[14]and Scott[970] have also reported transmission studies of vCJD, but using different hosts and less well established techniques. Hill studied transgenic and wild-type FVB mice inoculated with brain material from various forms of CJD and BSE in cattle.[14] The 'humanised' transgenic mice expressed only human PrP ($HuPrP^{+/+}$ $Prn\text{-}p^{0/0}$) and were codon 129 valine homozygous. In retrospect, it would perhaps have been more helpful if the mice were methionine homozygotes (as seen in all vCJD cases to date), but at the time the mice were constructed there was reason to believe that humans with valine homozygosity may be more susceptible to acquired forms of CJD, as this had been observed in iatrogenic CJD associated with pituitary hormones.[736,971] The transgenic mice were susceptible to sporadic and iatrogenic CJD with a short incubation period and almost all animals contracted disease, consistent with a lack of species barrier. However, with wild-type FVB mice only occasional transmissions, at longer and variable incubation periods, were seen, as would be expected due to the species barrier. In contrast, efficient transmission of vCJD to FVB mice was observed, although incubation periods were prolonged. The attack rate of vCJD in the transgenic mice was reduced in comparison to typical CJD cases, and incubation periods were generally more variable and prolonged. The clinical course in the transgenic mice inoculated with vCJD was much longer than in the mice inoculated with conventional CJD. Some of the mice showed the unusual feature of walking backwards, a characteristic not observed in transmission of several other human prion diseases. The transmission characteristics of BSE to FVB mice was reminiscent of those of vCJD: efficient transmission albeit with prolonged and variable incubation periods. BSE was transmitted to the humanised transgenic mice with a low attack rate similar to that seen with vCJD. However, in contrast to vCJD the incubation period was much longer (mean 602 v 228 days). Clinically these transmissions resulted in a phenotype similar to that with transmitted vCJD. Striking similarities in PrP deposition patterns between the BSE- and vCJD-inoculated mice were reported. The authors stated that detailed neuropathological studies would be published elsewhere. This is still awaited to the best of my knowledge. Hill's results show that vCJD has transmission characteristics that distinguish it from other CJD subtypes, and which would perhaps be more in keeping with a TSE agent of non-human origin. Furthermore these characteristics are shared, largely, by the BSE agent. Thus, Hill's findings are compatible with the hypothesis that the agent that causes vCJD is that same as that associated with BSE.

**Figure 41: Incubation times in mice with spongiform encephalopathies**



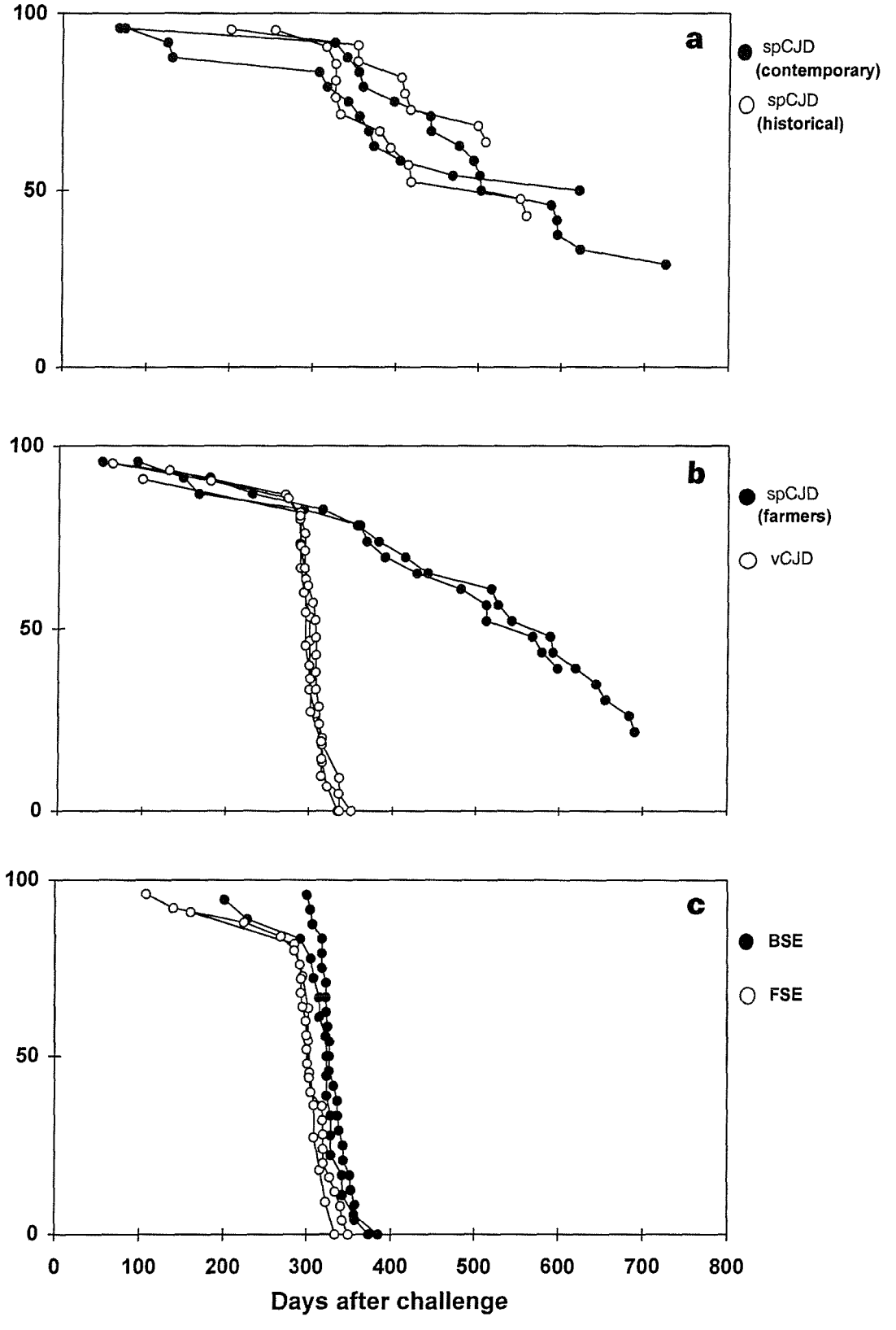

Incubation periods in RIII, C57BL, VM and C57BL x VM mice in transmissions of: **a**, natural scrapie from six sheep; **b**, BSE from eight cattle; **c**, FSE from two cats; and **d**, three cases of vCJD. C57BL x VM mice were not included in the first, third and fourth BSE transmission; missing symbols elsewhere indicate that no clinical disease was seen in these groups up to the natural lifespan of the mice. The result of vCJD transmission to C57BL x VM mice is awaited, but is known to be long.[13] Data are mean ± standard error of the mean.

**Figure 42: Lesion profiles for mice following transmission of spongiform encephalopathies**




Lesion profiles are for RIII mice in transmissions of: **a**, natural scrapie; **b**, BSE, **c**, FSE and TSEs from a greater kudu and a nyala; **d**, vCJD and **e**, sporadic CJD from two sources, a farmer and a contemporary case. The BSE profile is shown as a dotted line in **c–e**. Vacuolation was scored on a scale of 0-5 in the following scoring areas: 1, dorsal medulla; 2, cerebellar cortex; 3, superior colliculus; 4, hypothalamus; 5, thalamus; 6, hippocampus; 7, septum; 8, retrosplenial and adjacent motor cortex; and 9, cingulate and adjacent motor cortex. Data are mean ± standard error of the mean.

**Figure 43: Survival curves for mice following transmission of spongiform encephalopathies**




Survival curves are for female RIII mice in transmissions of: **a**, sporadic CJD from cases with no known occupational exposure to BSE; **b**, sporadic CJD from two farmers and vCJD from three sources; and **c**, BSE from the two cattle sources and FSE from two cats

Scott reported that transgenic (Tg) mice expressing bovine (Bo) PrP serially propagate BSE prions and that there is no species barrier for transmission from cattle to Tg(BoPrP) mice.[970] These mice were also highly susceptible to a vCJD and natural sheep scrapie. The incubation times, neuropathology, and disease-associated PrP isoforms in the Tg(BoPrP)$Prnp^{0/0}$ mice inoculated with vCJD and BSE brain extracts were indistinguishable and differed 'dramatically' from those seen in mice infected with natural scrapie. Scott suggests, rather boldly, that his findings provided the most compelling evidence at that time that prions from cattle with BSE have infected humans and caused fatal neurodegeneration. However, his study used sheep scrapie rather than non-variant CJD as a control group. Therefore, he cannot conclude from the results of his study alone that the properties of the vCJD agent observed were not simply a function of a human derived agent.[13]

## Molecular strain

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 the disease-associated PrP. PrP structural variations are reflected in different Western blotting patterns after limited protease digestion, and the various banding patterns observed can be used as markers of 'molecular strain'. PrP has two possible sites of glycosylation and the electrophoretic pattern shows three bands (corresponding to the di-, mono-, and unglycosylated forms).

Collinge performed PrP Western blotting on brain material from 10 cases of vCJD, 26 cases of sporadic CJD and seven patients with iatrogenic CJD. He found that the molecular weight of $PrP^{Res}$ after proteolysis was lower in vCJD and iatrogenic CJD than in the sporadic CJD, and that vCJD was associated with a higher proportion of diglycosylated $PrP^{Res}$. The pattern seen in the vCJD cases was highly consistent and has been termed type 4. A similar type 4 pattern was observed in cattle with BSE and in mice, domestic cats and monkeys, either experimentally or naturally infected by BSE (see Figure 44).[947] Collinge concluded that the identification of 'this distinct molecular marker' serves to support the proposal that vCJD is a new subtype of TSE resulting from BSE transmission to humans.[947]

Parchi later confirmed that PrP glycoform ratios distinguished the first French vCJD case from patients with sporadic or iatrogenic CJD and kuru, but in contrast to Collinge reported that vCJD shared the $PrP^{Res}$ electrophoretic mobility with a subset of sporadic CJD, namely type 2 (using the Parchi classification system).[972] Parchi proposed that the PrP type associated with vCJD should be called type 2B. An analysis of 24 vCJD cases and 74 sporadic CJD cases by the UK NCJDSU concurred with Parchi's findings: the $PrP^{Res}$ isoform pattern from patients with vCJD was indistinguishable in terms of mobility from one of the major isoform patterns seen in sporadic CJD. Furthermore, as found by Collinge and Parchi, vCJD samples showed a glycoform profile characterised by the predominance of di-glycosylated $PrP^{Res}$ in all cases. This pattern was not seen in any of the 74 sporadic CJD cases analysed,[920] but has been reported in familial CJD.[972]

Parchi and Prusiner criticised Collinge's work using PrP glycoform ratios to try and relate vCJD to BSE.[846,972] They note that glycosylation is a co- and post- translational event likely to be affected by the cell type of the species. Parchi reports that transmission of sporadic CJD type 1 to chimeric transgenic animals consistently reproduces the size but not the glycoform pattern of the original $PrP^{Res}$ inoculate.[972] Somerville's found that although molecular strains were the same when using inbred strains of mice, very different patterns could be

observed in different hosts, implying that the BSE strain identification in different animals might not be as straightforward as it might seem.[973] Bruce[12] notes that a BSE-like glycoform pattern has also been seen in experimental scrapie unrelated to BSE[973] and in FFI in humans.[974] She commented that it would therefore be premature to draw conclusions concerning causative links between TSEs in different species on the basis of glycoform ratio analysis alone.[12]

Aguzzi states[975] that the unique feature of the type 4 PrP - namely the high ratio of diglycosylated to unglycosylated PrP - may reflect increased susceptibility of the diglycosylated form of PrP to BSE-mediated conformational change. Alternatively, cells that preferentially produce the diglycosylated form may be more readily targeted by the BSE agent.

## Transmission to primates

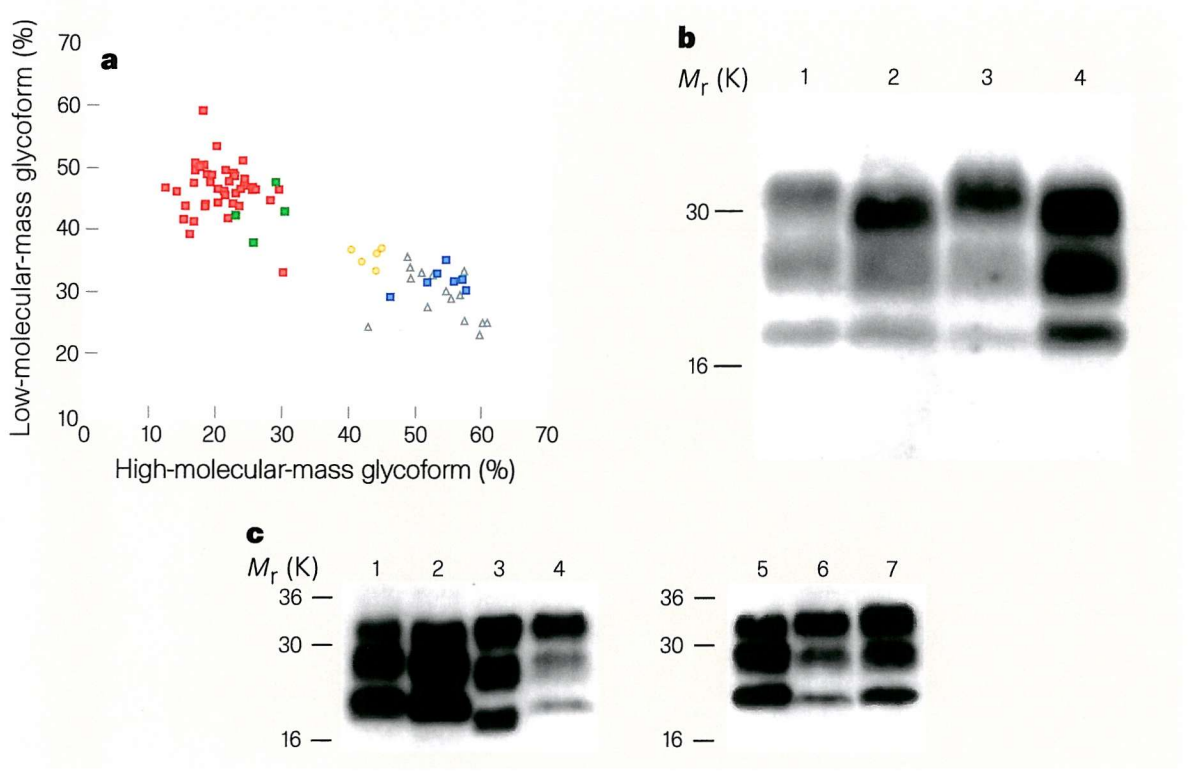
Three cynomolgus macaques inoculated by the intracerebral route with BSE brain homogenate all developed a spongiform encephalopathy after three years, with neuropathological features similar to vCJD, in particular the distribution of spongiform change and the typical morphology of the plaques (see Figure 45).[15] Although this supports the hypothesis that vCJD is caused by BSE,[976] its significance is tempered by the results of transmission studies of BSE in common marmosets, which did not show typical florid plaques.[977] Furthermore florid plaques are seen in TSEs unrelated to BSE, including Icelandic scrapie in mice,[921] and CWD in white-tailed deer.[922]

## A summary of the evidence indicating that vCJD is caused by the BSE agent

The geographical association between BSE and vCJD is striking and is not adequately explained by ascertainment bias. The temporal association between these diseases is entirely in keeping with known incubations periods for acquired CJD. Transmission studies using various murine hosts have convincingly shown that the properties of the vCJD and BSE agent are almost indistinguishable, but differ from those of scrapie and sporadic CJD. A novel technique of assessing 'molecular strain' through analysis of $PrP^{Res}$ glycoform ratios also shown that BSE and vCJD shares similar properties that differ from other forms of CJD. The utility of this technique has however been criticised. Macaque monkeys inoculated with BSE have a neuropathological appearance similar to vCJD lending further support to the hypothesis that the diseases are due to the same agent.

In summary scientific evidence strongly supports the hypothesis that vCJD is caused by the BSE agent. Whether this constitutes 'proof' is a matter of debate. Some have argued that it does,[963] whereas others have considered that only when a declining epidemic curve, similar to that seen in kuru, is observed, can an association be assured. More sceptical individuals might concur with the views of the philosopher of science Karl Popper who argues that whereas a theory can be falsified it can never be proved.[845]

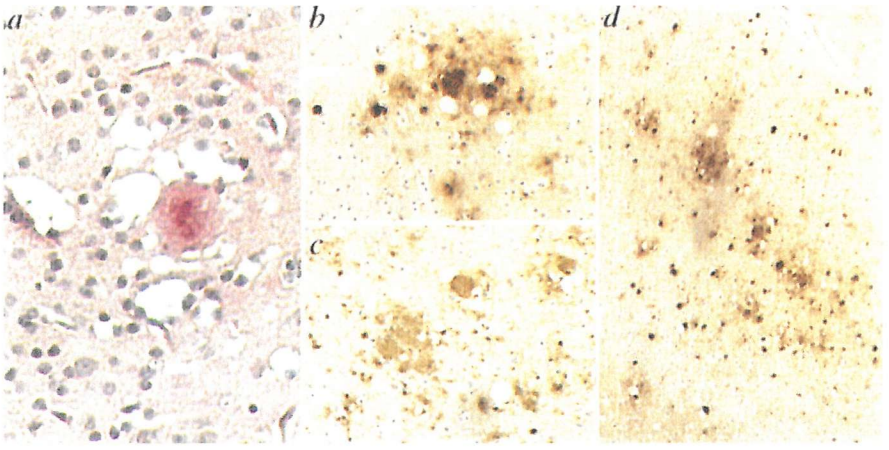
**Figure 44: Transmission of prion diseases to mice**




**a,** Scatter graph of proportions of protease-resistant PrP in the high-molecular-mass (di-glycosylated) and low-molecular-mass (mono-glycosylated) glycoforms in individual human cases and FVB mice with experimentally transmitted CJD, vCJD or BSE. Sporadic and iatrogenic CJD cases ($PrP^{Sc}$ types 1–3), red squares; vCJD, yellow circles; transmissions of typical CJD to FVB mice, green squares; BSE to FVB mice, blue squares. Transmissions of vCJD to FVB mice, open triangles. **b,c,** Western blots of brain homogenates after pre-treatment with proteinase K using anti-PrP polyclonal antibody 95-108 **(b)** or anti-PrP monoclonal antibody 3F4 **(c)**. **b,** Transmission of vCJD and BSE to non-transgenic (FVB) mice. Lane 1, human vCJD; 2, vCJD-inoculated FVB mouse (same case as lane 1); 3, BSE; 4, BSE-inoculated FVB mouse (same case as in lane 3). **c,** Transmission of vCJD to $HuPrP^{+/+}$ $Prn\text{-}p^{0/0}$ transgenic mice. Lane 1, human CJD, type-2 $PrP^{Sc}$; 2, transgenic mouse inoculated with CJD case from lane 1 showing type-2 pattern; 3, human vCJD case, type-4 $PrP^{Sc}$; 4, transgenic mouse inoculated with vCJD from lane 3 showing type-5 pattern; 5, human CJD case, type-2 $PrP^{Sc}$; 6 and 7, type-5 $PrP^{Sc}$ pattern in vCJD-inoculated transgenic mice.

**Figure 45: Pathology of BSE transmission to macaques**

**a**, PAS staining of a florid plaque; **b-d**, PrP immuno-histochemistry showing pericellular deposits, kuru-type PrP plaques associated with vacuoles (**b**) and larger uni- or multicentric plaques (**c**).



## Age

A striking feature of the vCJD patients is their young age. The lack of cases in older age groups could be due to a number of factors, including misdiagnosis, age-related exposure to the BSE agent or reduced susceptibility.[84] That vCJD may have been missed in a small number of very old people seems plausible but that it would be missed in a large number, or in patients in their late 40s and early 50s seems less so.[7] Indeed a recent case has been detected in an individual aged 74.[978] Therefore underascertainment is not likely to be responsible for the marked difference in the age-related incidence of vCJD.

Gore has postulated that age-related dietary factors may explain the young age of the vCJD cases.[27] She reviewed British dietary surveys of teenagers and adults conducted in the 1980s and 1990s to identify whether consumption of certain foods - in particular, those likely to contain mechanically-recovered meat (MRM), was related to age (see Table 54). She concluded that the surveys showed that consumption of beefburgers declines strikingly with age, and states that 'age-related exposure of patients to the agent of BSE should not be downplayed'. Verdrager concurs with this view, and suggests that most of the infections probably occurred via the oral route and argues that as, in the UK, hamburgers are eaten predominantly by young people this may explain the age distribution of vCJD.[316] He adds that 'conjunctival, nasal and skin contamination could also have occurred in children and young people scratching their impetigo, acne or herpes or cleaning their nose or eyes with fingers contaminated by highly infectious BSE-infected bovine brain pool homogenates used as binding agent for the preparation of hamburgers'.[963] Certainly burgers had the potential to act as a vehicle for transmission of BSE – the Phillips Inquiry was told by a quality assurance executive with Somerfield supermarkets that: 'If you want to buy the cheapest economy burger you can get, it can be made very largely out of MRM.'[979] However, although one of the studies cited by Gore showed that consumption of meat pies and pastries was slightly higher in the younger age groups the second study found no such difference. Furthermore, neither study provided evidence that other MRM-containing products were consumed more by younger people. This is particularly relevant as MRM was used in sausages, meat pies and various other meat products[979] and there is no evidence (as far as I am aware) that MRM was used to a lesser extent in these food stuffs compared to burgers. Even in the unlikely circumstance that dietary exposure to the BSE agent was mainly via beefburger consumption, the relative difference of consumption with age does not match the striking difference in vCJD incidence between young and old persons (see Table 55). That age-related dietary exposure alone explains the observed age distribution of vCJD therefore appears unlikely, but the possibility that it is a contributory factor can not be dismissed.

Exposure to the BSE agent in the UK is likely to have been greatest during 1985-1990, especially at the end of this period. At the beginning of 1985 the age range of the 14 vCJD cases was 6-39 years (mean 16) and 50% of the cases were aged 18-20. This would argue strongly against childhood vaccination as cause of the vCJD in the majority of cases.[980]

**Table 54: Consumption of meat products by age in two British dietary surveys**

| Age group (years) | Burgers* | Sausages | Meat pies & pastries† | Other meat products‡ | Beef§ |
|---|---|---|---|---|---|
| **Dietary and nutrition survey of British adults, 1986-7**[981] | | | | | |
| 16-24 | 45 (218) | 54 (162) | 61 (316) | 39 (254) | 72 (323) |
| 25-34 | 35 (185) | 52 (147) | 54 (246) | 46 (188) | 73 (404) |
| 35-49 | 26 (133) | 55 (131) | 52 (240) | 44 (202) | 78 (345) |
| 50-64 | 13 (120) | 49 (137) | 49 (223) | 50 (178) | 77 (294) |
| **EPIC food frequency questionnaire, Norfolk respondents,1993-5** | | | | | |
| 45-54 | 27 | 71 | 54 | 37 | 88 |
| 55-64 | 15 | 70 | 53 | 41 | 87 |
| 65-74 | 12 | 72 | 52 | 43 | 89 |

Consumption of meat products by age in two British dietary surveys. Values are percentages of subjects consuming product in seven day period (with mean consumption in grams over that period in parentheses) in first survey and percentages of respondents consuming product once a week or more in second survey.

EPIC=European prospective investigation of cancer.

*Burgers and kebabs in dietary and nutrition survey of British adults,[981] beefburgers in EPIC food frequency questionnaire.

†Savoury pies - for example, meat pie, pork pie, pasties, steak and kidney pie, sausage rolls—in EPIC food frequency questionnaire.

‡Corned beef, Spam, luncheon meat in EPIC food frequency questionnaire.

§Beef and veal in dietary and nutrition survey of British adults,[981] and beef in the form of roast beef, steak, mince, stew, or casserole in EPIC food frequency questionnaire.

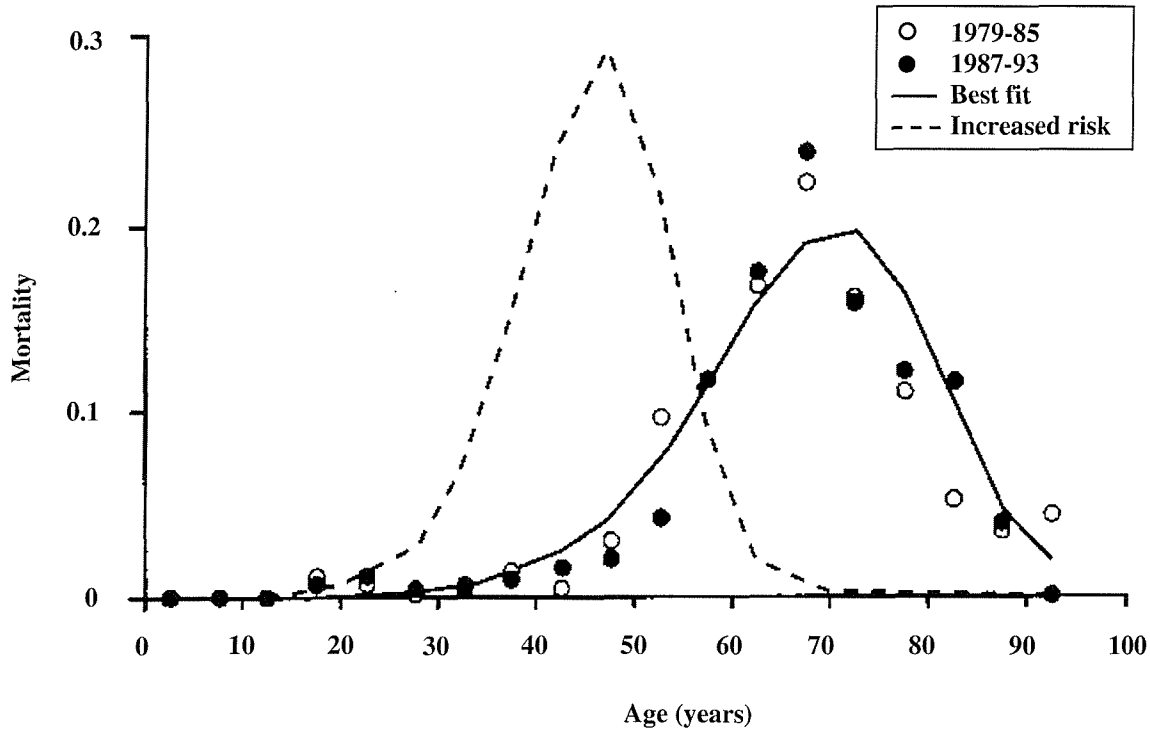
**Table 55: Cases of vCJD and consumption of burgers and kebabs from Gore's data by age group**

| Date of birth | Age on 1st January 1987 | Number of vCJD cases | Relative burger and kebab consumption* |
|---|---|---|---|
| 2-1-62 to 1-1-71 | 16-24 | 30 | 6.3 |
| 2-1-52 to 1-1-62 | 25-34 | 11 | 4.2 |
| 2-1-37 to1-1-52 | 35-49 | 5 | 2.2 |
| 2-1-22 to 1-1-37 | 50-64 | 1 | 1.0 |

* Figures derived from Gore's data[27] by multiplying percentages of subjects consuming product in seven day period by mean consumption in grams over that period and making data for 50-64 age group equal unity.

Neilson has postulated a radical hypothesis to explain the youth of the vCJD patients. He states that 'standard survival modelling techniques suggest that there is a small subpopulation susceptible to death due to CJD, whose relatively high mean age at death could potentially be reduced by exposure to a triggering agent derived from cattle' (I presume referring to the BSE agent).[16] He argues that the age-related mortality distribution of CJD is well fit by a multistage (Weibull) mortality distribution in which mortality rises as the seventh power of age (for all ages) and the risk of death is restricted to about 70 per million of the general population, or about 3500 people. Figure 46 shows the projected effect of a 50% increase in exposure to risk. Declining mortality after age 67 can be explained by the reduction in the number of susceptible people. Should Neilson's hypothesis be correct, the size of the vCJD epidemic in the UK will be limited to the persons who are currently expected to develop sporadic CJD in their lifetime. It is not stated in Neilson's article how a 'triggering agent' could speed up the development of CJD and I am not aware of any biological further evidence in support of this theory.

**Figure 46: Effect of 50% increase risk of CJD on age distribution theorised by Neilson**




Probability distribution of mortality from CJD in England and Wales during 1979-85 and 1987-93, best fitting Weibull distribution, and hypothetical curve based on 50% increase in risk. From Neilson.[16]

Another radical explanation for the relative young age of the vCJD cases is that older persons may acquire a resistance factor. MacKnight[982] and Deslys[960] postulate the following: it is known that infection with one strain of scrapie can protect against infection with other strains[844] and that some strains have been shown to have an incubation period exceeding a normal lifespan in some hosts.[983] If this is also true for humans, hypothetically most people could, at a young age, acquire a TSE agent with an extremely long incubation period that exceeds the normal lifespan and prevents infection with another TSE agent. As vCJD is presumed to be caused by a strain which first entered the human population in the mid-1980s, then it would be expected that the majority of cases would occur in people who were very young at that time as older persons would be resistant to infection. As a result, the exposure of the whole population to BSE could be apparent in an unusual form of CJD in a small group of the population under the age of 40.

A further possible explanation for the youth of the vCJD cases is that the incubation period of the BSE agent might increase in proportion to age. Mckinley found that the incubation period of scrapie in young hamsters increased with age over a period nil to 20 days after birth. He postulated that this observation could be related to the size of the animal's brain at the time of inoculation, i.e. the smaller the size of the brain the less time it would take for infection to spread.[984] Kimberlin performed a study in which mice were inoculated intraperitoneally with scrapie at age six or 30 days. He found that if the same dose per body weight (the younger mice weighed one-tenth that of the older animals) was used the incubation period was similar, but that if an identical dose was used the incubation period was shorter in the younger animals.[985] However, he cited other studies (Chandler[986] and Gibbs[987]) of experimentally-induced scrapie in mice that showed that the age of the animal at the time of intracerebral inoculation had little effect on the length of the incubation period even using the same infectious dose. Furthermore, Brown reported that in his series of 440 experimental attempts at transmission of human TSEs to primates (mostly intracerebral) age of the recipient was not found to affect incubation period.[70] In addition, Klitzman states that 'the natural incubation period of kuru... is not determined by age at exposure.'[988] The most compelling, and probably relevant, argument against age-related variation in incubation period as a sole explanation for the age distribution of vCJD comes from a study of statistical modelling by Ghani et al.[18] Their analysis of vCJD cases does not indicate an increase in median age over time and they suggest that the age distribution of vCJD cases could not have arisen from an age-dependent incubation period alone.

A final proposed explanation for the young age of the vCJD cases is age-related susceptibility. Donnelly reported that statistical analysis of BSE suggests a strong trend with age, with highest susceptibility occurring between six and 18 months of age.[989] However, Brown's series of 440 experimental attempts at transmission of human TSEs to primates (mostly via the intracerebral route) found no relation between age of the recipient and susceptibility.[70] This would suggest that if age-related susceptibility occurs its origins reside prior to the agent infection of the brain. Various hypotheses have been put forward. DeArmond has proposed that the increased propensity of young persons to inflamed throat mucosa due to tonsillitis facilitates infection when eating BSE-contaminated foods.[990] Grant boldly claims that 'the apparent preference of vCJD for young people... is surely because the victims were children between 1981 and 1989... and therefore shedding teeth. The resultant raw areas in their gums provided the organism with direct access to the bloodstream, thus shortening the incubation period.'[991] Shmakov states in an article for the journal Gut[992] that 'it is well known that increased permeability to macromolecules represents a normal feature of neonatal gut epithelium. The decreased number of Peyer's patches in older animals[993] may also contribute to their reduced susceptibility to oral TSE infection. In humans,

the number of Peyer's patches was reported to be higher in the intestines of teenagers than in young children or adults.[994] Assuming that particle uptake capacity is a function of the area occupied by the epithelium overlying Peyer's patches, these differences may explain susceptibility of young people to vCJD.' There is little evidence that I am aware of for or against any of these hypotheses, although it is worth noting that in 1981 11 of the 14 vCJD cases described in this thesis had past the age (12 years) at which the milk teeth are usually shed, and presumably if shedding teeth is a major risk factor for vCJD then the elderly would be more susceptible than those in their 20s and 30s.

In conclusion, failure of case detection in the elderly, a change in incubation period with age, exposure to vaccinations or shedding of milk teeth are not likely sole explanations for the observed age distribution of vCJD. Increased exposure in young people due to consumption of hamburgers or increased susceptibility of this group due to age-related changes in the gastro-intestinal lymphoid system are possible explanations, although there is no convincing evidence currently available to support either of these hypotheses. Perhaps it is most likely that a combination of factors are involved, including some of those above and others as yet unidentified.

## Symptoms and physical signs

Variant CJD is characterised clinically by presentation with psychiatric or painful sensory disturbance or both. Later progressive ataxia and dementia develop in addition to multifocal neurological signs and a movement disorder, often chorea. The patient becomes increasingly incapacitated, often leading to a state of akinetic mutism. The characteristic EEG of sporadic CJD is not seen and the illness duration is relatively prolonged in comparison to sporadic CJD. This is a clinical phenotype that is only rarely seen in non-variant CJD.[681,995,996] The various clinical features are discussed below.

### Psychiatric

Nine (64%) of the 14 vCJD cases presented solely with psychiatric features. Four other vCJD cases had psychiatric disturbance at the same time as, or within a few weeks of, other presenting symptoms, most often painful sensory disturbance. Only a single patient (case 480) had a delay to the development of psychiatric symptoms (five months after the onset of limb paraesthesia). In contrast surveys of non-variant (mostly sporadic) CJD report behavioural disturbance at presentation in only 15-49% of patients and during the illness course in 45-62% of patients (see Annexes 1 and 2). A study of Chinese sporadic CJD cases reports behavioural abnormality as an initial manifestation in 50% and during the illness course in 71% of cases.[997] However, this series consisted of only 14 cases. Its noteworthy that in sporadic CJD psychiatric disturbance is usually apparent for only a short period before progressive dementia and immobility dominate the clinical picture, whereas in vCJD the psychiatric phase typically lasts for a number of months. A recent update describing the psychiatric features of a total of 47 vCJD cases (including the 14 cases in this thesis) has shown that psychiatric symptoms remain a consistent feature occurring in 69% of cases at onset and in all but one during the illness course.[285]

Nine (64%) of the 14 vCJD cases had depression early in their illness, four (29%) as an initial symptom, and four were diagnosed as having a severe or major depressive illness by a psychiatrist. Seven (50%) of the patients received antidepressant medication. Three of the 14 vCJD patients were diagnosed as suffering from a psychosis. Case reports of other forms of CJD with prominent early depression or psychosis are rare.[567,649,995,998-

[1000] Three CJD case series have documented the frequency of depression as a presenting feature as 3-16%.[196,210,1001] However, other studies reported by Masullo[206] and Zerr[1002] found that 45% and 42% of cases respectively had depression as an early feature. These values are at odds with an analysis of non-variant CJD cases in the UK (Table 37) which found that only 25% of cases were depressed at some point in their illness and, furthermore, in contrast to vCJD, only 22% had received an antidepressant medication. One possible explanation for the high incidence of depression in Zerr's series from Germany is that neurologists in this country are trained in both neurology and psychiatry and thus may tend to emphasise psychiatric features.

Fleeting delusions occurred in 86% of the 14 vCJD cases. In contrast, in series of non-variant CJD (including from the UK - Table 37) delusions were reported as an initial symptom and during the illness course in only 6% and 12-37% of patients respectively.[210,256] Delusions may occur in a wide range of other organic disorders,[1003] including Alzheimer's disease.[1004] However in Alzheimer's disease, the delusions are usually simple and sustained, whereas those occurring in vCJD were usually fleeting and complex.[1004]

Visual hallucinations were reported in 57% of the 14 vCJD patients and in only 16-29% of non-variant CJD cases.[196,210,253,997] In sporadic CJD visual hallucinations may actually be more often misperceptions, and are usually associated with cortical blindness, whereas in vCJD descriptions of the visual hallucinations are often more suggestive of true hallucinations rather than misinterpretation and cortical blindness was not a feature at the time hallucinations developed. The incidence of auditory hallucinations at any time during the illness has not been reported in cases series of non-variant CJD, but this was described as a presenting symptom in one of 32 cases in one study[210] and in two of 49 cases in another.[1001] Table 37 shows that auditory hallucinations were much more common in vCJD (36%) compared to non-variant CJD (2%) cases in the UK.

The possibility of a functional or hysterical illness was raised in three (cases 474, 485, 582) (21%) of the 14 vCJD cases. Lundberg found that in 11% of cases in his series of sporadic CJD initial symptoms were described in patient's notes as 'functional' or 'hysterical'.[196]

Nine (64%) vCJD cases had early insomnia and most had excessive daytime sleepiness. In contrast 'disordered sleep', insomnia and somnolence were reported as early features in only 14-34%,[254,1002] 6-7%[210,254] and 7%[254] of patents respectively in case series' of non-variant CJD. Weight loss was noted early in the course of the illness in 11 (79%) of the 14 vCJD cases but Brown found that only 15% of non-variant CJD cases in his study had weight loss in the illness prodrome.[254]

All but one of the 14 vCJD patients saw a psychiatrist during their illness, which contrasts to only 36% of the non-variant CJD cases in Table 37. At the time of initial psychiatric assessment the lack of significant organic features led to a functional diagnosis in many patients (see Table 25). Forgetfulness and unsteadiness of gait, although common at this stage, were mild and occasionally fluctuating, and considered either part of the functional disorder or a side effect of medication. For the eight vCJD cases that saw a neurologist after initial psychiatric assessment the delay between assessments ranged from one week to eight months (median two months). Part of this delay may relate to the infrequency that psychiatrists see patients with CJD. Fleminger estimates that only one in a hundred psychiatrists see a case of CJD over a five-year period.[1005] It is perhaps therefore unsurprising that none of the psychiatric notes mention CJD as a possibility and none of the cases were referred to the NCJDSU by a psychiatrist.

### Sensory

Sensory symptoms were reported in 57% of the 14 vCJD cases and in 29% this was a presenting symptom. In case series of non-variant CJD, 3-6% of patients had sensory symptoms at onset or on presentation[70,196,208,251,253] and up to 18% during the illness course (see Annexes 1 and 2 and Table 37).[70,196,251,997] A study of 509 CJD cases in Europe reported a higher incidence of sensory symptoms at onset (15%), but the proportion who had this feature at any point during the illness was not stated.[211] The first French patient with vCJD had marked painful sensory disturbance and clinical evidence of polyneuropathy, confirmed by EMG. A small number of sporadic and familial CJD cases are also reported with peripheral neuropathies.[426,428-430,720,759,1006-1009] Furthermore, $PrP^{Res}$ has been identified in dorsal root ganglia (although not peripheral nerve[1010]) of patients with vCJD. In the UK vCJD cases, the lack of lower motor neuron signs, the distribution of the sensory disturbance (including hemi-sensory and face), and normal nerve conduction and EMG studies would argue for a central origin, possibly thalamic, to explain the sensory symptoms.[937]

### Clinical features during illness course

Many clinical features occur at a similar frequency in sporadic and variant CJD, particularly those characteristic of the later stages of the disease, e.g. cerebellar signs, primitive reflexes, myoclonus, akinetic mutism, etc (see Annex 2 and Table 37). Tremor was noted in 29% of the 14 cases and although from personal experience this is a unusual feature in sporadic CJD, the only case series that has commented on the frequency of this reported it in 25% of patients.[256] A number of other features are remarkable due to their relatively high frequency in vCJD. Dystonia was described in five (36%) cases but was found in only 16% of sporadic CJD cases in the only series that has reported this feature.[252] Upgaze paresis was noted in seven (50%) of the 14 vCJD cases, but was found in only 5% of Brown's case series of non-variant CJD patients[70] and in only 13% of the UK non-variant cases in Table 37. Seven (50%) of the vCJD cases had chorea in contrast to only 12% of non-variant cases in Table 37 and 13% of UK sporadic CJD cases from 1990-4 reported by de Silva.[252] Why chorea should be more common in vCJD is unclear, but possibly reflects a greater degree of basal ganglia involvement. Interestingly, all three cases of vCJD initially diagnosed as psychotic developed chorea, raising the possibility that these psychiatric features may be due to basal ganglia damage also.

### Are the clinical features of vCJD similar to peripherally acquired iatrogenic CJD?

One possible explanation for the discrepancy between the clinical features of vCJD and sporadic CJD is that the former is peripherally acquired whereas the latter is thought by many to arise as a spontaneous event in the CNS. If this is so then the clinical features of peripherally acquired iatrogenic CJD may be expected to more closely resemble those of vCJD. This, however, does not appear to be supported by Lang's analysis of 22 dura mater cases, in whom behavioural disturbance was reported at onset in only 27% and sensory disturbance in 9%.[351] The situation with hGH CJD is less clear. Billette de Villemeur reported on the clinical characteristics of 34 such cases from France and noted 'mood disturbance' in 62% at onset and symptoms of 'sensory and visual loss' in 73% at the time of clinical examination.[364] However, mood disturbance was not further elaborated upon and whether 'sensory and visual loss' should really have read 'sensory or visual loss' is unclear. One unambiguous distinction between variant and hGH CJD is the presence of visual disturbance and gait ataxia at

presentation. These features are reported to occur in 94% and 88% of hGH cases[364] compared to 7% and 0% of vCJD patients respectively.

**Are the clinical features of vCJD similar to sporadic CJD in young people**

The above paragraphs compare the prevalence of various clinical features in vCJD with case series' of non-variant CJD. The latter consist almost entirely of patients older than 30 whereas the majority of vCJD cases were aged less than 30. Prior to 2001 clinical details of only 19 sporadic CJD cases aged 30 or less had been published (those listed in Table 21 and others[202,325,690,916]). For two of these cases information is extremely limited and only a summary of the clinical features is available in English for a further six. Psychiatric disturbance was described in 10 of the 19 cases, depression in one, delusions in two, emotional lability in three, apathy/withdrawal in three, aggression in two and hallucinations in five cases. Sensory disturbance was described in only one case, two cases were noted to have chorea and none were found to have upgaze paresis. A recent report by Zerr summaries the clinical characteristics of 23 sporadic CJD cases under the age of 50 in Germany.[1011] Although 'psychiatric conditions were often diagnosed initially', in contrast to vCJD 16 patients (70%) presented with progressive dementia and seven (30%) with ataxia. Therefore, the limited data that is available does not suggest that the clinical features of vCJD are due to young age. The results of a large study of transmission of human TSEs to primates supports this conclusion, as age of the recipient was not found to affect clinical features.[70]

Strain of the agent has been shown to influence clinical characteristics in experimental transmission of TSEs.[1012,1013] The most plausible explanation for the phenotypic difference between variant and non-variant CJD is that the former arises (presumably) due to a single strain of agent that differs from those associated with other forms of human TSE.

## Duration of illness

The median and mean duration of illness in case series of non-variant CJD is consistently reported as approximately four-and-a-half and eight months respectively. About 10% of patients live longer than one year and 5% longer than two years.[70] This contrasts with a median and mean duration in vCJD of 15.5 months and 17.5 months respectively, with 57% of cases surviving beyond a year and 14% longer than two years. In part the extended duration of vCJD is due to the prodrome of psychiatric or sensory disturbance and it is possible that the inclusion of coincidental minor psychiatric features has led to a falsely prolonged estimate of disease duration. However, even if the disease onset is taken as the time forgetfulness or unsteadiness developed (the most common presenting symptoms of sporadic CJD), the median disease duration is still more than double that of sporadic CJD. Furthermore, Wientjens' study of over 400 mostly sporadic CJD cases in Europe found that sensory or psychiatric symptoms at onset did not lead to a significant increase in median illness duration.[211] There are a number of possible explanations for the relatively prolonged duration observed in vCJD including an effect of age, route of infection or agent strain. These are discussed below.

### Effect of age

An inverse correlation between age and length of illness was found in a large study of CJD cases (mostly sporadic but including a small number with vCJD) in Europe. Median durations of four, five and 10 months were reported for the age groups >64, 40-64 and <40 years respectively.[211] In keeping with this observation, a series of 23 sporadic CJD cases aged less than 50 (youngest 24, median 44) identified in Germany had a mean disease duration of 14 months (range 3-31). Furthermore, reviewing the length of illness of the previously reported cases of sporadic CJD aged 30 or less in the world literature (Table 21 and others[202,325,690,916]) shows a range of 3-55 months (median 10, mean 19, and 29% <6 months). These results are in keeping with the hypothesis that the long illness course of vCJD is a function of the age of the patients. Although the observation that age was not correlated with duration in the 14 vCJD reported in this thesis appears at odds with this hypothesis, a recent analysis of over 100 vCJD cases does indicate an inverse correlation between age and illness duration (personal communication Bob Will). Two factors that might explain the relatively prolonged illness in young patients is that they are better able to survive the complications of immobility and may tend to be treated more intensively. This alone still does not completely explain the prolonged course of vCJD as the median time from onset of forgetfulness or unsteadiness to becoming bedbound in vCJD exceeds the median total disease duration of sporadic CJD.

### Effect of peripheral infection

Iatrogenic CJD is associated with a longer clinical course compared to sporadic CJD, with the exception of cases due to direct intracerebral inoculation (see Table 56). Hornabrook's study of 123 kuru patients reported that none died in less than four months, only 19% had a duration less than six months and 38% survived over one year.[28] These results are in keeping with the hypothesis that the long illness course of vCJD is due peripheral infection. This statement needs to be interpreted with caution as the age of patients with kuru and iatrogenic CJD are young compared to sporadic CJD (see Table 56) and the relatively long duration may be a function of age (see above). However, the data on kuru and iatrogenic CJD does not support this explanation. A review of 25 iatrogenic CJD related to dural homografts and corneal grafts patients (age 19-67 mean 40) for whom both age and disease duration were known found that the mean and median duration of the 13 youngest patients (all <40) vs. the 12 oldest (all >40) was 10 and eight months respectively vs. 13 and nine months respectively.[351] Hornabrook's study of kuru also showed that older cases had a longer duration (see Table 57).[28]

**Table 56: Age and duration (in months) of iatrogenic CJD**

| Iatrogenic risk | Number of cases | Mean age | Duration (mean) | Duration (median) | Duration <6 months |
|---|---|---|---|---|---|
| Corneal transplanatation[71,340,341] | 3 | 55 | 21 | 14 | 0 |
| Neurosurgery or EEG electrodes[57,324,326] | 5 | 44 | 5 | 5 | 60% |
| Dural homograft[351] | 25 | 38 | 10 | 7 | 41% |
| Human growth hormone[364] | 34 | 21 | 17 | Not stated | ~5-10% |

**Table 57: Duration of illness in months by age group for 123 cases of kuru**

| Age | <6 months | 6-12 months | 12-18 months | 18-24 months | Total number |
|---|---|---|---|---|---|
| **5-10** | 67% | 33% | - | - | 6 |
| **11-15** | 58% | 42% | - | - | 12 |
| **16-20** | - | 50% | 50% | - | 10 |
| **21-30** | 17% | 50% | 17% | 15% | 52 |
| **31-40** | 9% | 38% | 32% | 21% | 34 |
| **>40** | - | 22% | 56% | 22% | 9 |

### Effect of agent strain

One factor that appears to distinguish vCJD from peripherally acquired iatrogenic CJD, kuru and sporadic CJD in young people is that vCJD is consistently associated with an illness course in excess of six months (see Tables 28, 56 and 57). The reason for this discrepancy is unclear but theoretically could arise if vCJD was due to a single strain of agent whereas the other forms of TSE are each due to more that one strain, including one or some with a short incubation period. Furthermore, although the above paragraphs argue that the relatively long duration of vCJD may be a function of peripheral acquisition of the infectious agent and, arguably to a lesser extent, age, an alternative hypothesis is that the main determinant of the prolonged duration in vCJD is that this is an effect of the strain of the BSE infectious agent.

## Diagnostic tests

Progressive dementia in a young person is a rare occurrence, but one with a wide differential diagnosis. As the possibility of CJD was not usually raised until late in the illness course in most of 14 vCJD cases it is not surprising that the patients underwent intensive investigation. This is reflected in the large number of tests listed in Tables 30-34. In essence there is no evidence that vCJD is associated with any abnormality of haematology, blood or urine biochemistry, immunology or microbiology. In particular, liver function tests were normal in all cases at least once and although seven cases had abnormalities these were minor, transient or both. Such abnormalities are most likely explained by intercurrent infection or medication rather than hepatic involvement in the disease process. It is noteworthy that case 480 (with normal LFTs) had a normal liver biopsy.

### Cerebrospinal fluid analysis

CSF did not show a raised leucocyte count or oligoclonal bands, in keeping with the vast majority of sporadic CJD cases. A raised protein up to 1.0g/L was present in just under one-third of patients, again in-keeping with

the findings in sporadic CJD. The cause of the raised protein is unknown, but may reflect blood-brain barrier dysfunction as a non-specific consequence of debility (personal communication Alison Green).

CSF 14-3-3 protein was positive in two of five cases. A more recent analysis of 45 vCJD and 34 controls (suspect vCJD with final alternative diagnoses) found that the sensitivity was ~50% and specificity was 93%. Although this specificity is comparable with that of 14-3-3 testing in sporadic CJD the sensitivity is much lower (~90% in sporadic CJD). The reason for this discrepancy is unclear, but may relate to the relatively prolonged illness in vCJD, as sporadic cases with a long illness course are also less likely to have a positive 14-3-3 test (personal communication Alison Green).

**Electroencephalography**

The EEG has traditionally been the most reliable non-invasive diagnostic test for CJD, with the majority of sporadic and centrally inoculated iatrogenic cases demonstrating a characteristic periodic pattern. Despite repeat recordings (up to two days before death in case 476) this pattern was not seen in vCJD. Most cases had abnormal EEG tracings but these showed non-specific slow-waves changes only. One possibility for this observation is that the failure to develop a periodic recording is purely a function of age. However, this is not supported by the limited data available for young sporadic CJD patients, although this does suggest that a periodic EEG is less common in younger patients. A periodic EEG was seen in five (22%) of 23 cases under the age of 50 reported by Zerr and six (46%) of 13 cases aged 30 or less reported in the literature prior to 2001 for whom the results of EEG were documented (Table 21 and others[202,325,690,916]).

The EEG in kuru does not show the classical periodic pattern either[631] nor do the recordings of the vast majority of patients with hGH-related CJD.[364] This suggests that the EEG findings in vCJD may reflect peripheral inoculation of the infective agent. An alternative hypothesis is that the failure to develop a periodic EEG in vCJD is an effect of the strain of infective agent, perhaps due to targeting of particular cell types.

Goto[609] postulates that the periodic synchronous discharges characteristic of sporadic CJD 'develop when the loss of cortical inhibition over subcortical structures brings about hypersynchronisation of the neuron group in the central grey matter. Such abnormal discharges are conducted upward by the relatively uninvolved diffuse thalamocortical projection system and fire the remaining cortical neurons.' If this mechanism is correct then the lack of periodic discharges in vCJD may relate to a greater degree of thalamic dysfunction compared to sporadic CJD, in keeping with the observed distribution of pathological changes. The fact that periodic discharges are also uncommon in FFI[451] supports this hypothesis.

**Cerebral imaging**

In keeping with other forms of CJD, cranial CT was generally unremarkable in vCJD. The pattern of posterior thalamic high signal on MRI documented in the hospital radiology reports of cases 474 and 499 is unusual and prompted the MRI study. This is discussed later.

A single patient underwent PET scanning and this was normal. Despite several reports mentioning CJD cases that have had PET studies I am not aware of any with a normal appearance. However, this may reflect bias due

to cases with normal PET being less likely to be published. Furthermore, the vCJD patient had the normal PET scan at an early stage of her illness before any physical neurological signs had occurred.

Two vCJD patients had SPECT studies and both were abnormal. De Silva suggested that the occurrence of these abnormalities in the context of a normal EEG or cerebral MRI raised or supported the diagnosis of an organic encephalopathy.[10] He added that although the perfusion abnormalities were non-specific and could not be claimed to be diagnostic of vCJD, they were more marked and widespread than those associated with depression. Therefore if SPECT has a role in vCJD diagnosis, it may be through raising the possibility of the disease in young patients presenting with unusual psychiatric or neurological syndromes, with normal or unhelpful results from routine investigations.

**Tonsil biopsy**

Although the precise role of lymphoreticular tissues in the pathogenesis of TSEs remains unclear, the pre-clinical involvement of various lymphoid organs has long been recognised. The diagnostic implications of this feature has been investigated and $PrP^{Res}$ has been detected in lymphoid follicles in palatine tonsil taken from presymptomatic sheep with a genetic susceptibility to scrapie.[1014] This prompted study of palatine tonsillar tissues in human TSEs. Positive results were first demonstrated in tissue taken at necropsy from a vCJD patient (case 571).[923] A subsequent analysis of various lymphoid tissues, including palatine tonsil, obtained at necropsy failed to identify $PrP^{Res}$ in a small number of CJD and GSS cases.[943] A more recent and larger study has confirmed the previous findings: all nine vCJD cases showed the presence of $PrP^{Sc}$ in tonsillar tissue obtained at necropsy, but post-mortem assessment of 16 sporadic CJD cases and a single iatrogenic cases all proved negative.[942] Ante-mortem tonsillar biopsies were analysed in 20 cases. Nine were positive and three of these cases were subsequently confirm as vCJD on neuropathology. A diagnosis of vCJD was considered clinically likely in the other six cases who are either alive, awaiting the results of neuropathology or died without autopsy being performed. Three of the cases with negative biopsies have been confirmed neuropathologically, two were familial CJD cases and one sporadic CJD. One of the negative cases had clinically probable sporadic CJD but in the remaining seven negative cases a diagnosis of CJD was clinically unlikely. The reason why tonsillar tissue contains $PrP^{Res}$ positivity in vCJD but not other forms of CJD is unknown, but presumably reflects either a property of the agent strain in humans or the peripheral route of infection. The absence of $PrP^{Res}$ in tonsillar tissue from hGH cases would argue against the route of infection as the sole cause of the observed $PrP^{Res}$ detection in vCJD tonsil tissue.

The above results suggested that ante-mortem tonsil biopsy could be used as a diagnostic test for vCJD and a positive result has now been incorporated into the criteria for a 'probable' case of vCJD (see Annex 3). However, the use of this procedure in patients suspected to have vCJD is controversial and concern has been expressed regarding potential morbidity[1015] (e.g. bleeding, infection and the risk of general anaesthesia). The most common differential diagnoses in cases of suspect vCJD that have died are sporadic CJD, Alzheimer's disease and cerebral vasculitis.[285] Tonsil biopsy cannot provide specific information on these diagnostic possibilities and there is insufficient current data to exclude the possibility of a false negative or false positive tonsil biopsy. On the basis of the published data, tonsil biopsy cannot be recommended as a routine diagnostic procedure in the investigation of vCJD, not least because most suspect cases will not have vCJD.[1016] For some suspect cases without bilateral pulvinar high signal on MRI (discussed later), tonsil biopsy may have a role.

## Codon 129 and other genetic factors

Codon 129 genotype influences susceptibility to sporadic CJD: in the UK 79% of cases are methionine homozygous compared to 37% of Caucasian controls. All 14 vCJD cases were found to have this genotype and a recent update has shown that all 91 vCJD cases for whom PrP gene analysis had been performed are also methionine homozygous.[1017] The possibility that this is a chance observation can be dismissed[1018] as can the hypothesis that it is solely due to a peripheral route of inoculation, as just over half of hGH cases tested were not methionine homozygotes.[25] Other explanations are that, 1) BSE has caused CJD in persons with other codon 129 genotypes, but that this has not been recognised, 2) individuals with the MM genotype are more susceptible to BSE, 3) methionine homozygosity is associated with a shorter incubation period compared with other genotypes, and 4) persons with the MV or VV genotypes are resistant to BSE.

### Has BSE caused CJD in persons with codon 129 genotypes other than MM?

The possibility that BSE has transmitted to humans with other codon 129 genotypes causing a pathological phenotype distinct from vCJD is worth considering. Indeed, a number of young valine homozygous sporadic CJD cases have been identified in the UK since 1995, many with clinical features in keeping with vCJD, in particular long duration and absence of a periodic EEG appearance (see report of case 527 above).[466] However, a comparable excess of young valine homozygous CJD cases, with similar clinical characteristics, has been reported in other European countries.[466] This observation argues against BSE as the cause of the young valine homozygous cases in the UK, and this argument will be further strengthened if the results of conventional strain typing studies do not show the transmission characterises of the BSE agent.

### Are methionine homozygotes more susceptible to BSE?

The results of some animal transmission studies (although not all[1019]) argue that the ability to transmit a TSE between species is, in part, determined by the homology of their PrP structures, with the region containing codon 129 thought to be particularly important.[184,1020-1026] Unlike humans, bovines have not been found to carry a polymorphism at the equivalent site to codon 129,[462] - they only code for methionine. Therefore if vCJD is due to infection with the BSE agent it could be hypothesised that methionine homozygosity would confer an increased susceptibility.[1018] In support of this, Raymond has shown a greater efficiency of in-vitro PrP interactions between bovine prions and human PrP with methionine at residue 129 compared to valine.[1027] However, Brown reported the codon 129 genotype of the four iatrogenic CJD cases (two EEG electrode related and one each associated with neurosurgery and corneal grafting) for whom the 'donors' codon 129 status was also known. All the donors and two of the recipients were methionine homozygotes but the other two recipients were heterozygotes.[1028] This observation appears at odds with the above hypothesis (although the number of cases is small), but one possible explanation is that Brown's study could not include cases with peripherally acquired disease. Thus, theoretically, peripheral interaction of bovine prions with host $PrP^{129M}$ prior to neuroinvasion, perhaps in the gut or lymphoreticular system, could be the crucial factor.

**Does methionine homozygosity predisposed to shorter incubation period?**

The observation that all vCJD cases to date are methionine homozygotes does not necessarily mean that individuals with other genotypes are less susceptible or immune to BSE. In theory, such persons could be just as (or even more) susceptible, but have yet to be observed because of a relatively prolonged incubation period determined by their codon 129 genotype. Indeed, studies of acquired human TSEs and animal transmission experiments have indicated that PrP polymorphisms can influence incubation period. A polymorphism at codon 136 of the ovine PrP gene affects time to onset of disease after intracerebral inoculation with BSE, with heterozygotes having almost twice the incubation period of homozygotes.[1029] A similar observation is seen in goats inoculated with BSE: bovine PrP codes for isoleucine (Ile) at codon 142 whereas goats have a Ile/Met polymorphism and goats homozygous for Ile have a shorter incubation period compared to goats with the Ile/Met or Met/Met genotypes.[1030] With regard to human TSEs, codon 129 genotype may influence incubation period in hGH-related CJD[1031,1032] and methionine homozygosity is reported to have predisposed to the development of kuru (see introduction section on codon 129).[471]

Goldmann has shown that the presence of arginine at codon 171 in Cheviot sheep resulted in none of the animal developing disease five years post inoculation (the end of the study) with BSE, whereas glutamine homozygotes developed disease within three years.[1029] Thus, although humans with codon 129 genotypes VV and MV may be susceptible BSE with a relatively long incubation period, it is possible that either this exceeds the human lifespan or that these individuals are resistant to infection.

**Would the clinicopathological of BSE in valine homozygotes or heterozygotes resemble vCJD?**

If BSE can transmit to humans with codon 129 genotypes other than MM, one issue is whether the clinicopathological phenotype of vCJD would be maintained. In sporadic CJD, patients with the VV genotype, in comparison to methionine homozygotes, have a relatively younger age at onset, more prolonged illness, decreased incidence of a periodic EEG and are more likely to have PrP plaques. In contrast, clinical and pathological features do not seem to be influenced by codon 129 genotype in hGH-related CJD[374,375,472] and in kuru although a shorter duration of illness was observed in homozygotes other clinical characteristics were similar for all genotypes.[472] Furthermore, McLean found that the neuropathology of three valine homozygous and two methionine homozygous kuru cases was similar[1033] (although another less rigorous study had reported that the presence of histologically recognisable plaques was limited to cases carrying at least one methionine allele[472]). Therefore, the available data does clearly indicate whether BSE transmission to humans with VV or MV genotypes is likely to cause disease with a clinicopathological phenotype resembling vCJD.

Western blotting of $PrP^{Res}$ from $HuPrP^{+/+129VV}$ $Prnp\text{-}p^{0/0}$ transgenic mice inoculated with vCJD showed a pattern considered distinct from variant and other forms of CJD and designated type 5.[14] Hill has suggested that this could be used to distinguish sporadic CJD in valine homozygotes from CJD due to BSE in persons with this genotype.[14] However, when the experiment was performed using BSE rather than vCJD as the inoculum (a model more analogous to human infection with BSE) Western blotting failed to detect $PrP^{Res}$, questioning the usefulness of this technique. Traditional biological strain typing may therefore be the best method for establishing whether BSE infection has occurred in valine homozygous or heterozygous persons with CJD.

### Other genetic factors

A gene or genes outwith the PrP gene has been shown to influenced clinical phenotype and incubation period in murine scrapie.[1034,1035] This would support the hypothesis that genes other than the PrP gene may influence the characteristics of BSE transmission to humans. Indeed, a greater number of the 14 vCJD cases had a family history of dementia compared to controls, raising the possibility that other 'dementia-susceptibility' genes may be relevant to developing vCJD. However, to date no such gene or genes have been identified. A prion doppel gene has recently been discovered, but a study of 41 cases of vCJD did not find evidence that polymorphism of this gene was associated with susceptibility to or phenotypic expression of vCJD.[481] Jackson reported a significantly reduced frequency of HLA-DQ7 in number of patients with vCJD,[1036] but this result has yet to be confirmed in a larger sample and may represent a chance finding.

## Is the clinicopathological phenotype of vCJD similar to kuru?

Claims have been made that the clinical features and pathology of vCJD and kuru are similar.[316] How valid are these? In kuru the presenting symptoms are characteristically gait disturbance (69%) or leg tremors (21%),[28] features that were observed at onset in one and none of the 14 vCJD cases. The frequency of the characteristic presenting features of vCJD, behavioural and sensory disturbance, in kuru is unclear, but 'headache and leg pains' were reported as initial symptoms in 10% of cases and 'severe depression' was noted during the illness in 7%.[28] Chorea occurs in kuru, and although its frequency is not described, it was seen less often than dystonia in a video series of kuru,[1037] in contrast to vCJD in which chorea is more prevalent. Upgaze paresis was reported in only 7% of kuru patients[1038] but was observed in half of the vCJD cases. Strabismus and pathological laughter, other characteristic features of kuru, were not seen in any of the 14 vCJD cases. In contrast to vCJD, dementia was only present in advanced stages of kuru and was not documented in the majority of patients.[28] EEG appearance and illness duration have been discussed above.

Two studies have compared the neuropathological features of vCJD and kuru. McLean[1033] analysed 11 kuru and 11 vCJD cases and found distinct differences: immunohistochemistry showed a much greater PrP load in all brain areas in vCJD, with the exception of the cerebellar granular layer, and although all the kuru cases had plaques, these did not have the morphological characteristics of the florid plaques seen in vCJD. A study by Lantos[1039] of four vCJD cases (two biopsies) and two kuru cases concurred with the observation that PrP deposition was more copious in vCJD and reported that florid plaques were only occasionally seen in kuru, noting that 'this configuration might have been a chance concurrence of PrP deposition and spongiform degeneration.' Lantos reported some differences from the other study, in particular that the pathological changes in the frontal cortex, hippocampus and the cerebellum were more marked in kuru.

In conclusion, clinical and pathological analysis of kuru cases has shown a pattern distinct from vCJD. This is in keeping with the hypothesis that the clinicopathological phenotype of vCJD can not be explained solely by route of infection and is more likely to be a function of agent strain.

## Differential diagnosis

The majority of cases referred to the NCJDSU with suspected vCJD during the study had an alternative final diagnosis (see Table 35). A recent update on the differential diagnosis has been produced (see Table 58) and shows that the most frequent conditions mimicking vCJD are sporadic CJD, Alzheimer's disease and cerebral vasculitis.[919] The diagnosis in the 19 patients (over one-third) with clinical improvement or recovery is uncertain, but, in the absence of any evidence of an inflammatory disorder, these should probably be classified as idiopathic encephalopathy, a clinical differential diagnosis reported in sporadic CJD.[285] Alternative clinical diagnoses include peripheral neuropathy, perhaps reflecting awareness of the sensory symptoms described in some patients with vCJD, and causes of cognitive impairment in younger patients, including Wilson's disease and vitamin $B_{12}$ deficiency. The latter conditions and cerebral vasculitis are of particular importance because they are potentially treatable. Due to the great public concern regarding BSE and CJD it is perhaps not surprising that patients with psychiatric conditions (neurosis[1040] and psychosis[1041]) incorrectly convinced that they have developed BSE have been reported.

**Table 58: Outcome in suspect vCJD cases with an alternative final diagnosis (n=51)**

| | |
|---|---|
| **Degenerative** | **Hereditary** |
| Sporadic CJD (n=9) | Huntington's disease (n=1) |
| Alzheimer's disease (n=6) | Probable familial spinocerebellar ataxia (n=1) |
| Corticostriatonigral degeneration (n=1) | |
| Multi-system degeneration (n=1) | **Metabolic** |
| | Wilson's disease (n=2) |
| **Infective/inflammatory** | ?Metabolic disorder (n=1) |
| Cerebral vasculitis (n=4) | |
| Encephalitis (n=1) | **Other** |
| Limbic encephalitis (n=1) | Cerebrovascular disease (n=2) |
| Post-viral encephalopathy (n=1) | Peripheral neuropathy (n=2) |
| Multiple sclerosis (n=1) | Vitamin B12 deficiency (n=1) |
| PML (n=1) | Normal brain (n=1) |
| Possible encephalitis lethargica (n=1) | No neuropathological diagnosis (n=1) |
| | Clinical recovery (n=4) or improvement (n=15) |

## Clinical and diagnostic features of vCJD: conclusions

Table 59 shows the percentage of various clinical features in vCJD, sporadic CJD and patients initially suspected to have vCJD but with an alternative final diagnosis. There is no single clinical characteristic that clearly distinguishes these groups, but certain features may be helpful discriminators. In particular, persistent sensory symptoms, early psychiatric disturbance, chorea and upgaze paresis are much more common in vCJD, whereas early forgetfulness and ataxia are relatively uncommon. Prolonged illness course and lack of a periodic

EEG are other features that appear to distinguish variant and sporadic CJD, although this disparity is less marked when vCJD is compared to young sporadic cases. Furthermore, some caution is required in comparing the frequency of clinical characteristics of vCJD with other CJD cases in the literature, in particular from large cases series, as the degree of scrutiny of the patient's records is unlikely to have been as intensive in the non-variant CJD patients. Certainly the records of the non-variant CJD cases in Table 37 were in general much less detailed than those of the vCJD patients. Thus, ascertainment bias may be contributing to the perception that certain characteristics have a greater prevalence in vCJD cases.

Psychiatric symptoms are a consistent feature of vCJD and there is a delay in the evolution of neurological signs in comparison with sporadic CJD. Can any clinical 'red flags' be identified that may point to, or away from, a diagnosis vCJD in the numerous patients that present with depression, anxiety and the other non-specific psychiatric symptoms observed in the vCJD cases? Formal psychometry was performed on six patients (cases 433, 466, 467, 480, 497 and 582), in some early in the illness course, and all showed clear global or multifocal cognitive impairment. Thus, normal psychometric testing may argue against a diagnosis of vCJD. Clearly this needs to interpreted with caution given the small number of cases and the lack of data on formal psychometry during the very early stages of the illness prior to the development of other neurological features. Conversely, psychometry indicating global cognitive impairment in a patient presenting with psychiatric symptoms suggestive of vCJD may support the diagnosis. Again caution would be required in interpreting such findings, which may not be readily distinguishable from those of pseudodementia in functional psychiatric illness.

Eight patients described paraesthesia or dysaesthesia or both early in their clinical illness and in four, sensory symptoms were the initial feature. These symptoms are very unusual in sporadic CJD or conditions that may mimic vCJD and their persistent and unremitting nature is distinct from the transient and usually painless sensory phenomena that occur in patients with anxiety and hyperventilation. Similar sensory symptoms may occur exceptionally in psychotic disease.[1042] The combination of recent-onset depression or psychosis and persistent paraesthesia/dysaesthesia may be a useful indicator of vCJD, but rarely sporadic CJD cases with both psychiatric and sensory disturbance do occur (see report of case 527).

Routine biochemistry and haematological tests were typically unremarkable, in keeping with other forms of CJD. Most EEG recordings showed non-specific slowing or, particularly early in the illness, a normal appearance. A periodic tracing was not seen in any case, possibly a consequence of peripheral infection or an effect of strain of the infectious agent or both. CSF analysis showed a raised protein in about one-third of patients, but oligoclonal bands or pleocytosis were not observed. Only five of the 14 cases has CSF studies for the 14-3-3 protein making it impossible to draw any firm conclusion about the utility of this test. A recent study of brain specific proteins in 45 vCJD cases found that the positive predictive value of CSF 14–3–3 was 86% and the negative predictive value was 63%, suggesting that this tests is not as useful a marker for vCJD as it is for sporadic CJD.[1043] An increased CSF tau had a positive predictive value of 93% and a negative predictive value of 81%, and therefore shows more promise as a diagnostic indicator of vCJD.[1043]

Cranial CT and PET did not show any significant abnormalities in the vCJD patients, but two cases had SPECT abnormalities, a finding that may possibly be a helpful indicator of an organic syndrome in some individuals with suspected vCJD.[10] MRI is discussed later.

**Table 59: Clinical features of vCJD, sporadic CJD and suspect vCJD cases with alternative final diagnoses**

| Feature | Variant CJD (n=47)* | Sporadic CJD† | Non-cases‡ |
|---|---|---|---|
| Psychiatric symptoms | 98 (69) | 55 (40) | 90 |
| Sensory disturbance | 70 (19) | 10 (5) | 20 |
| Ataxia | 100 (9) | 85 (40) | 65 |
| Forgetfulness | 85 (17) | >95 (50) | - |
| Involuntary movements | 100 (5) | 90 (15) | - |
| Myoclonus | 70 (0) | 80 (<2) | 65 |
| Chorea | 55 (0) | 10 (1) | 25§ |
| Dystonia | 30 (4) | <15 (0) | 20 |
| Upgaze paresis | 36 (0) | 5 (0) | 7 |
| Dementia | 100 (0) | >95 | 80 |
| Akinetic mutism | 51 (0) | 55 (0) | 30 |

Values are percentage of patients with the feature during the illness course and at onset in parenthesis.

*Details of all the clinical features were not available for some cases[285]

†Values are an approximate and subjective estimation from the literature and personal experience

‡Suspect vCJD cases with an alternative final diagnosis (n=27) – see Table 35

§Includes patients described as fidgety.

## Residence

Clusters of CJD had been reported in the UK and elsewhere prior to the epidemic of BSE, most likely as a result of chance, genetic susceptibility or improved case detection in a particular area. Analysis of the residential distribution of the 14 vCJD cases in this thesis does not provide evidence of clustering within the UK. However, this number is small and further analyses (discussed below) have subsequently been conducted as cases have increased. Apparent clusters of vCJD cases within UK have been reported, mostly by the news media, in Leicester (five cases),[1044] Armthorpe (two patients in the same street), Adswood area of Stockport (two cases were apparently near neighbours) and Glasgow. The Leicester cases were investigated in detail by Bryant and Monk who concluded that four out of the five cases may have been exposed to the BSE agent through the purchase and consumption of beef from a butcher's shop where the meat could be contaminated with brain tissue.[1045] The significance of these 'clusters' is uncertain and continued surveillance may ultimately suggest that they are most likely to be chance occurrences.

### Rendering plants and 'the Kent cluster'

In 1997 concern was raised about the occurrence of several vCJD cases around the Ashford area in Kent. This concern was heightened by the fact that the first BSE case was reported in this area and Kent was one of the three counties with the highest incidence of BSE by 1988.[1046] Furthermore, Kent had some of the early cases of BSE among captive exotic ungulates.[1047] Various explanations for this apparent cluster of vCJD were postulated, including drinking-water contamination by effluent from local feed mills[1048] and an unusually restricted distribution of products from locally reared beef resulting from Kent being geographically 'cut-off' from other parts of Britain.[1046,1047]

To address the hypothesis that cases of vCJD had resulted from exposure to the BSE agent via rendering plants involved in the production of MBM Cousens performed a statistical analysis of the proximity of the cases to rendering plants.[1049] The observed and expected number living within a specified distance of any rendering plant up to 50 km were almost the same. However, two plants in the county of Kent each had four cases within 50 km in 1988, significantly more than expected. Cousens emphasised that this should be interpreted with caution as 156 significance tests were performed during the analysis, so some would be expected to appear significant by chance alone. To address this issue computer simulations were undertaken and these suggested that the observation of four vCJD cases living in an area with a population of 1.5 million (the size of Kent) was not unexpected. Cousens concluded that there was no evidence that people with vCJD tended to live closer than the population as a whole to rendering plants in the 1980s. He added that the reported cluster of vCJD cases in Kent was most probably a chance finding.

A number of authors were critical of this analysis.[1046,1050] Colchester highlighted that the study had shown a statistically significant ($p<0{\cdot}007$) excess of cases living within 50 km of a rendering plant in Kent and that this should not be played down because of a lack of an a priori hypothesis.[1050] Indeed Colchester stated that he had

drawn 'attention to deficiencies in the regulation of rendering and the disposal of its products, and to the poor standard of practice at the particular [rendering] factory [in Kent], as early as April 1996'. He added that another vCJD case had lived within 10km of the rendering plant in the 1980s but Cousens had excluded the patient from his study because the case was not resident at that location on the arbitrary index date of 1 January 1988. Colchester repeated the statically analysis with this additional case and reported that this led to the geographical link becoming highly statistically significant (p=0.001). Furthermore, using the Bonferroni correction he estimated that the likelihood of any area with a population of 1·5 million in Britain containing five or more cases was significant at the 5% level.[1050]

In response to these criticisms Cousens updated his statistical analysis with data from 11 additional cases of vCJD, none of whom had lived in Kent during the 1980s.[1051] The excess of cases living within 50km of the rending plant in 1988 was still significant (p=0.02). However, Cousens emphasised that this p-value could only be taken at face value by someone who had postulated that the particular rendering plant might be a transmission source for the vCJD agent without knowing that any cases had been identified in individuals who had lived near the plant. Colchester had indicated that he first raised concern after two cases of vCJD had been identified in the vicinity of the rendering plant. Cousens agreed that including cases who had lived close to the rendering plant at times other than 1998 may increase the statistical evidence for a cluster, but he noted that analysis would be difficult as it was not known what proportion of the normal population (to whom the vCJD cases were compared) had ever lived near the plant.[1051]

### The North-South divide

In April 2001 the NCJDSU published the results of an investigation to assess if regional incidence of vCJD correlated with dietary data.[1044] The study involved the 84 cases that had been identified in Great Britain prior to November 10 2000. Incidence was found to be higher in the north of Great Britain than the south, with a ratio (north vs. south) of 1.94 (95% CI 1.27-2.98). The mean Carstairs' deprivation score for areas of residence of people with vCJD was -0.09 (-0.73 to 0.55), which is close to the national average of zero. Regional rates of vCJD correlated with consumption of 'other meat or meat products' as classified and recorded by the Household Food Consumption and Expenditure Survey (r=0.72), but not with data from the Dietary and Nutritional Survey of British Adults. The study concluded that regional differences in vCJD incidence were unlikely to be due to ascertainment bias, and that it was difficult to determine whether diet might explain the observation, since the results of dietary analyses were inconsistent.

## Occupation

Only one of the 14 vCJD cases described in this thesis worked in what might be considered a high-risk occupation with regard to BSE. The patient was employed in a butcher's shop in 1985 and 1987. Whether this is coincidence or of aetiological relevance is unknown. A subsequent analysis of lifetime occupations of 60 vCJD cases and 60 controls by the NCJDSU does not provide evidence to suggest that there is an increased risk of vCJD associated with occupation.[29]

It could be considered surprising that an excess incidence of vCJD has not been reported in slaughtermen or other occupations with a high risk of exposure to the BSE agent. Scrimgeour has discussed the potential risk of

slaughtermen contracting BSE.[1052] He noted that scrapie had been experimentally transmitted via the conjunctivae,[1053] the nasal mucosa[1054] and cutaneous abrasions,[1055] and added that prior to 1991 part of the slaughtering procedure in cattle entailed opening the cranium with a power saw to remove the brain for processing, with the unavoidable accompaniment of an aerosol. Furthermore, he reported that when the policy of discarding the brain was adopted, instead of opening the skull, a high-pressure air hose was frequently introduced into the foramen magnum to force out the intracranial contents, and again, the production of an aerosol was inevitable. As abattoir workers would often not wear gloves they might be considered at risk of exposed via abrasions. Other occupations could also be considered at risk through parenteral infection, such as those working in the food manufacturing industry (in particular animal feed) and butchers. Individuals working with infected cattle may also be considered at an increased risk, although a mechanism of contact with CNS tissue is difficult to identify. Transmission studies have not found evidence of infectivity in any other tissues of naturally infected bovines, so assisting with calving, dehorning or even more invasive general surgical procedures is not likely to pose a significant risk. Arguably the highest risk to farmers may come from exposure to cattle feed, either through touching, inhalation or eating pellets.

A possible explanation for the failure to identify an occupational risk for vCJD has been postulated by McPherson: 'Diseases for which the epidemiology is poorly understood are seen mostly in individuals with apparently low or moderate risk levels, because these represent the bulk of the population, albeit at lower risk. Individuals at apparently high risk who present with such diseases, will be correspondingly uncommon, because being at high risk is uncommon... Thus a lack of obvious exposure to BSE among patients with [variant] CJD is not strong evidence against an important association...'[1056] An alternative argument is that the amount of infectivity resulting from the likely modes of occupational exposure could be insufficient to transmit disease whereas dietary exposure may be associated with a relatively high dose, albeit via a relatively inefficient route.

## Past medical and surgical history

### Exposure to medicines and medical products

Bovine materials have been are used in a wide variety of medicines and medical products (see Table 60), and concern has been expressed that vCJD may have arisen as a result.[1057] Measures aimed at minimising exposure to TSE agents via medicinal products were introduced soon after the report of the Southwood Committee in 1988, in guidelines for manufacturers issued by Britain's Committee on Safety of Medicines in 1989, and these were essentially adopted by the European Committee for Proprietary Medicinal Products in 1992.[1058] Materials were to be sourced from cattle aged under six months from countries free of BSE or from countries where a low number of cases had been reported, provided the disease was notifiable in that country and the carcasses of affected animals were destroyed and their progeny not used.[1059] Therefore the greatest risk of exposure to the BSE agent in medicines or medical products is likely to have been during the 1980s, and particularly toward the end of that decade.

Analysis of the case-control data for the 14 patients in this thesis does not indicate that the vCJD cases were more exposed to medicines or medical products than controls. As 'catgut' sutures could potentially have contained infectivity past surgical history may be relevant, but a recent analysis of the of 51 vCJD cases and 27

controls found that 61% of cases were reported to have had some form of operation/surgical procedure (other than dental procedures) prior to the onset of their illness compared with 70% of controls.[284] Although this analysis may have been biased by the use of hospital controls, similar results were reported in a later study using community controls.[29]

**Table 60: Medicines and surgical products with bovine content**

| Material or tissue | Use |
|---|---|
| Lung | Aprotinins, heparins, surfactant |
| Heart | Heart valves, pericardium patches |
| Stomach | Stabiliser in latex production |
| Small intestine | Surgical sutures |
| Liver | Vaccines, multivitamins |
| Pancreas | Glucagons, insulin, vaccines |
| Adrenal glands | Steroids and other hormones. Blood volume expanders, capsules, pastes, tablets, suppositories, pastilles |
| Bone | Calcium gluconate tablets, gelatin coatings or impregnantions of medical devices, dressings. Bone substitutes. Blood volume expanders, capsules, pastes, tablets, suppositories, pastilles |
| Tendons and ligaments | Ankle/knee support products |
| Skeletal and cardiac muscle | Vaccines, antibodies, recombinant proteins |
| Hide | Collagen implants |
| Tallow | Excipient, ointments, capsules, pastes, tablets, suppositories, pastilles |
| Bile | Vaccines, plasma-derived medicinal products, antibodies, recombinant proteins |
| Serum | Vaccines, antibodies, recombinant proteins |
| Milk | Vaccines, antibodies, recombinant proteins, hormones, enzymes, lactulose |

### Other medical factors potentially predisposing to infection

Aguzzi raised the possibility that lesions of the digestive tract may predispose to BSE infection in humans by aiding the penetration of the infective agent.[1060] One of the 14 vCJD cases had a family history of polyposis coli but none of the others were known to suffer from any particular digestive disorder. Carp[1061] showed that gingival scarification of mice challenged with scrapie via the oral route led to increased take up of infection and shorter incubation period. This raises the possibility that dental disease and receding gums may predispose individuals to the oral transmission of BSE. Although a detailed dental history was not taken for the vCJD cases, as a group the 14 vCJD cases were reported to have had a similar incidence of dental extractions as the controls.

## Diet

If vCJD is caused by the BSE agent, and the most important mechanism of exposure is via the diet, which foodstuffs are most likely to be implicated? The highest titres of infectivity are found in the CNS of cattle affected by BSE, although in cattle challenged orally by 100mg of BSE brain (but not naturally affected cattle) infectivity was also identified in cervical and thoracic dorsal root ganglia, the trigeminal ganglia, retina, distal ileum and bone marrow.[283] Various other tissues and fluids (see BSE subsection of the Introduction) have not been shown to transmit the disease using the mouse assay, although this is a relatively insensitive method for the detection of infectivity (as discussed above). Thus, consumption of products containing bovine CNS tissue is likely to represent the highest risk of infection.[1062]

### Brain consumption

In other countries, and in the past in the UK, overt brain consumption is likely to have been common. However, this has been a rare occurrence in the UK in recent years, and is more likely to be a habit of old rather than young persons (an assumption supported by unpublished data on controls in the NCJDSU database). Until November 1989 it would have been legal to supply cattle brains for human consumption in the UK. Collee[1063] argues that it would have been easier to supply brain from calves rather than adult cattle, because in the method used to stun calves (electronarcosis) the brain is not mutilated, as it would be by a captive bolt,[1064] the method used for adult cattle. Calf brains would also have been much more easily removed, since the skull is easier to split. As infectivity has not be detected in cattle brain 22 months post oral infection with BSE,[283] it seems unlikely that consumption of calf brain could have been a major risk for the transmission of BSE to humans.

### Mechanically-recovered meat

Which food products were most likely to have contained bovine CNS tissues in the UK during 1980s is not clear.[1062] To address this issue further, Will investigated the contemporary use of bovine CNS tissues in food products in Australia and New Zealand.[1062] No legislative action has been necessary in these countries with regard to food products and TSEs, as the countries are considered to be free of animal TSEs. Therefore, they

may provide some indication of past practices in the UK. It was found that with rare exceptions, bovine brain did not enter the human food chain in either Australia or New Zealand. However, a detailed investigation in New Zealand revealed that cattle remains, including vertebral column, were used in the production of MRM. Spinal cord was removed from the majority of carcasses, but spinal remnants, including occasion whole cords, were present in a significant proportion of vertebral columns used in the production of MRM.[1062]

The BSE Inquiry has discussed the issue of MRM in detail.[979] The Inquiry reported that historically if a butcher's knife was used to remove meat from a carcass, quite a lot of meat was left on the bone, especially on the ribs. In the 1950s mechanical hand tools were developed to minimise wastage by recovering this meat and by the early 1960s automatic machines were being employed. These machines had been in use since then to recover residual meat attached to the bone 'which would otherwise be difficult or uneconomical to remove'. Dr Tim Render of MAFF's Animal Health (Disease Control) Division told the Inquiry: ' [MRM] can be used in any product containing chopped or minced meat. But in practice it is used in very few fresh, raw meat products and in few fresh cooked products. The main use is, apparently, in products at the bottom end of the market, such as frozen sausages, burgers and pies etc.' The concentration of MRM in such products was reported to be typically about 10% by weight. However, it was noted that some contained a higher proportion of MRM. Mr Stephen Ridge, Quality Assurance Executive with Somerfield supermarkets, told the Inquiry: 'If you want to buy the cheapest economy burger you can get, it can be made very largely out of MRM.' The Inquiry also heard that MRM was widely used in institutional catering, including in schools and that a report undertaken for MAFF in 1997 also found evidence of the use of MRM in baby food. During 1986–96, about 5% of all MRM manufactured (5000 tonnes) was from bovine material. The vertebral column, together with the ribs, was the major source of bovine MRM. Because of the difficulty of deboning it by hand, the vertebral column was the part of the skeleton which had the most commercially attractive amount of meat still attached. Parts of the spinal cords were also inadvertently incorporated into MRM when processing the vertebral column. The inquiry noted that a report prepared for MAFF in 1997 estimated the possible extent of contamination of beef MRM with spinal cord prior to the SBO controls: 'The average contamination of beef MRM with spinal cord would therefore be about 0.04%. However, there would be likely to be considerable variation about this figure; the worst-case (where all of the vertebral columns contained an intact spinal cord) would give about 2.8% contamination. A 100g meat product, containing 10% of such beef MRM, would contain 0.28g spinal cord.' Heads were said to never be included in MRM because the enamel of the teeth could damage the machines.

The above figures suggest that between 1985 and 1996 about two tonnes of CNS tissue could have been consumed in human food containing MRM manufactured in the UK. Mathematical modelling by Anderson has indicated that considerable numbers of cattle incubating BSE, but clinically unaffected, have been slaughtered for human consumption since the late 1980s. Together these data suggests that a large number of the people in the UK are likely to have been exposed to foods contaminated by the BSE agent in the form of MRM and that rarely some products may have contained over 0.5g of bovine CNS - the quantity (of brain) that has transmitted disease via the oral route to sheep, i.e. across a species barrier.

If 0.5g of spinal cord is considered to be equivalent to one infectious unit in humans (there is no data to support this guesstimate) and if one in 50 cattle slaughtered for human consumption between 1985 and 1996 is considered to be infected with BSE (a rough approximation from Anderson's data[962]) then 80,000 infectious

units may have entered human food over this period. The actual figure could easily be orders of magnitude higher or lower than this estimate, which relies on multiple unsubstantiated assumptions.

**Infectivity in other tissues and from non-bovine animals**

Arguably, organs and tissues outside of the CNS may also have contained bovine brain and been used in human food. Garland found grossly visible brain tissue (confirmed microscopically) in the pulmonary arteries of 2.5-5% of cattle after slaughter with a captive bolt.[1064] The fragment sizes ranged from several millimetres to 14cm.[1064] Bovine lungs were legally permitted in certain meat products 'sold for consumption without further cooking', such as salami and pates.[1065,1066] However, in Taylor's response to Garland's article he implied that the type of captive bolt gun used in the UK differed from the compressed-air gun used in Garland's experiment. Furthermore, when Taylor repeated Garland's study with the other type of gun, brain emboli was not detected in the lungs of the 10 animals assessed. Garland responded by providing evidence that the compressed air-type captive bolt gun had been available in the UK and argued that Taylor's experiment was too small.

Natural BSE infection has not been identified in any species used for human consumption other than bovines. However, it would be premature to conclude that BSE has not caused cases of sheep scrapie, as the detection of this occurrence may be difficult, particularly if rare. Furthermore, it can not be certain that should BSE transmit to sheep, then humans would be any more or less susceptible to the this novel ovine TSE than to conventional scrapie or BSE in cattle.

Although experimental transmission of BSE to pigs (via the oral route)[6] and poultry[89] have been negative, this does not exclude the possibility that these animals may be asymptomatic carriers of infection and thus still pose a risk to humans. Although it is somewhat reassuring that no infectivity has been detected in neural or non-neural tissues from pigs killed two years after oral BSE challenge, this has be interpreted with caution due to sensitivity of the mouse bioassay. Furthermore, the results of bioassays done at the termination of the porcine studies are still awaited as are those from poultry.[1067]

**Case-control analysis**

The results of the analysis of the case-control dietary data in this thesis do not provide evidence of a dietary risk factor for vCJD. A more recent analysis of 60 vCJD cases and 60 hospital controls[29] found that a higher proportions of cases than controls were reported to have ever consumed burgers (p=0.02) and meat pies (p=0.08). Furthermore, when assessing the frequency of consumption, cases were reported as having eaten two foodstuffs (out of 14 foodstuffs that were analysed) more frequently than their matched hospital controls: beef, p=0.008 and burgers, p=0.002. Forty-five percent of cases were reported to have eaten beef more than once per week compared with 23% of hospital controls; and 66% of cases were reported to have eaten burgers at least once per week compared with 41% of hospital controls. There was no evidence that the reported frequency of consumption of sausages or meat pies differed between cases and hospital controls (p=0.6 and 0.1 respectively). There was weak evidence that reported consumption of products likely to contain MRM was more frequent among cases than among hospital controls (p=0.07). The positive results of this analysis should not be over-interpreted as a large number of statistical comparisons were performed, increasing the probability of observing some 'statistically significant' associations, which reflect nothing but chance. In addition to this problem, there

was also considerable scope for recall bias with respect to dietary histories. In order to examine this possibility, a further comparison was undertaken of cases (n=90) with a group of controls (n=31), who were people referred to the NCJDSU with suspect vCJD who were subsequently determined to have an alternative diagnosis. Cases were reported to have eaten beef and burgers more frequently than controls. However, no statistically significant differences were found between cases and controls in the frequency of consumption of beef, burgers, sausages, meat pies or MRM containing products (p= 0.5, 0.1, 0.6, 0.7, 0.9 respectively) (see Table 61). It is possible, therefore, that the differences observed between cases and hospital controls with regard to the consumption of certain food items result from recall bias.

An analysis of the case-control data in the NCJDSU 1999 Annual Report[284] found that three of the 51 vCJD cases (6%) and three (15%) controls had been vegetarians for a period of at least one year ($p > 0.4$). One of the vCJD patients had been a vegetarian since the mid 1980s. As BSE was only reported in 1986 the occurrence of vCJD case may be considered as an argument against a causative link between vCJD and BSE. However, the patient may have been exposed to the BSE agent in her diet prior to 1986, as it is estimated that up to 54,000 infected animals were slaughtered for human consumption between 1980 and 1985.[1068] Moreover, any food product containing <18% meat can be classified as vegetarian.

**Table 61: Frequency of consumption of food items more than once a week for vCJD cases and controls**

| Foodstuff eaten | Frequency | % of cases (n= 90) | % of controls (n= 31) | Odds ratio (95% C.I.) | P-value |
|---|---|---|---|---|---|
| Beef | ≤1 per month | 22 | 26 | 1.0 | 0.5 |
| | 1 per week | 34 | 39 | 1.0 (0.3, 2.9) | |
| | >1 per week | 44 | 35 | 1.4 (0.5, 4.1) | |
| Burgers | ≤1 per year | 13 | 24 | 1.0 | 0.1 |
| | Several times a year to 1 per month | 29 | 31 | 1.8 (0.5, 6.0) | |
| | ≥1 per week | 59 | 45 | 2.5 (0.8, 7.7) | |
| Sausages | ≤1 per month | 40 | 32 | 1.0 | 0.6 |
| | 1 per week | 34 | 42 | 0.6 (0.3, 1.7) | |
| | >1 per week | 26 | 26 | 0.8 (0.3, 2.3) | |
| Meat pies | ≤1 per year | 17 | 22 | 1.0 | 0.7 |
| | Several times a year to 1 per month | 33 | 30 | 1.4 (0.4, 5.0) | |
| | ≥1 per week | 49 | 48 | 1.3 (0.4, 4.1) | |
| MRM* | ≤2 per month | 12 | 14 | 1.0 | 0.9 |
| | >2 and <8 per month | 21 | 21 | 1.2 (0.3, 5.0) | |
| | ≥8 per month | 67 | 66 | 1.1 (0.3, 4.0) | |

In five cases and five controls the dietary history was recorded from 1985 onwards. In the remainder it was taken from 1980.

* MRM - burgers, meat pies & sausages used in this analysis

## A summary of vCJD epidemiology and case-control data

The most important epidemiological risk factor for the development of vCJD is residence in the UK. No other clear occupational, dietary or past medical or surgical factors have been identified. The disease appears to be more common in the north of Britain, although this significance of this is uncertain, and the possibility remains that this may be a chance observation.

The generally held view is that the most likely exposure was through eating beef products that included infected offal before this was banned from human food in late 1989.[948] As this ban may not have been completely effective in preventing dietary exposure to BSE it is possible that persons may have become infected after this date. The case-control study has major limitations, due largely to the small number of cases and the potential for bias, and can not exclude the rare occurrence of disease transmission by other routes or from other sources.

# THE FUTURE NUMBER OF VCJD CASES: DETERMINING FACTORS AND ESTIMATES

Concern regarding a huge plaque of BSE in humans was expressed in a fictional article by Bruce Sterling in Omni magazine in 1993. This predicted the death of '90% of Britain, 30% of Western Europe [and] 20% of jet-setting America...'[1069] The news media echoed these concerns following the announcement of a new variant of CJD by the UK authorities in March 1996 and have continued to do since. But can we predict what will happen in the future, and if so what factors are likely to influence such predictions? The variables that have been proposed as relevant in this regard are discussed below, followed by a review of the small number of scientific studies that have estimated the size of the vCJD epidemic.

## The species barrier

A 'barrier' to transmission of a TSE between species is well documented. This means that a smaller infective dose is required and disease occurs quicker when a TSE is transmitted between animals of the same, compared to different, species. The size of the species barrier between bovines and humans has implications for the susceptibility of people to the BSE agent. Although the human/bovine species barrier can not be measured directly, techniques are available to help assess this. A major determinant is thought to be the ease with which donor and host PrP molecules interact (see discussion on genetics above). This in turn may relate to the similarity of the donor and host PrP amino acid structure. Different species have different PrP gene sequences and hence different PrP structure. Bovine and ovine PrP usually differ at seven positions, whereas bovine and human PrP differ at more than 30. However, the central codons 96-167 are arguably more important for PrP interactions and in this region there are five amino acid differences between humans and bovines compared to six between sheep and bovines.[907,1021] These findings could be interpreted as suggesting that the species barrier for the transmission of BSE to humans is equivalent, or less, than that for the transmission of BSE to sheep. This would be highly concerning as sheep have developed BSE after oral challenge with as little as 0.5g of infected bovine brain.[890] However, the use of PrP sequence homology to predict species barrier requires qualification. The number of sequence differences may not be as important as the site of the differences: one difference at a crucial codon may have a dramatic effect on transmission, whereas multiple differences at other sites may have no or minimal effect.

The efficiency of in-vitro PrP interaction between different species has been investigated by Raymond and colleagues.[1027] They found that the conversion of human PrP by BSE prions was much less efficient than the conversion of bovine PrP by BSE prions. Previous in-vivo transmission studies had shown that sheep with the A136 R171 PrP genotype and hamsters appear to be resistant to BSE. The in-vitro conversion of ovine A136 R171 PrP and hamster PrP by BSE prions was found to be less efficient than the conversion of human PrP by BSE prions. These results imply that the species barrier between bovines and humans is considerable, but is smaller than that seen between bovines and certain animals that appear to be resistance to BSE. However, these observations need to be interpreted with caution, as the study also showed that human PrP was as efficiently converted by scrapie as BSE prions. This contradicts epidemiological evidence that argues against scrapie as a risk factor for CJD, and questions the validity of using in vitro PrP conversion studies as a model to accurately predict the size of the species barrier. Alternative interpretations of this apparent contraction are that either BSE does not cause vCJD or that scrapie is a human pathogen, albeit one that causes CJD so rarely that this is beyond the limited power of epidemiological studies to detect or that the dose of scrapie that humans are potentially exposed to is insufficient to cause infection.

Another model to assess the susceptibility of humans to the BSE agent used 'humanised' transgenic mice. These mice had their PrP gene substituted by the human equivalent.[14] Because the PrP gene is thought to be an important determinant of the species barrier this theoretically allowed mice to be used as surrogates for testing human susceptibility to BSE. When inoculated with the BSE agent the humanised transgenic mice were shown to be clinically affected with a neurological disease, but after a much longer incubation period and lower incidence of successful transmission than when inoculated with sporadic CJD.[14] On pathological examination immunocytochemistry was negative for $PrP^{Res}$, although such appearances have rarely been documented in TSE before. The authors of this study interpreted their results as suggesting human susceptibility to the BSE agent, but with a considerable species barrier.[14] However, this reassurance requires qualification, as the humanised transgenic mice were valine homozygous at codon 129, in contrast to all the cases of vCJD tested to date who are methionine homozygotes. It is possible that if the experiment had used humanised transgenic mice homozygous for methionine at codon 129 these would have shown greater susceptible to BSE. The latter experiment has been conducted but the results are unpublished.

## Incubation period

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 vCJD is kuru, as the oral route is assumed to be the an important method of infection in both conditions. The average incubation period of kuru has not been clearly established, but the youngest patient was aged four at onset of symptoms and cases have been described over 40 after the cessation of cannibalism. Collinge cites Alpers as estimating that the mean incubation period of kuru is about 12 years, although no data was given.[1070] Ridley, also citing Alpers, states that 'for kuru... the median [incubation period] ranged from less than five years to nine years (judged by the minimum ages at onset at the height of the epidemic).' Hornabrook's book 'Essays on kuru' published in 1976 gives values for the incubation period of 8-9 years, but adds that this could be an underestimate.[1071] It is also helpful to consider hGH and hPG-related iatrogenic CJD when assessing the possible incubation period for the BSE agent in human as this is also a peripherally acquired TSE. The mean incubation period for hGH-

related CJD is estimated to be 13 years (personnel communication Dr Paul Brown), although this may be an under estimate as cases with longer incubation periods may yet appear. In keeping with Brown's figure, Cochius estimates that the incubation period for hPG cases is 12-14 years.[371,372] It may be reasonable to assume that the average incubation period of the BSE agent in humans is going to exceed this figure because of the species barrier effect. Baker showed that the incubation period for transmission of BSE to marmosets injected intracerebrally (and concurrently intraperitoneal) was 49 months as opposed to 17 months for transmission of marmoset TSE (second passaged CJD).[1072] Collinge argued that 'if we assume... that the barrier [of BSE transmission to humans] is similar to that observed in mice, extrapolation would suggest mean incubation periods ... of perhaps 30 years, with a range of 10 years to longer than a normal human lifespan.'[1070] However, other unknown factors will also effect the incubation period, in particular the dose of the agent, route of exposure and possibly as yet unidentified genetic factors. These make it hard to draw any firm conclusions as to the average incubation period of the BSE agent in humans.

## How many people were exposed and susceptible to BSE?

The most likely method of exposure of the vCJD cases to the BSE agent is the oral route, probably through the consumption of contaminated meat products in the UK during the period 1985-1990. Case-control data does not indicate any modes of occupational or iatrogenic infection, although these can not be excluded. Furthermore, it seems likely that cattle incubating BSE were slaughtered for human consumption for a few years prior to 1985 and that contaminated meat products may have still entered human food for a number of the years after the SBO ban. Thus the population of individuals potentially exposed to the BSE agent in their diet is likely to be nearly all of those persons living in (or visiting) the UK since the early 1980s. In addition, a significant amount of the human 'risk' of BSE is likely to have been exported from the UK, as either live cattle, contaminated MBM, bovine carcasses or meat products. Medicines and blood were also exported and these are discussed below. The exact proportion of the BSE risk exported is unknown, but one unpublished guesstimate is that this may be up to 50%. Therefore the size of the population exposed to the BSE agent is likely to be large and extend to Europe and beyond.

But what proportion of the exposed individuals are both susceptible and have received a high enough dose to cause disease? Analysis of the characteristics of the vCJD cases suggests that young age (<50 years) and codon 129 methionine homozygosity predispose people to BSE infection. However, over 20% of the UK population have both these factors and with time it is possible that BSE will be identified as a cause of CJD in other codon 129 genotypes and a higher incidence may occur in older age groups. The amount of BSE agent required to transmit disease to humans via the oral route is unknown. BSE has been experimentally transmitted to sheep by feeding as little as 0.5g of infected bovine brain.[890] However, it can not be inferred from this that a similar dose will be required to infect humans as the effect of the species barrier is unknown and other factors such as genetic influences, gastrointestinal absorption and the effect of the food manufacturing process and cooking may be important. Furthermore, the amount of bovine CNS material that could be expected to contaminate a food product that may be consumed in a single sitting is not precisely known (although see above for estimates). In conclusion the number of people that are likely to have been exposed to the BSE agent in their diet is large, but the proportion of such individuals that are susceptible and have been exposed to a dose high enough to transmit the disease is a matter of conjecture.

## Secondary transmission of the BSE agent in humans (iatrogenic and maternal transmission)

A factor that will influence the number of people developing CJD as a result of BSE is whether secondary transmission of the BSE agent in humans will occur. The three main methods by which secondary cases could be anticipated to arise are via contaminated surgical instruments, blood products or maternal transmission.

### Surgical instruments

Infectivity is largely confined to the CNS in patients with sporadic CJD and although iatrogenic CJD is known to have arisen via surgery, only instruments used during donor and recipient brain operations have been implicated. Because of a likely oral route of infection and a novel strain of infectious agent, the distribution of tissue infectivity in vCJD 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 vCJD (but not other forms of CJD), including in the vermiform appendix eight months prior to the onset of symptoms of vCJD in one case.[923,942,1073] Resistance of the TSE agent to standard hospital decontamination regimes coupled with the observation of lymphoid tissue involvement in vCJD has raised the concern that the potential for iatrogenic transmission via surgical instruments used outside of the CNS may exceed that for other CJD subtypes. This concern is heightened by the possibly large number of people incubating vCJD, who are potentially 'infectious', but unidentifiable as such. In this regard it is noteworthy that infectivity in the lymphoreticular system (spleen) of hamsters inoculated with scrapie can be detected at less than half of the incubation period, suggesting that hypothetically a person infected with BSE in 1990, who will develop vCJD in the year 2010, could harbour infection for over a decade. It is of some reassurance that a UK study to detect PrP in a retrospective series of over 3000 tonsil and appendix specimens taken post 1995 from individuals aged 15 to 54 years found no positives.[1074] A similar prospective study is currently in progress. The concerns relating to the potential iatrogenic transmission of vCJD via surgical instruments led the UK Department of Health to start introducing single use instruments for tonsil surgery during 2001 and to consider extending this to other procedures.

### Blood transfusion

The normal form of PrP is widely distributed in human peripheral blood, including B and T lymphocytes, monocytes and platelets[1075,1076] suggesting that blood may have the potential to transmit TSE infection.[1077,1078] About 15% of patients with sporadic CJD are reported to have previously acted as blood donors, a proportion which is similar to that of the population as a whole. Therefore a significant number of people must have received blood components or plasma products from donors in the preclinical phases of sporadic CJD.[1079] Although there are a handful of clinical case reports in which transmission of sporadic CJD has been raised as a possibility,[385,1080-1083] epidemiological case-control,[229,231,233-235,250,637] look-back[1079,1084,1085] and surveillance studies[1086-1088] have shown no evidence of an increased risk of CJD in those who have received blood components or plasma products. Of further reassurance, a study of 33 HIV positive patients who had received clotting factor concentrate did not find $PrP^{Res}$ on post mortem examination of brain tissue.[1088] The consensus appears to be that if sporadic CJD is transmissible by blood products, this occurs very rarely.

However, vCJD raises new concerns because, unlike other forms of human TSE, PrP is detectable in the lymphoreticular system, raising the possibility that significant infectivity may be present in circulating blood. To add to these concerns a paper in Nature in 1997 showed that B lymphocytes are required for neuroinvasion following peripheral inoculation in experimental TSEs, implying that B lymphocytes may 'carry' infection.[181] The UK government therefore commissioned an assessment of the risk of vCJD transmission via blood and blood products. This concluded that based on estimates from animal models, up to 0.8 persons might develop vCJD from each infected donation, half predicted to be due to blood transfusion and half due to plasma derivatives.[1089] The UK authorities have subsequently adopted leucodepletion of donated blood and importation of plasma for use in plasma derivatives from outside of the UK.[1090] In retrospect, data implying that B cells carry infection may have been over-interpreted. The lymphoid cell type that stains for $PrP^{Res}$ in vCJD is the non-circulating follicle dendritic cell, and although B-cells appear to be required for neuroinvasion they may do this via a maturation role on follicular dendrite cells rather than by actually 'carrying' infection themselves.[181,182] However, a recent study lends support to the cautious measures adopted by the UK authorities; Houston transmitted BSE to a sheep by transfusion with whole blood taken from another sheep during the symptom-free phase of an experimental BSE infection.[1091] Animal transmission studies using blood from patients with vCJD are ongoing.

**Maternal transmission**

The media and some scientists have raised much concern about the transmission of CJD to children of vCJD victims. Are these justified? The issue of maternal transmission of TSEs is controversial.[1092] The best evidence that this occurs comes from studies of scrapie in sheep. However, these studies have been criticised.[1093] The exact mode of the apparent maternal transmission of sheep scrapie is unclear and may not be through pregnancy, but via exposure to placental material following birth or inheritance of a PrP 'susceptibility' genotype.[1094] Another experiment cited as evidence for maternal transmission is the cohort study of dam to calf transmission of BSE. This showed that dams who developed BSE had a 10% higher incidence of calves with the disease compared to unaffected dams. However, the study has to be interpreted with caution: because the calves were fed with potentially contaminated feed the results do not exclude the possibility that the affected animals acquired BSE through a combination of genetic susceptibility and feed-borne exposure, rather than during pregnancy.[882,1095] Studies of maternal transmission in mice have been negative[1096] and 10 progeny born to primates incubating or clinically ill with an experimental TSE did not acquire the disease despite follow-up of up to over 11 years.[1097]

Is there evidence that maternal transmission has ever occurred in any human TSE? The largest group of mothers affected by, or incubating, a TSE during pregnancy was in Papua New Guinea as a result of kuru. Cannibalism stopped in the late 1950s,[321] but as kuru has a long incubation period many women went on to develop the disease after this time and delivered babies. All of the accumulated evidence indicates that vertical transfer of infection did not occur, most pointedly illustrated by the fact that none of more than 1000 children who were born of affected mothers following the cessation of cannibalism ever developed kuru.[1098-1101]

Three women are reported to have conceived and successfully completed a pregnancy during the evolution of CJD. I am aware of two other unpublished cases, one a mother who became unwell with dura mater associated CJD during pregnancy and case 476 in this thesis. None of the five offspring (oldest >22 years) subsequently

developed CJD.[326,732,1102] Of further reassurance PrP immunocytochemistry on placenta from case 476 was negative for $PrP^{Res}$. This contrasts with the report by Tamai of infectivity in human placenta (and colostrum) identified by intracerebral inoculation of mice. However, Will noted that there were inconsistencies in Tamai's data which seriously question the study's validity, in particular the finding of equivalent levels of infectivity in placenta and brain.[1103]

In summary, animal studies suggest that maternal transmission of TSEs may occur, but the evidence is not conclusive. The possibility that vCJD could be transmitted vertically can not be excluded (and is likely to remain a concern for decades to come), but it is reassuring that there is no evidence for this from any other form of human TSE.

## Estimates for the number of future cases

Deaths due to vCJD have increased from three in 1995 to 27 in 2000 (see Figure 47). The underlying rate of deaths from vCJD has been calculated by Andrews and appears to be increasing, with a doubling time estimated at three years (see Figure 48).[17]

**Figure 47: Deaths due to vCJD 1995-2000**

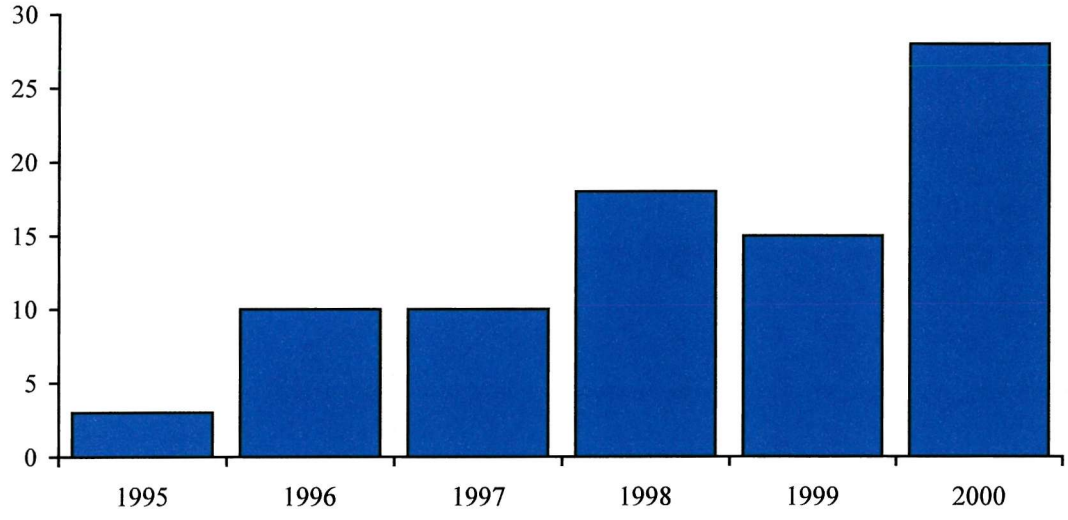
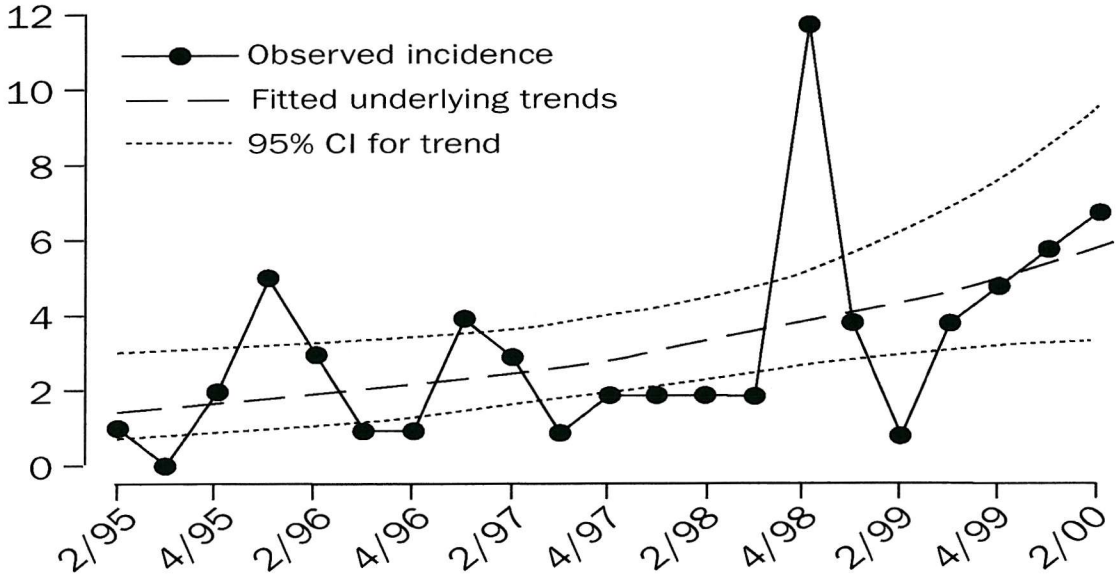



**Figure 48: Deaths due to vCJD 1995-2000 and underlying trend**



Three groups have published predictions for the total size of the vCJD epidemic. The first study involved projections based on the 14 cases of vCJD identified up to the end of 1996, of whom 13 had onset in 1994 or 1995.[8] The authors performed calculations using simple mathematical models, making assumptions about the distribution of exposure of the human population to the BSE agent over time, and about incubation period. The models estimated, by back calculation, how many human infections eventually resulting in disease would have been required to produce 13 cases of vCJD with onset in 1994/95, and how many cases might be expected to arise over the subsequent three years. The models used assumed that, until 1989, the number of persons infected with the BSE agent in each year was proportional to the number of cases of BSE with onset in that year. Two sets of figures for the number of BSE cases were used. The first set, the number of confirmed cases of BSE, assumed that the degree of under-reporting of BSE changed little over time (exposure pattern A). The second set (exposure pattern B) assumed that under-reporting was greatest early in the epidemic. Assumptions were also made regarding the length (10-25 years) and distribution (lognormal, gamma or Weibull) of the incubation period. The range of variability of the incubation period that were considered (90% of individuals developing disease within 1.5-2 times the mean incubation period) corresponded closely with the range of 'dispersion factors' reported for a variety of acute infectious diseases and was consistent with that used by Anderson[962] to model the BSE epidemic. The estimated number of infections was extremely sensitive to the assumptions made about the shape of the incubation period distribution, its mean and its variability, and ranged from less than 100 to many thousands (see Table 62). The authors were hesitant to draw sweeping conclusions based on such limited data and unverifiable assumptions. However, they tentatively stated it would be premature to conclude that because only 14 vCJD cases had been reported at that time that any subsequent epidemic would necessarily be small. They added that the number of cases occurring over the following few years might provide a better indication of how large any epidemic might eventually be, but considerable uncertainty might remain even after four years.

In a study published in 1999, Thomas and Newby predicted that the size of the vCJD epidemic would not exceed a few hundred cases, and would most likely be limited to one hundred or less. However, they estimated that the mean mortality period (time from infection to death) lay in the range six to 16 years, arguably very conservative values, given approximate mean incubation periods for kuru and hGH-related CJD of 12-13 years and the effect the species barrier (as discussed above). Furthermore, the methodology adopted by Thomas and Newby was criticised by Ferguson and colleagues who claimed that the authors misused a test statistic and as a consequence arrived at an estimate for the upper bounds of disease incidence that was too low.[1104] Using the same data Ferguson determined that only very wide bounds could be placed on future vCJD incidence.[1104] The epidemiology subcommittee of SEAC also felt that the analyses carried out by Thomas and Newby were flawed and therefore rejected their conclusions.[6]

Ghani and coworkers used extensive scenario analyses to relate the number of infected cattle slaughtered for consumption to the observed incidence of vCJD at the end of 1999 stratified by time and age.[18] The authors made the supposition that only individuals with codon 129 methionine homozygotes were at risk. They explored a wide range of assumptions regarding the distribution of the vCJD incubation period, the relative infectivity of cattle by incubation stage, and the effectiveness of control measures at reducing human exposure to infected material. The study concluded that the upper bound on the vCJD epidemic was about 136,000 and the data suggested that, on average, no more than two cases of vCJD could arise from the consumption of one maximally infectious bovine. Furthermore, they added that a large number (>6000) of cases could only arise if the mean

incubation period is about the same length as average life expectancy. Finally they stated that if the average annual incidence of vCJD over the following three years was fewer than 15 cases, then the maximum total number of cases would fall to approximately 20,000 (see Table 63 and Figure 49).

## A summary of the prediction for the future number of vCJD cases

Multiple factors are likely to influence the future number of cases of vCJD, in particular the size of the human/bovine species barrier; the amount of exposure of the UK and other populations to the BSE agent; the variability and average length of the incubation period and the potential for secondary cases. All these factors are unknown, but using various estimates has allowed predictions for the future size of the vCJD epidemic to be made. Values have ranged from just a handful of cases to 100,000 or more. Thus, there is great uncertainty, and as the microbiologist Gerald Collee stated in 1997 'we must wait for a further decade or two of continuing surveillance of both CJD and BSE until we know the likely extent of the problems posed by BSE for man.'[1063]

**Table 62: Predicted number of vCJD cases under various possible scenarios by Cousens et al 1997**

| Mean incubation period (years) | Ninetieth centile of incubation period distribution (years) | Effectiveness of ban on specified bovine offals | Lognormal incubation period distribution | | | | | | | | | | Gamma incubation period distribution | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Exposure Pattern A | | | | | Exposure Pattern B | | | | | Exposure Pattern A | | | | | Exposure Pattern B |
| | | | Predicted cases with onset | | | | Total number of human infections | Predicted cases with onset | | | | Total number of human infections | Predicted cases with onset | | | | Total number of human infections | Total number of human infections |
| | | | pre-'94 | in '96 | in '97 | In '98 | | pre-'94 | in '96 | in '97 | In '98 | | pre-'94 | in '96 | in '97 | In '98 | | |
| 10 | 15.0 | 90% | 3 | 12 | 15 | 18 | **213** | 5 | 10 | 12 | 13 | **151** | 4 | 11 | 14 | 17 | **211** | **156** |
| | | 100% | 3 | 11 | 12 | 12 | **99** | 5 | 9 | 10 | 9 | **83** | 4 | 10 | 11 | 11 | **103** | **88** |
| | 20.0 | 90% | 13 | 8 | 8 | 7 | **104** | 17 | 7 | 7 | 7 | **101** | 15 | 7 | 8 | 7 | **117** | **114** |
| | | 100% | 19 | 6 | 5 | 4 | **75** | 23 | 5 | 5 | 4 | **79** | 22 | 6 | 6 | 5 | **92** | **94** |
| 15 | 22.5 | 90% | 1 | 19 | 32 | 48 | **1595** | 3 | 15 | 22 | 30 | **801** | 3 | 14 | 22 | 30 | **1001** | **620** |
| | | 100% | 1 | 18 | 29 | 40 | **714** | 3 | 14 | 21 | 27 | **430** | 3 | 13 | 19 | 24 | **469** | **342** |
| | 30.0 | 90% | 10 | 8 | 9 | 10 | **174** | 13 | 8 | 8 | 9 | **158** | 13 | 8 | 8 | 9 | **188** | **177** |
| | | 100% | 13 | 7 | 6 | 6 | **107** | 16 | 6 | 6 | 6 | **107** | 19 | 7 | 7 | 6 | **137** | **137** |
| 20 | 30.0 | 90% | 1 | 26 | 54 | 97 | **12,000** | 2 | 19 | 34 | 54 | **5000** | 2 | 16 | 27 | 41 | **4000** | **2179** |
| | | 100% | 1 | 25 | 51 | 89 | **5000** | 2 | 19 | 33 | 51 | **2421** | 2 | 15 | 24 | 35 | **1800** | **1189** |
| | 40.0 | 90% | 8 | 9 | 10 | 11 | **284** | 11 | 8 | 9 | 10 | **244** | 13 | 8 | 9 | 9 | **276** | **255** |
| | | 100% | 10 | 7 | 8 | 8 | **162** | 13 | 7 | 7 | 7 | **156** | 18 | 7 | 7 | 7 | **195** | **191** |
| 25 | 37.5 | 90% | 1 | 32 | 79 | 166 | **80,000** | 2 | 22 | 45 | 83 | **24,000** | 2 | 18 | 31 | 50 | **13,000** | **7000** |
| | | 100% | 1 | 31 | 76 | 156 | **35,000** | 2 | 22 | 45 | 80 | **13,000** | 2 | 17 | 28 | 44 | **6000** | **4000** |
| | 50.0 | 90% | 7 | 10 | 11 | 13 | **450** | 9 | 9 | 10 | 11 | **370** | 12 | 8 | 9 | 10 | **380** | **346** |
| | | 100% | 8 | 8 | 9 | 9 | **245** | 11 | 8 | 8 | 8 | **228** | 17 | 7 | 7 | 8 | **263** | **256** |

Numbers greater than 2500 have been rounded to the nearest 1000. Number greater than 25,000 have been rounded to the nearest 5000.

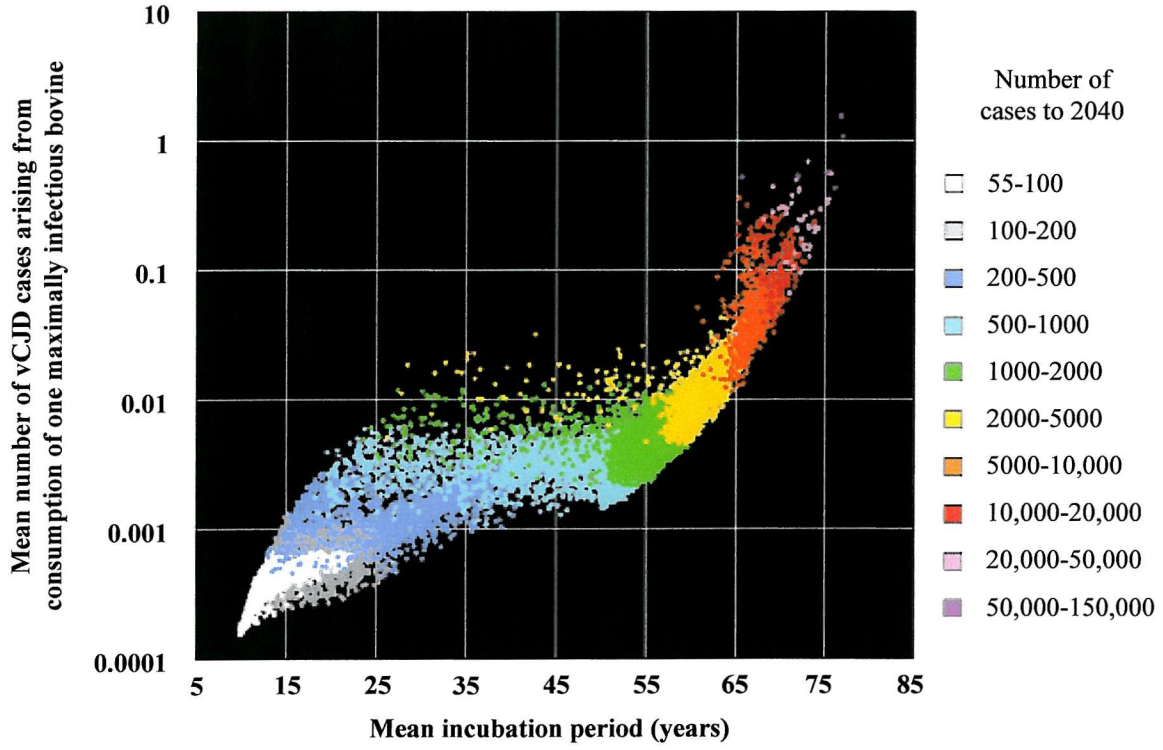
Actual number of onsets is now know to be eleven in 1996, fourteen in 1997 and sixteen in 1998.

**Table 63: Projections for the eventual total number of vCJD cases, published by Ghani et al 2000**

| Mean IP (years) | Number of cases in 2000 | | | Average number of cases in 2000-2 | | | |
|---|---|---|---|---|---|---|---|
| | 10-14 | 15-19 | 20+ | <10 | 10-14 | 15-19 | 20+ |
| <20 | 70-630 | 75-630 | 80-630 | 63-350 | 90-530 | 105-630 | 120-630 |
| 20-30 | 70-2900 | 87-2900 | 110-2800 | 70-900 | 90-1500 | 105-2900 | 120-2900 |
| 30-60 | 150-5500 | 150-6000 | 150-6000 | 150-1500 | 150-4500 | 150-5500 | 200-6000 |
| ≥60 | 1300-136,000 | 1300-136,000 | 1300-136,000 | 1300-1700 | 1300-20,000 | 1300-20,000 | 1300-136,000 |

The range of the total number of cases is given that is consistent with the number of cases recorded in 2000 and with the average annual number of cases in the next three years, calculated by the conditional mean incubation period. Results include concentrated sampling of parameter combinations that result in large epidemics.

**Figure 49: Epidemic scenarios produced by Ghani consistent with vCJD mortality data to the end of 1999**



## Historical basis of the MRI study

In January 1996 a letter was published in the BMJ reporting a case of CJD with high signal abnormalities in the basal ganglia on MRI.[1105] The author noted that although such changes had been reported before in CJD, their prevalence was unknown and suggested that this would be worth investigating. In response a review was undertaken of the MRI reports from all cases of definite or probable CJD identified by the NCJDSU since 1990 and all those suspect cases with a final alternative diagnosis (controls). This review, published in March 1996, found that basal ganglia abnormalities were reported in only four of the 96 cases for whom MRI reports were available and in none of the six controls.[706] This led to the conclusion that although basal ganglia high signal might be a relatively specific sign of CJD, this was unlikely to be a sensitive finding.[706]

In June 1996 Finkenstaedt reported a detailed review of MRI films from 29 cases of CJD and found that 79% had basal ganglia high signal, contradicting the earlier conclusion that MRI was an insensitive test for CJD.[707] The explanation for the presumed under ascertainment in the NCJDSU study was probably that this relied on a review of MRI reports from local hospitals, whereas Finkenstaedt personally scrutinised the MRI films.

In September 1996 a young women (case 580 - Annex 8) with clinical features suggestive of vCJD was referred to the NCJDSU from the same centre as a previous patient (case 499). The local neuroradiologist commented that both cases had similar posterior thalamic high signal on MRI. This prompted a review of the previous MRI reports of the vCJD cases up to that time and three[343,898,937,940] were noted to have had posterior thalamic abnormalities. None of these were reported to have striatal high signal, the most frequently described distribution of basal ganglia hyperintensity in sporadic CJD. The latter findings, and the results of the two MRI studies,[706,707] raised the possibility that posterior thalamic high signal might be a specific sign for vCJD, and that this may not have been noted on MRI reports from the patients' hospitals. This led to the MRI study in vCJD described above, which was published in April 2000.[771] Preliminary findings were reported at neuroradiology conferences in 1997 and 1998.[1106,1107]

## Main findings

The MRI study in this thesis shows that the most important MRI feature in vCJD is bilateral thalamic high signal, in particular in the pulvinar, on T2-weighted or PD-weighted images or both. In those patients with the most prominent pulvinar high signal, medial thalamic high signal was also usually seen, giving a characteristic 'hockey-stick' appearance on axial images (see Figure 30B). The other notable MRI features were the presence of periaqueductal grey high signal and absent or minimal cerebral atrophy. PD-weighted images were superior to standard T2-weighted images, though the abnormalities were most conspicuous on the FLAIR images (where available). The latter finding has been confirmed in a follow-up report of MRI in a further 16 vCJD cases: six had FLAIR imaging and in all of these the high signal abnormality was more marked than on T2 or PD-weighted sequences.[1108] T1-weighted images showed no signal abnormalities in any vCJD case in the study. Normal T1 signal is also a characteristic of sporadic CJD, with only a single case reported with T1 hyperintensity (in the globus pallidus bilaterally).[763] A recent review of MRI in vCJD identified mild T1-

weighted high signal in the putamen, but only in a single case.[1109] The plane of scanning does not seem to be critical, though axial and sagittal planes are best orientated for assessing differences in basal ganglia signal and delineating the anterior margin of the thalamic high-signal changes. The thalamic changes in vCJD were always symmetrical, in contrast to the basal ganglia in sporadic CJD which may, albeit rarely, show unilateral or asymmetrical hyperintensity.[691,711,752,762,764]

In the first part of the study MRI scans were assessed blind by two radiologists, and in the second part the scans from all histologically proven vCJD cases and any other cases with reported pulvinar high signal were openly reassessed to reach a consensus on the radiological features. Similar results were produced from each observer. The sensitivity and specificity of radiological changes for the diagnosis of vCJD were 78% or better and 95% or better, respectively. These findings also had good intraobserver repeatability (kappas >0·8).

## Controls

In terms of assessing the discriminatory ability of high pulvinar signal for the diagnosis of vCJD, the most useful controls for the study were those cases of suspect vCJD that were later confirmed not to have the disease. High pulvinar signal was reported at only one of the 56 blind MRI assessments from the 14 cases in this group, and this was graded as only minimal/equivocal. As only a limited number of scans were available from this group, scans from patients with other forms of CJD (mostly sporadic) were used because this is the most common condition that resembles vCJD.[285] As a group, the 57 controls were older than the histologically confirmed vCJD cases. However, when the controls were restricted to those less than age 52 years (the age of the oldest case of vCJD in this study), the specificity of a radiological diagnosis of vCJD remained high, at 92% or more. Thalamic T2-weighted signal intensity was not found to show a significant change with age in a group of normal individuals aged 30-85 years, [1110] further arguing against an age-related cause of the pulvinar high signal found in the vCJD cases.

## The 'pulvinar sign'

One hundred-and-twenty-four of 144 blind assessments from histologically confirmed vCJD cases were classed radiologically as vCJD, but nine of 124 were graded as only minimal/equivocal changes. Seven of 228 blind assessments in controls were diagnosed radiologically as vCJD and in these assessments the changes were considered minimal/equivocal in six cases and moderate in only one. To diagnose vCJD radiologically with confidence, and hence limit the number of false-positive assessments, it is proposed that only scans with the most prominent changes (moderate or marked) should be considered positive. This degree of abnormality correlates with pulvinar signal graded +++ or ++++ on the consensus review. In retrospect, this approximates to bilateral pulvinar signal intensity greater than all other basal ganglia on PD-weighted images, or greater than or equal to all other basal ganglia on T2-weighted images. It is proposed that this degree of abnormality is called the 'pulvinar sign'. Coulthard and co-workers'[1111] quantitative analysis of MRI abnormalities in three patients with vCJD and 14 controls supports this qualitative definition of the pulvinar sign. Since 28 of 36 of the cases but no controls in the study had prominent pulvinar high signal (+++ or ++++) on the consensus review, it is estimated that the pulvinar sign has a sensitivity of 78% (95% CI 61-90%) and a specificity of 100% (94-100%) for the diagnosis of vCJD. Although these estimates must be treated with caution, since the consensus review

was done with the assessor knowing the patient's true status, these estimates are comparable to those obtained from the initial blind assessments.

The study does not show if the pulvinar abnormalities may be present preclinically. The prevalence of pulvinar high signal in the normal population and those without vCJD, but who complain of sensory or psychiatric disturbance, is also not known. Therefore, there is no evidence that MRI can be used as a presymptomatic test for vCJD or as a diagnostic screen in individuals with possible but non-specific symptoms of the disease. Two patients with vCJD had pulvinar MRI changes that changed from very slight to very prominent throughout their clinical course, suggesting that the pulvinar sign might be a late feature in the disease process.

Although prominent increased pulvinar signal (+++ or ++++) was present in 78% of histologically-confirmed vCJD cases on consensus review in the study, pulvinar high signal was noted at the referring hospitals in only 12 (33%) cases. A similar disparity has been seen in sporadic CJD,[706,707] as described above. The reason for these discrepancies is unclear, but may reflect the often subtle appearance of the basal ganglia hyperintensities, which may not be considered of significance to a radiologist whose main concern is to exclude structural pathology and identify recognised diagnostic abnormalities.

## Comments on the study design

The study is open to several criticisms. MRI films were not obtained for a number of vCJD cases and suspects with alternative final diagnoses. However, attempts (often multiple) were made to obtain scans from all such cases by contacting the relevant clinician. Films were reviewed blind to clinical information, but were not masked to cover the patients details as the large number of images made this impractical. Thus it was possible that the neuroradiologist may have recognised the patients' name, the centre at which the scan was performed or have been influenced by the patients age. The definition of the pulvinar sign was made *post hoc*, and in retrospect it would have been better if this had be defined *a priori* and used as the criterion for a positive scan in the blinded assessments. Unfortunately at the beginning of the study it was not known which MRI features could be used as defining characteristics. Scans are continuing to be collected by the NCJDSU, and it would be helpful to perform a further and larger blinded evaluation in the future, enabling a more objective assessment of sensitivity and specificity of the pulvinar sign.

## Differential diagnosis of MRI thalamic high signal

Bithalamic high signal abnormalities on T2-weighted images have also been described in the following conditions: encephalopathy of unknown cause,[1112] Japanese encephalitis,[1113] deep cerebral vein thrombosis,[1114] a patient with 'transient dysaesthesia of unknown cause',[1115] 'top of the basilar' syndrome,[1114] bithalamic glioma,[1116] thalamic germ cell tumours,[1117,1117] reversible posterior leukoencephalopathy syndrome in polyarteritis nodosa,[1118] thalamic infarction,[1119] Wernicke's encephalopathy,[1120] and carbon monoxide poisoning.[1121] However all of the thalamic abnormalities reproduced in the reports of the above cases differed from the typical changes vCJD, being more diffuse or located more anteriorly. Most of the reports also documented focal abnormalities on CT scanning or T1-weighted MRI or both.

The pulvinar sign is not entirely specific for vCJD: MRI posterior thalamic high signal abnormalities on T2-weighted or PD-weighted images similar to those of vCJD have been reported in benign intracranial hypertension,[20] Alpers' syndrome,[19] post-infectious encephalitis,[22] and cat-scratch disease[21] (see Figure 50). However, the clinical syndromes were all distinct from vCJD in these reports. Furthermore, other imaging abnormalities not noted in vCJD were present in the latter three cases: occipital lobe high signal in Alpers' syndrome; hypointensity in the thalamus on T1-weighted images in post-infectious encephalitis; and meningeal enhancement with gadolinium in cat-scratch disease.

The most common differential diagnosis of vCJD is sporadic CJD.[285] Schröter reported MRI thalamic hyperintensity in 12 (7%) of 162 sporadic CJD cases and Grisoli found high signal in the medial and posterior thalami in 13 (42%) of a series of 31 mostly sporadic CJD cases. Others have also reported high signal in the thalamus in sporadic or familial CJD,[534,682,707,709,723,749,755,756,758,759,766] but in Schröter's paper and all the other reports in which the images are clearly reproduced signal changes are more substantial in the caudate and/or corpus striatum, the reverse of the findings in vCJD. It is noteworthy that more than 280 MRI examinations from non-vCJD patients have been reviewed as part of CJD surveillance in Germany and none has shown the pulvinar sign (personal communication Michael Finkenstaedt).

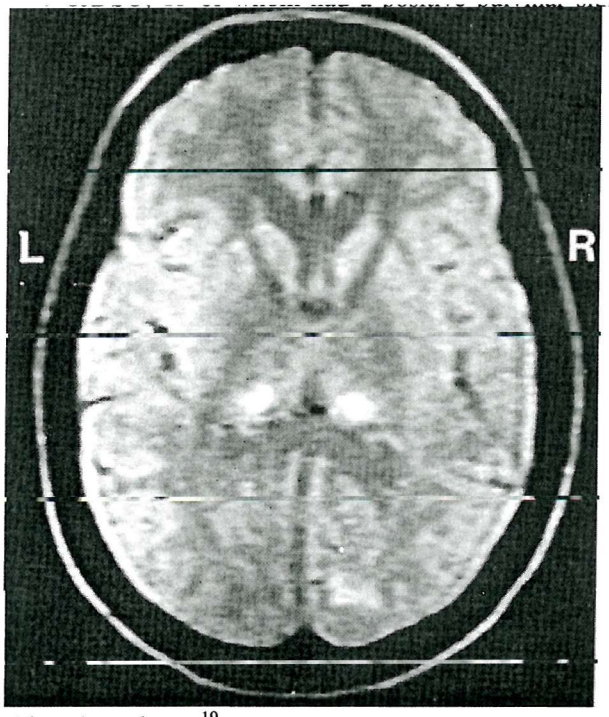
## Proposed pathological basis of the pulvinar high signal

The distribution of the MRI changes identified in the study prompted a detailed review of the distribution of thalamic neuropathology in cases of variant and sporadic CJD. Thalamic gliosis is a characteristic feature in vCJD, the overall severity of which is more substantial than in other grey matter regions.[897] In the pulvinar, gliosis was accompanied by severe (in some cases almost total) neuronal loss, but spongiform change, PrP deposition, and amyloid plaques were less prominent. Thalamic gliosis is a prominent feature in other human prion diseases, including FFI[451] and sporadic fatal insomnia.[459] However, in contrast to vCJD, the anterior and medial thalamic nuclei are most affected, and the pulvinar is relatively spared. MRI of the brain has not been reported to show specific abnormalities in patients with FFI or sporadic fatal insomnia, although a detailed radiological assessment of these conditions has not been published.[281,457,505,507,508,700] The findings of the MRI study in vCJD and previous reports correlating MRI and pathological changes in sporadic CJD and hamster scrapie (see Introduction section on MRI) argue that astrocytosis is likely to be the most important cause of the high signal found in the pulvinar in vCJD.

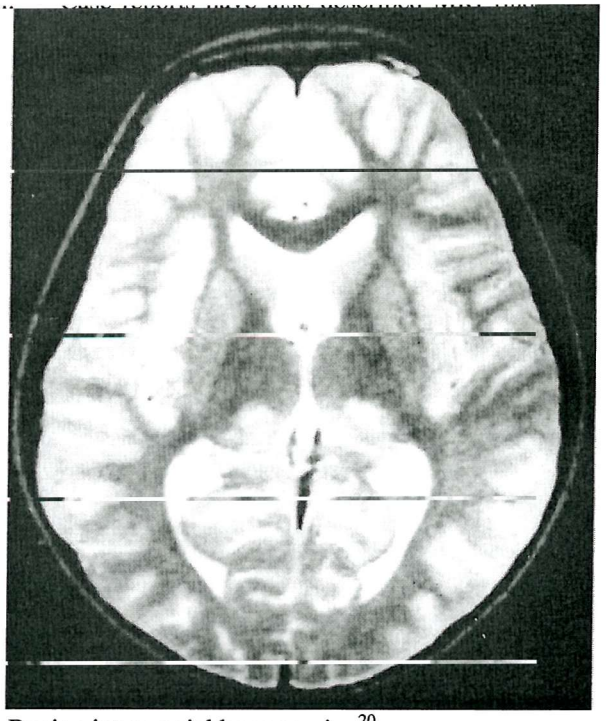
## Conclusion of MRI study

The study findings indicate that in the appropriate clinical context, symmetrical high signal in the pulvinar of the thalamus on T2-weighted or PD-weighted MR images or both is strongly suggestive of a diagnosis of vCJD. A periodic EEG and positive 14-3-3 cerebrospinal-fluid test are included in established clinical diagnostic criteria for sporadic CJD[194] and have estimated sensitivities of 66% and 94% and estimated specificities of 86% and 93%, respectively.[579] The presence of increased pulvinar signal on MRI has a comparable sensitivity and specificity in vCJD and should facilitate the diagnosis of this form of CJD during the clinical course, avoiding the need for more invasive diagnostic procedures.

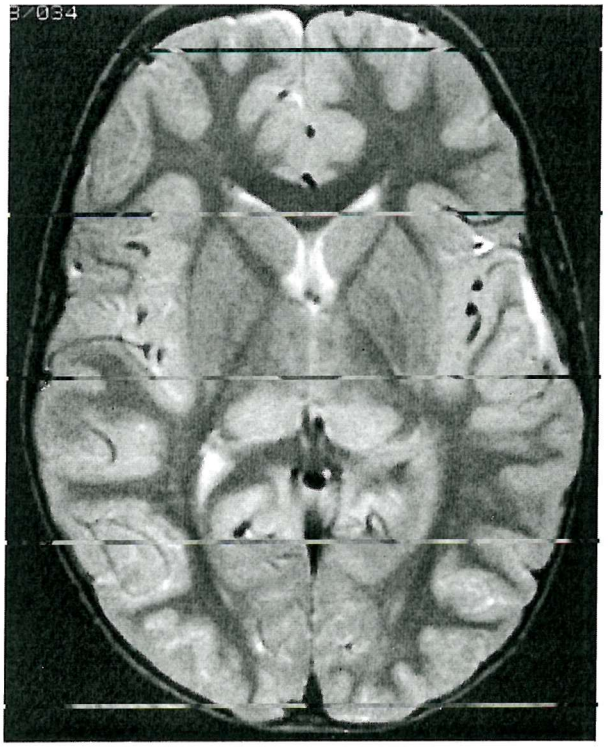
Since this study was published the MRI appearances of an additional 16 vCJD patients have been reported by the CJDSU, 13 of whom had a positive pulvinar sign.[1108] Case reports have also described MRI findings in recent vCJD cases from France and the Republic of Ireland and both had pulvinar high signal.[1122,1123] Clinical diagnostic criteria for vCJD have been formulated which incorporate the pulvinar sign and allow patients to be classified as having 'probable' vCJD when pathological examination is not available (see Annex 5).[285]



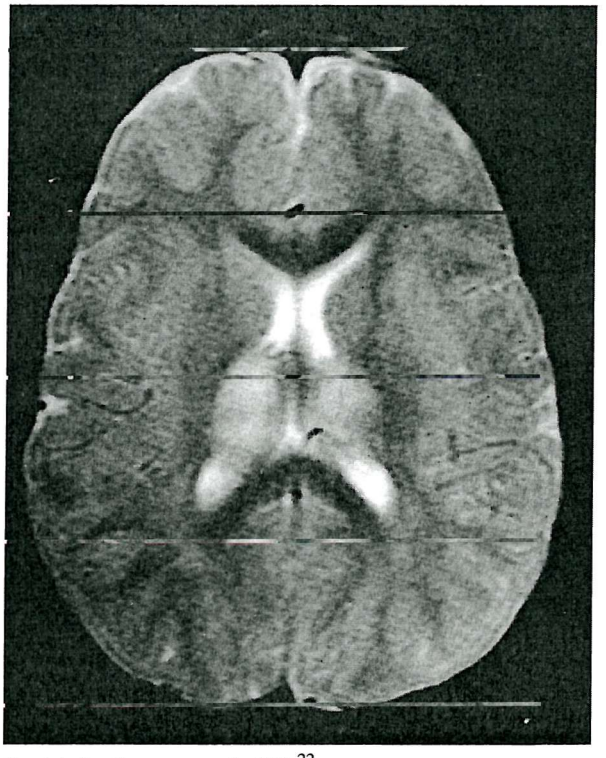

Alpers' syndrome[19]



Benign intracranial hypertension[20]



Cat-scratch disease[21]



Post-infectious encephalitis[22]



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## CONCLUSIONS OF THESIS

Between mid-1995 and the end of 1996 14 cases of CJD were identified in the UK with a novel clinicopathological phenotype. The patients characteristically presented with psychiatric or painful sensory symptoms, or both, and many developed features considered unusual for CJD: fleeting delusions, chorea, dystonia and upgaze paresis. Compared to sporadic CJD the cases were also remarkable because of their youth and lack of a periodic EEG. The presence of certain neuropathological features, in particular florid plaques, was highly consistent between cases and distinct from other forms of CJD. All the patients were methionine homozygous at codon 129 of the PrP gene, in contrast to only 41% of the normal population.

An analysis of case-control data did not identify an clear risk factors, although this has to be interpreted with caution given the number of cases. The temporal and spatial link with BSE provided initial evidence for a causal association. This has been strengthened by the demonstration that the vCJD agent and the BSE agent share biological and molecular strain characteristics, in keeping with the hypothesis that they are the same. The most plausible explanation for the appearance of these 14 cases is they have been infected with the BSE agent through dietary exposure.

A study of cranial MRI in 36 vCJD patients and 57 controls found that the presence of bilateral pulvinar high signal on T2 or PD-weighted sequences is a sensitive and highly specific diagnostic sign of vCJD. This abnormality has been incorporated into the WHO criteria for probable vCJD.

**Table 64: Series of clinical features in CJD used in Annex 1 and 2**

| Study | Population | Dates* | Non-sporadic cases | Number of cases† | Path confirmed | Criteria for non-path cases |
|---|---|---|---|---|---|---|
| Wientjens[211] | European Union‡ | 1993-5 | 7% familial<br>2% iatrogenic<br>2% variant | 606 | 56% | Probable (Budka)[895] |
| Brown[70] | NIH series§ | 1963-93 | All sporadic | 232 | >95% | Transmitted |
| Brown[251] | France | 1968-82 | 4-8% familial‖ | 230 | 100% | - |
| Poser[255] | Germany | 1993-6 | All sporadic | 201 | 51% | Probable (Budka¶)[895] |
| de Silva[252] | UK | 1990-4 | All sporadic | 143 | 84% | Probable (Budka)[895] |
| Will[253] | England & Wales | 1970-9 | ~6% familial | 137** | 77% | Probable (Masters)[200] |
| Satoh[256] | Japan | 1961-83 | ~10% familial | 127 | 100% | - |
| Brown[254] | France | 1968-77 | 5-10% familial‖ | 124 | 100% | - |
| Lundberg[196] | Sweden | 1985-96 | All sporadic | 122 | 58% | Probable (WHO 1996)[1124] |
| Masullo[206] | Italy | 1972-86 | All sporadic | 78 | ~67% | Transmitted or clinically definite (Brown)[251] |
| Zerr[1002] | Germany | 1993 | All sporadic | Not stated | Not stated | Not stated |
| Tsuji[208] | Japan | 1975-8 | ~6% familial | 63 | ~50% | Probable (Masters)[200] |
| Kovanen[210] | Finland | 1974-89 | All sporadic | 32 | 100% | - |
| Satishchandra[209] | India | 1971-90 | All sporadic | 30 | 67% | Probable (Masters)[200] |

* Some cases are included in more than one case series

† In some series details on all clinical features not available for every case

‡ France, Germany, Italy, Slovakia and UK

§ Cases referred to the National Institutes of Health, USA, mostly from the Americas and Europe

‖ Lower and upper values represent those with a family history of possible and definite CJD respectively

¶ Additionally, 14-3-3 CSF protein equivalent to a positive EEG in defining a probable case

** All cases 'subacute'. Intermediate cases with prolonged clinical course and amyotrophic cases not included.

# ANNEX 1: PRESENTING FEATURES OF CJD

| Presenting clinical feature* | | Percent* | Range† |
|---|---|---|---|
| **Cognitive** | Mental deterioration[70] | 69 | 63-64[251,254] |
| | Memory impairment[211] | 60 | 19-50[70,210,252] |
| | Rapid cognitive decline[211] | 57 | - |
| | Dementia[251] | 31 | 21-50[196,208,253,254] |
| | Confusion[210] | 19 | - |
| | Defect of higher cortical function[70] | 16 | 15-18[251,254] |
| | Disorientation[252] | 15 | - |
| | Aphasia/apraxia[196] | 8 | - |
| | Dysphasia/dysgraphia[253] | 5 | - |
| | Dysphasia[252] | 4 | 6[210] |
| | Expressive dysphasia[252] | 4 | - |
| | Dysgraphia[210] | 3 | - |
| | Dyspraxia[252] | 3 | 6[210] |
| | Receptive dysphasia[252] | 0 | - |
| **Psychiatric** | Psychogenic symptoms or personality change[208] | 36 | - |
| | Behavioural abnormalities[70] | 29 | 15-49[251-254,1002] |
| | 'Psychiatric'[211] | 26 | - |
| | Asthenia[254] | 23 | - |
| | Personality change[252] | 14 | - |
| | Depression or emotional lability‡[253] | 9 | - |
| | Delusions[210] | 6 | - |
| | Depression[1002] | 42 | 3-45[196,206,210] |
| | Anxiety[210] | 3 | - |
| | Auditory hallucinations[210] | 3 | |
| | Tearfulness[210] | 3 | - |
| | Aggressivity[210] | 3 | - |
| | General fatigue[208] | 1 | 6[210] |
| | Abnormally tired and exhausted[1002] | 56 | |
| **Abnormal movements** | Involuntary movements[211] | 20 | 0.4-16[70,208,251,253,254] |
| | Myoclonus[211] | 18 | 0-2[70,196,251,252] |
| | Tremor[210] | 16 | |
| | Involuntary movements other than myoclonus[70] | 3 | 0.4[251] |
| | Chorea[252] | 1 | |
| | Dystonia[252] | 0 | |

*Reference and percentage relate to the largest study reporting the feature. †Range refers to the percentage of the feature in other studies. ‡Leading to psychiatric referral

| Presenting clinical feature* | | Percent* | Range† |
|---|---|---|---|
| **Cerebellar** | Disturbance of balance and gait[210] | 63 | - |
| | Gait disturbance[211] | 61 | - |
| | Cerebellar signs[211] | 48 | - |
| | Gait ataxia[208] | 41 | - |
| | Ataxia/vertigo[196] | 39 | - |
| | Cerebellar incoordination[252] | 39 | - |
| | Cerebellar symptoms/signs[70] | 33 | 34[251] |
| | Cerebellar symptoms[254] | 29 | - |
| | Ataxia[253] | 19 | - |
| **Extrapyramidal** | Extrapyramidal[211] | 13 | 0.5-3[70,251,254] |
| | Rigidity/tremor[196] | 2 | - |
| | Parkinsonism[252] | 1 | - |
| | Extrapyramidal rigidity[251] | 0 | - |
| **Visual** | Visual symptoms[211] | 40 | 17[254] |
| | Visual/oculomotor symptoms/signs[211] | 36 | 17-19[70,251] |
| | Visual (non oculomotor)[251] | 14 | |
| | Visual problems[208] | 13 | |
| | Cortical blindness[252] | 9 | |
| | Visual disturbance[253] | 9 | |
| | Visual blurring[210] | 6 | |
| | Oculomotor[251] | 6 | 1-2[196,252] |
| | Diplopia[210] | 3 | |
| | Visual manifestation[196] | 2 | |
| | Visual hallucinations[252] | 1 | 1-3[210,253] |
| **LMN features** | LMN signs[70] | 3 | - |
| | Amyotrophy/muscle wasting[211] | 2 | - |
| | LMN signs/symptoms[70] | 0.5 | 0.4[251] |
| **Sensory** | Sensory[211] | 15 | 2-6[70,196,251,253,254] |
| | Leg parasthesias[208] | 6 | |
| **Other motor** | Hemiparesis[210] | 9 | - |
| | Pyramidal[211] | 9 | 2-3[70,251,252,254] |
| | Paresis of extremities[196] | 6 | |

*Reference and percentage relate to the largest study reporting the feature. †Range refers to the percentage of the feature in other studies.

| Presenting clinical feature* | | Percent* | Range† |
|---|---|---|---|
| **Miscellaneous** | Speech disturbance[211] | 34 | - |
| | Vertigo/dizziness[211] | 30 | 13[70] |
| | Dysarthria[208] | 24 | - |
| | Weight loss[254] | 15 | - |
| | Disordered sleep[1002] | 34 | 14[254] |
| | Headache[211] | 13 | 3-11[70,208,210,251,253,254] |
| | Dizziness[253] | 11 | 19[210] |
| | Pseudobulbar[211] | 10 | 0.5[70] |
| | Vertigo[251] | 7 | 1-8[208,254] |
| | Insomnia[254] | 7 | 6[210] |
| | Somnolence[254] | 7 | - |
| | Epileptic seizures[211] | 5 | 0-3[70,208,210,251] |
| | Hypothalamic (vegetative) [251] | 3 | - |
| | Stuttering[210] | 3 | - |
| | 'Blackout attacks'[253] | 1 | - |
| | Cranial nerve signs/symptoms[251] | 0 | - |
| | Epilepsia partialis continua[252] | 0 | - |
| | Nystagmus[252] | 0 | - |

*Reference and percentage relate to the largest study reporting the feature. †Range refers to the percentage of the feature in other studies.

# ANNEX 2: FEATURES DURING COURSE OF CJD

| Clinical feature during illness course* | | Percent* | Range† |
|---|---|---|---|
| **Cognitive** | Mental deterioration[70] | 100 | 100[206,251,254] |
| | Memory impairment[70] | 100 | 64-100[196,210,252] |
| | Dementia[251] | 96 | 87-100[196,208-210,253-256] |
| | Aphasia/apraxia[196] | 92 | - |
| | Disorientation[252] | 78 | - |
| | Defects of higher cortical function[70] | 73 | 39-47[251,254] |
| | Confusion[210] | 63 | - |
| | Dysphasia[253] | 62 | 41[210] |
| | Dyspraxia[252] | 55 | - |
| | Expressive dysphasia[252] | 38 | - |
| | Disturbance of consciousness[256] | 32 | - |
| | Receptive dysphasia[252] | 20 | - |
| | Perserveration[256] | 8 | - |
| **Psychiatric** | Anxiety/depression/aggression[196] | 59 | - |
| | Behavioural abnormalities[70] | 57 | 45-62[251,252,254] |
| | Personality change[252] | 53 | - |
| | Fear[253] | 18 | - |
| | Hallucinations[256] | 17 | 8-25[196,206,210] |
| | Insomnia[256] | 17 | - |
| | Delusions[256] | 12 | 37[210] |
| | 'Psychiatric manifestations'[209] | 10 | - |
| | Psychomotor excitement[256] | 8 | - |
| **Abnormal movements** | Involuntary movements[70] | 91 | 90-91[251,254] |
| | Myoclonus[70] | 78 | 73-92[196,206,208-210,251-256] |
| | Tremor[210] | 38 | - |
| | Startle response[210] | 38 | - |
| | Involuntary movements other than myoclonus[70] | 36 | 11-53[196,251,253,254] |
| | Dyskinesia[210] | 32 | - |
| | Hand tremor[256] | 25 | - |
| | Dystonia[252] | 16 | - |
| | Chorea[252] | 13 | - |
| | Choreo-athetoid movement[256] | 10 | - |

*Reference and percentage relate to the largest study reporting the feature. †Range refers to the percentage of the feature in other studies.

| Clinical feature during illness course* | | Percent* | Range† |
|---|---|---|---|
| **Cerebellar** | Unsteady gait[210] | 100 | - |
| | Gait ataxia[255] | 86 | - |
| | Cerebellar incoordination[252] | 85 | - |
| | Cerebellar symptoms/signs[70] | 71 | 61[251] |
| | Ataxia[253] | 62 | 24-80[196,206,256] |
| | Gait disturbance[256] | 60 | |
| | Cerebellar symptoms[254] | 56 | |
| | Cerebellar signs[253] | 42 | 19-85[206,208,209,252,256] |
| | Nystagmus[252] | 20 | 28[210] |
| **Extrapyramidal** | Rigor or other extrapyramidal signs[255] | 73 | - |
| | Extrapyramidal[70] | 56 | 60-67[251,254] |
| | Extrapyramidal rigidity[251] | 51 | 31[210] |
| | Parkinsonism[252] | 34 | |
| | Extrapyramidal signs[253] | 3 | 40-91[206,208,209,252] |
| **Visual** | Visual disturbance[255] | 54 | 25-33[209,210,253,256] |
| | Visual/oculomotor symptoms/signs[70] | 42 | 42[251] |
| | Visual signs[206] | 40 | - |
| | Visual symptoms[254] | 40 | 25[206] |
| | Visual (non oculomotor) [251] | 31 | - |
| | Visual defects[196] | 18 | 8[210] |
| | Visual hallucinations[253] | 17 | 32[252] |
| | Oculomotor[251] | 16 | 27-28[196,252] |
| | Cortical blindness[253] | 13 | 52[252] |
| | Diplopia[256] | 13 | - |
| | Supranuclear gaze palsy[251] | 9 | - |
| | Upgaze paresis[70] | 5 | - |
| **LMN features** | Amyotrophy/muscle wasting[252] | 17 | 27-44[208,209] |
| | LMN signs/symptoms[70] | 12 | 11-12[251,254] |
| | Fasciculations[210] | 9 | |
| | LMN signs[253] | 3 | 6-8[206,256] |

*Reference and percentage relate to the largest study reporting the feature. †Range refers to the percentage of the feature in other studies.

| Clinical feature during illness course* | | Percent* | Range† |
|---|---|---|---|
| **Sensory** | Sensory defect on examination[210] | 16 | - |
| | Sensory features[70] | 11 | 5-16[196,251,254,256] |
| | Romberg's sign[256] | 6 | - |
| **Other motor** | Pyramidal signs[253] | 79 | 29-90[206,208-210,252,256] |
| | Paresis of extremities[196] | 66 | - |
| | Muscular rigidity[256] | 64 | - |
| | Pyramidal[70] | 62 | 43-62[251,252,254] |
| | Rigidity/spasticity[196] | 61 | - |
| | Paresis and spasticity[255] | 52 | - |
| | Paratonic rigidity[252] | 38 | - |
| | 'Motor disturbance'[253] | 27 | - |
| | Focal signs[256] | 18 | - |
| | Facial weakness[252] | 16 | - |
| **Miscellaneous** | Mutism[210] | 100 | - |
| | Akinetic mutism[255] | 53 | 39-90[206,208,252,253,256] |
| | Incontinence[252] | 39 | - |
| | Primitive reflexes[253] | 30 | 18-58[252,256] |
| | Weight loss/nausea[253] | 26 | - |
| | Dysarthria[256] | 25 | 16[210] |
| | Vertigo/dizziness[70] | 19 | - |
| | Seizures[70] | 19 | 8-38[196,206,209,210,251-256] |
| | Headache[70] | 18 | 8-16[206,251,253,254] |
| | Dysphagia[256] | 14 | 32[196] |
| | Respiratory depression[252] | 15 | 100[210] |
| | Vertigo[251] | 10 | 11-35[206,254] |
| | Bulbar dysfunction[252] | 8 | - |
| | Hypothalamic (vegetative)[251] | 7 | 3[254] |
| | Pseudobulbar[70] | 7 | - |
| | Epilepsia partialis continua[251] | 0.5 | 1-4[252,254] |
| | Cranial nerve signs/symptoms[251] | 0.4 | 2-6[206,254] |

*Reference and percentage relate to the largest study reporting the feature. †Range refers to the percentage of the feature in other studies.

# ANNEX 3: DEFINITION OF CJD SUBTYPES

## SPORADIC CJD

### Definite:

- Spongiform encephalopathy in cerebral and/or cerebellar cortex and/or subcortical grey matter;
  and/or
- Encephalopathy with immunocytochemically and/or Western blot confirmed protease-resistant PrP;
  and/or
- Presence of scrapie-associated fibrils.

### Probable:

- Progressive dementia,
  and
- At least two out of the following four clinical features:
  - Myoclonus
  - Visual or cerebellar disturbance
  - Pyramidal/extrapyramidal dysfunction
  - Akinetic mutism;

  and
- A typical EEG

### Possible:

Same as for probable CJD but without EEG or with atypical EEG and duration of less than two years

## IATROGENIC CJD

- Progressive cerebellar syndrome in a pituitary hormone recipient;
  or
- Sporadic CJD with a recognised exposure risk, e.g. antecedent neurosurgery with dura mater graft.

## FAMILIAL CJD

- Definite or probable CJD plus definite or probable CJD in a first degree relative;
  and/or
- Neuropsychiatric disorder plus disease-specific PRNP mutation.

# ANNEX 4: CJD STUDY QUESTIONNAIRE

1. Patient number

2. a) Case Source:
   b) Control

3. Date of interview

4. Place of interview

5. Patient (Maiden Name) Interviewee

   Name:

   Address:

   Tel:

   Relationship:

6. Sex

7. Date of birth

8. Hospital:

   Ward:

   Hospital No:

   Consultant:

9. GP:

   Address:

   Tel:

10. Country of origin

11. Skin Colour

**PRODROMAL PHASE**

12a. Has the patient ever seen a doctor in the last year?

Reason:

Date:

Doctor:

12b. Has the patient ever seen a dentist in the last year?

Reason:

Date:

Dentist:

13. Had the patient, prior to this admission been:

a) Depressed

b) Tired and exhausted

c) Unable to sleep

d) Behaving oddly; specify:

e) Sweating abnormally

f) Eating abnormally

g) Losing weight

h) Gaining weight

14. In the last 3 months prior to this admission, had the patient had:

a) Cough

b) Cold

c) "Flu"

d) Diarrhoea

e) Other illness; specify:

15. When did the patient first see the doctor about the present illness?

Date:

Reason:

Doctor:

## PAST MEDICAL HISTORY

16. Has the patient every been in hospital prior to this illness?

| If so: | Date | Reason | Hospital |
|---|---|---|---|
| 1. | | | |
| 2. | | | |
| 3. | | | |
| 4. | | | |

17. Does the patient either regularly attend hospital or GP?

| Date | Practitioner | Reason |
|---|---|---|

18. Has the patient ever had an operation?

| If so: | Date | Type of operation | Reason | Hospital |
|---|---|---|---|---|
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |

19a. Has the patient ever had an eye operation?

If so: Reason:

Date:

Hospital:

19b. Has the patient ever been tested for glaucoma?

20. Has the patient ever had a head injury (with loss of consciousness or skull fracture)?

If so: Details:

21. Has the patient ever been jaundiced?

If so: Details:

22. Has the patient ever had an epileptic fit?

If so: Details

23. Has the patient ever attended a psychiatrist?

If so: Date:

Doctor:

Reason:

24a. Has the patient ever had a blood transfusion?

If so: Details:

24b. Has the patient ever been a blood donor?

If so: Details:

25. Has the patient ever had an organ transplant

If so: Specify organ and give details:

26. Has the patient ever been in contact with any person with a serious dementing illness?

If so, details

27. Is the patient left or right handed?

28. Has the patient ever suffered from:

a) Glandular fever?

b) Polio?

c) Shingles?

d) Herpes Simplex?

e) Rheumatoid arthritis

f) Diabetes Mellitus

g) Allergies

29. What medications has the patient been exposed to:

| | Name (including dose and freq) | Reason | Duration |
|---|---|---|---|
| (a) Prescribed | | | |

Hormone supplement:

Please specify type and route of administration:

(b) OTC
Purchases

(c) Homeopathic/Herbal

(e) Eyedrops

30a. What injectable therapy, or vaccinations has the patient received in the past?

| Name | Date | Reason | Frequency |
|---|---|---|---|

30b. Has the patient ever had an EMG

If yes, age at first EMG:
If yes, age at last EMG:
If yes, specify reason:
(eg myopathy, neuropathy etc)

31 Smoking habits:

| | | |
|---|---|---|
| Never | = | 1 |
| Ex (>12/12) | = | 2 |
| Current cigs | = | 3 |
| Current pipe/cigar | = | 4 |
| Not Known | = | 9 |

Drinking habits:

| | | |
|---|---|---|
| Never | = | 1 |
| Ex drinker (>12/12) | = | 2 |
| Current | = | 3 |
| Not Known | = | 9 |

Tattooing:

Ear piercing:

Acupuncture:

## FAMILY HISTORY

| 32.1 | | Father | Mother | Spouse |
|---|---|---|---|---|
| a) | Country of birth? | | | |
| b) | Is relative alive now? | | | |
| c) | If no: Age at death: | | | |
| | Cause of death | | | |
| | Place of death | | | |
| | Prior to death was relative confused or unconscious? | | | |
| | Did relative suffer any other disease or illness? | | | |

| 32.2 | | Paternal Grandfather | Paternal Grandmother | Maternal Grandfather | Maternal Grandmother |
|---|---|---|---|---|---|
| a) | Country of birth? | | | | |
| b) | Is relative alive now? | | | | |
| c) | If no: Age at death: | | | | |
| | Cause of death | | | | |
| | Place of death | | | | |
| | Prior to death was relative confused or unconscious? | | | | |
| | Did relative suffer any other disease or illness? | | | | |

33. Siblings

| | 1st name | Age | Full/half sib | Alive/Dead | Age at death | Cause of death |
|---|---|---|---|---|---|---|
| 1. | | | | | | |
| 2. | | | | | | |
| 3. | | | | | | |
| 4. | | | | | | |
| 5. | | | | | | |

34. Patient's marital status

35. Has the patient been married more than once?

36. Children

| | Name | Age | Sex | Alive/Dead | Cause of Death |
|---|---|---|---|---|---|
| 1. | | | | | |
| 2. | | | | | |
| 3. | | | | | |
| 4. | | | | | |
| 5. | | | | | |

## SOCIAL HISTORY

37. Residential history

| | Address | Dates | Local characteristics (eg proximity to farms, hospitals, factories etc.) |
|---|---|---|---|
| 1. | | | |
| 2. | | | |
| 3. | | | |
| 4. | | | |
| 5. | | | |
| 6. | | | |
| 7. | | | |
| 8. | | | |
| 9. | | | |
| 10. | | | |

38. Occupational history

| | Type of job | Employer | Location | Dates |
|---|---|---|---|---|
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |
| 5. | | | | |
| 6. | | | | |

39. Occupation of partner:

40. Occupation of parents:

41. Educational history

| | Institution | Town | Dates |
|---|---|---|---|
| 1. | | | |
| 2. | | | |
| 3. | | | |

42. Eating habits

a) How often does the patient eat the following

| | | |
|---|---|---|
| Never | = | 1 |
| < 1 yr | = | 2 |
| Several times/year | = | 3 |
| > 1/12 | = | 4 |
| > 1/week | = | 5 |
| Not Known | = | 9 |

| | | Ever | After 1985 |
|---|---|---|---|
| (i) | Lamb/mutton | | |
| (ii) | Pork/bacon/ham | | |
| (iii) | Beef | | |
| (iv) | Venison | | |
| (v) | Veal | | |
| (vi) | Poultry | | |
| (vii) | Fish | | |
| (vii) | Shellfish | | |

---

-

| | | |
|---|---|---|
| (i) | Sausages | |
| (ii) | Tripe | |
| (iii) | Liver (state origin) | |
| (iv) | Kidneys (origin) | |
| (v) | Sweetbreads | |
| (vi) | Tongue | |
| (vii) | Brains | |
| (viii) | Trotters | |
| (ix) | Puddings | |
| (x) | Eyes | |
| (xi) | Haggis | |
| (xii) | Heart | |
| (xiii) | Raw fish | |
| (xiv) | Beefburgers | |
| (xv) | Meat Pies | |
| (xvi) | Faggots | |

c) Does the patient ever eat rare or undercooked meat?

d) Does the patient have any dietary restrictions or eccentricities?
(NB: If vegetarian, did they ever eat meat, and if so, for how long?)

e) Does the patient consume dairy products?

| | Ever | After 1985 |
|---|---|---|
| Milk | | |
| Cheese | | |

## EXPOSURE TO ANIMALS

43. Has the patient ever had personal contact with the following animals:

| | | Y/N | Age first exposed | Duration | Ever Bitten | Age Bite | No. of Bites |
|---|---|---|---|---|---|---|---|
| (i) | Cats | | | | | | |
| (ii) | Ferrets | | | | | | |
| (iii) | Live mink | | | | | | |
| (iv) | Cattle | | | | | | |
| (v) | Sheep | | | | | | |
| (vi) | Deer | | | | | | |
| (vii) | Horses | | | | | | |
| (viii) | Pigs | | | | | | |
| (ix) | Rabbits | | | | | | |
| (x) | Rodent/hamster | | | | | | |

44. Has the patient had contact with fur or leather other than normal contact with everyday clothes?

45. Has the patient ever lived or worked on a farm?

46. Hobbies:

47. Has the patient ever had contact with the following:

(a) Fertiliser
(b) Bonemeal
(c) Hoof and Horn
(d) Dried blood

48. Has the patient any domestic animals now or has he/she had any in the past?

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| a) Species | | | | | | |
| b) Was animal allowed to mix freely with other animals outside the house? | | | | | | |
| c) Did animal sleep in house? -bed? | | | | | | |
| d) Did animal ever have a serious illness? | | | | | | |
| e) Age of patient when animal in house? | | | | | | |
| f) Cause of animal's death? | | | | | | |

1. Always

2. Usually

3. Rarely

4. Never

49. Has the patient ever travelled abroad?

If so: Location and dates

50. Does the patient regularly travel within Britain?

If so: Location, frequency and reasons

51. Has any other relative had a similar disease? (OMIT THIS QUESTION IN THE CASE OF CONTROLS)

CLINICAL HISTORY

## EXAMINATION ON ADMISSION

1. General appearance:

2. Mental state/speech functions:

3. Cranial nerves:

4. Motor system:

5. Reflexes:

6. Sensory system:

7. General examination:

## REVIEW OF PROGRESSION OF PHYSICAL SIGNS

**EXAMINATION**

1. General appearance:

2. Mental state/speech functions:

3. Cranial nerves:

4. Motor system:

5. Reflexes:

6. Sensory system:

7. General examination:

## INVESTIGATIONS

a) Abnormalities on routine biochemical/haematological investigation:

b) LFT's

c) CSF:

d) EEG results:

e) CT scan:

f) MRI scan:

e) Other investigations:

## TREATMENT

**OUTCOME**

a) Date of death:

Place of death:

Cause of death:

b) Review of clinical course:

c) EEG progression:

d) Abnormalities in other investigations:

e) Post-mortem: Yes/No

Histology

f) Blood taken for genetic studies? Yes/No

Analysing Centre:

## CLASSIFICATION

| | | | Present | Absent |
|---|---|---|---|---|
| 1. | Rapidly progressive dementia: | | | |
| 2. | a) | Myoclonus | | |
| | b) | Cortical blindness | | |
| | c) | Pyramidal/extra-pyramidal/ cerebellar signs | | |
| | d) | Akinetic mutism | | |
| | e) | Early onset of neurogenic muscle wasting | | |
| | f) | Characteristic EEG | | |
| 3. | Histology: | | | |

Classification 1. CJD
- Definite
- Probable
- Possible

Other

# ANNEX 5: CONSENT FORM FOR GENETIC ANALYSIS

I, ..... consent to a blood sample being taken from myself/my relative ..... for the purpose of research on the disorder Creutzfeldt-Jakob disease.

Signed .....

The research on the blood sample will principally involve studies that will not have any direct implications for individuals, although we hope that they will help us understand the disorder better in future. However, in a few cases, Creutzfeldt-Jakob disease may result from a change in a genetic factor that could give a risk to family members. In view of this,

1. Would you like to know any test result that might suggest a risk to other family members?

   **YES / NO**

2. It is possible that information from this research that does not appear to be important now, might become so in the future. If this were to occur, would you like to be informed?

   **YES / NO**

Signed .....

Signed ..... Research Registrar

Date .....

# ANNEX 5 CONTINUED

## INFORMATION TO BE GIVEN TO RELATIVES OF CJD PATIENTS WHEN CONSENT IS BEING OBTAINED FOR BLOOD TO BE TAKEN FOR GENETIC STUDIES

1. The cause of CJD in the great majority of patients is unknown.
2. A small proportion of cases are hereditary in nature due to a faulty gene.
3. In nearly all the hereditary cases, the family are already aware of other affected family members. In these families, about half the family members can be affected by CJD and the disease may occur from generation to generation.
4. The chances of finding a faulty gene in a case of CJD without any other affected family members is very small, probably less than 1 in 50.
5. We wish to take blood from cases of CJD in order to look for abnormalities in the gene and we also store blood for future research.
6. In this way we hope to advance knowledge of CJD which may, in the future, lead to a better understanding of the disease.
7. If you do not want to now the result of this test, we will not inform you, your family doctor or the hospital doctor of the result.
8. If you do want to know the result of the test, this will done through the local genetic counselling clinic even if the test is negative which is the most likely outcome.
9. The chances of finding an abnormality in the genetic test are very low and in the great majority of cases there is no increased risk of developing this disease in family members. It is particularly important to know that CJD is not infectious and there is no risk from contact with patients during their illness.

10. **In Summary,**
    - There is no risk of developing CJD by contact.
    - Only a small proportion of cases are hereditary.
    - The blood sample will help research.
    - The result of the genetic test will only be made available **if you want it to be.**

# ANNEX 6: WHO DEFINITION OF VARIANT CJD

| | | |
|---|---|---|
| I | A | Progressive neuropsychiatric disorder |
| | B | Duration of illness > 6 months |
| | C | Routine investigations do not suggest an alternative diagnosis |
| | D | No history of potential iatrogenic exposure |
| | E | No evidence of a familial form of TSE |
| II | A | Early psychiatric symptoms[a] |
| | B | Persistent painful sensory symptoms[b] |
| | C | Ataxia |
| | D | Myoclonus or chorea or dystonia |
| | E | Dementia |
| III | A | EEG does not show the typical appearance of sporadic CJD[c](or no EEG performed) |
| | B | MRI brain scan shows bilateral symmetrical pulvinar high signal[d] |
| IV | A | Positive tonsil biopsy[e] |

| | |
|---|---|
| **DEFINITE:** | I A **and** neuropathological confirmation of vCJD[f] |
| **PROBABLE:** | I **and** 4/5 of II **and** III A **and** III B<br>**OR**<br>I **and** IV A[d] |
| **POSSIBLE:** | I and 4/5 of II and III A |

a. Depression, anxiety, apathy, withdrawal, delusions

b. This includes both frank pain and/or dysaesthesia

c. Generalised triphasic periodic complexes at approximately one per second

d. Relative to the signal intensity of other deep grey matter nuclei and cortical grey matter

e. Tonsil biopsy is not recommended routinely, nor in cases with EEG appearances typical of sporadic CJD, but may be useful in suspect cases in which the clinical features are compatible with vCJD and where MRI does not show bilateral pulvinar high signal

f. Spongiform change and extensive PrP deposition with florid plaques, throughout the cerebrum and cerebellum

# ANNEX 7: VCJD PATHOLOGICAL CHARACTERISTICS

The pathological aspects typical of TSE (spongiosis, neuronal loss, reactive astrocytosis and the accumulation in the affected brain of $PrP^{Sc}$ ) are present in vCJD as well.

However, the following key features are characteristic of vCJD:

3. Abundant kuru-type fibrillary PrP plaques, often surrounded by a halo of spongiform change (the 'florid' plaque).

4. Multiple small PrP plaques occurring in cluster within the cerebral and cerebellar cortex, not related to spongiform changes.

5. Amorphous PrP deposits around neurons and blood vessels in the cerebral and cerebellar cortex.

Immunocytochemistry for PrP is an invaluable aid to diagnosis, although the large fibrillary plaques are easily visualised by haematoxylin and eosin staining. Plaques can also be identified by periodic acid-Schiff or Gallyas silver stains, but the amorphous PrP deposits are best visualised by immunocytochemistry.

Additional neuropathological characteristics are:

1. Spongiform change most prominent in the basal ganglia, with dense perineuronal and periaxonal PrP deposition

2. Severe thalamic astrocytosis and neuronal loss, particularly involving the dorsomedial and posterior nuclei (including the pulvinar)

3. Massive accumulation of PrP, often with focal distribution, in the cerebellar cortex including the molecular and granular layer with occasional plaques in the white matter.

4. Punctate neuronal staining for PrP in the pontine nuclei.

# ANNEX 8: CASE 580 ('PROBABLE' VCJD)

In the summer of 1994 a 17-year-old student achieved her expected A' level results and after leaving school she was employed in office. Around Christmas 1995 she became uncharacteristically tired and quiet. The tiredness persisted and over the following two months she would return home from work and go to bed at 7.30 in the evening. She would sleep for 12 hours and still complain of feeling tired in the morning. Her excessive sleepiness continued and in March 1996 she was crying when getting up in the morning because she felt so tired. On one occasion she fell asleep within minutes of entering a night-club. She consulted her GP because of these symptoms at the beginning of March and again in mid-April. At the latter appointment a history of 'TATT 5/12' was noted, in addition to some frequency of micturition and polydipsia. No abnormality was detected on physical examination.

During April she appeared to be depressed, which she said was because she had too much work to do. Her family also noted that from this time she was occasionally uncharacteristically aggressive. In May she became increasingly apathetic and stopped driving. Her handwriting became untidy and the content of her letters was confused. At the end of the month her GP also noted a history of emotional lability, anxiety, decreased concentration and early morning wakening. A diagnosis of depression was made and she was started on dothiepin 75 mg at night. She subsequently left her job because of difficulty concentrating.

The dothiepin was stopped after three weeks because of drowsiness and she was started on paroxetine 20 mg/day. Her GP felt that the diagnosis at this time was chronic fatigue syndrome and made a referral for a medical opinion. She saw a consultant physician toward the end of June, who noted that she had felt 'rather better' since starting antidepressants. A physical examination, including the nervous system, was reported to be normal and it was concluded that there was no indication of any specific organic disorder.

At the beginning of July she started to experience nightmares, e.g. of sharks, and would go and sleep with her parents. She incorrectly, at times, thought that there were men in the room with her. Later that month she very uncharacteristically lost her temper with her mother and swore at her for the first time ever. Toward the end of July she went with her parents on holiday to Majorca and started to became unsteady on her feet, with her parents having to hold on to her arm to stop her falling about at times, especially going up stairs or hills. Occasionally she appeared 'fidgety'. Her memory was considered to be normal during the holiday, although at times she was confused, e.g. answering 'pizza' when asked what she wanted to drink, and she was having difficulty comprehending simple messages. When she returned home she told her friends that she had been (incorrectly) to Tenerife. At this time she was having difficulty washing and dressing and needed to be supervised. The paroxetine was considered to be unhelpful and was stopped.

Her confusion and memory deteriorate further toward the end of August and she was occasionally experiencing visual hallucinations, e.g. seeing things in the garden. A specialist in 'myalgic encephalomyelitis' was consulted at this time, and he noted that she was experiencing cold sensations in her feet. This led to her subsequently wearing socks in bed at night. On examination she was unable to recall where she had been on holiday and her conversation was described as 'intermittently halted and unsteady'. When asked by her mother how she was feeling she replied 'Mum, I don't know what the fuss is about'. Choreiform movements of the arms and legs, but not the head, were evident and she had a wide-based unsteady gait, but no ataxia of her arms. Deep tendon

reflexes were generally brisk and her plantars bilaterally upgoing.

Subsequently swallowing difficulties and choking were noted and her speech became slurred 'as if she had been drinking too much alcohol'. Her behaviour became more childish - e.g. when having her hair washed she would cry and ask for a flannel over her eyes. She was noted by her mother to 'visibly jump when surprised'.

At the beginning of September her GP became increasingly concerned by her deterioration, raised the possibility of CJD and admitted her to a local hospital. Two days later she was transferred to a neurology unit for two weeks' assessment. She was now disorientated in place and was confabulating, saying she had not taken any A-levels because her school had burnt down. No history of topographical disorientation, agnosia, apraxia, dysphasia or dyscalculia was apparent. Choreoathetoid movements had become more prominent and were ameliorated by tetrabenazine. On examination she was unable to give the day or the date and incorrectly stated that she was in a hotel in Blackpool. She knew the month and year; her name, address, post code, age and date, but not year of birth. Her concentration was poor, she was unable to recall any of three items after five minutes, but knew the name of the Prime Minister. Reading and comprehension appeared intact. She had poor proverb interpretation and had difficulty with the Luria three-stage test. She had no constructional apraxia and correctly drew a clockface. Cerebellar dysarthria, decreased coordination in all limbs and strong bilateral grasp reflexes were noted, but she had no other frontal release signs. There were no long tract, extrapyramidal or sensory signs. CSF examination was performed twice during the admission and did not reveal any abnormal biochemistry, oligoclonal bands or white cell count. The 14-3-3 CSF assay was performed on the first sample and was positive. A CT brain scan showed no abnormality, but cranial MRI revealed mild atrophy, especially involving the cerebellar vermis. The proton density and T2-weighted sequences showed abnormal hyperintensity involving the posterior aspects of the thalami bilaterally. An EEG was reported as abnormal, with diffuse slowing of the background with an excess of delta and theta activity.

She was discharged in mid-September and continued to deteriorate, becoming less alert and responsive. Her weight was decreasing as she was eating less. She was readmitted in early October for further investigation. Her speech was now more slurred and less spontaneous. She was still emotionally labile and sleeping excessively. Her condition was noted to fluctuate from day to day. On examination she would not respond to commands, was disoriented in time and place and she was unable to count to 20 or recite the alphabet. She had a mask-like face and was drooling saliva. Tone was markedly increased in her legs and the right plantar reflex was extensor. Plasma IgA was decreased to just below the lower limit of normal. Serum IgG subclasses 1, 2 and 4 were normal but subclass 3 was raised at 1.52 (NR 0.5-0.9). A Paul Burnell test had been positive in 1995 and in May 1996 her Epstein-Barr virus IgM and IgG titres were both increased, raising the question of a 'chronic infection'. However, the titre in September 1996 was not elevated. All other routine haematological, biochemical, immunological and microbiological investigations had been unremarkable. Two further EEGs were performed a week apart and showed a deterioration since the previous record, with gross diffuse slowing, and in the latter tracing, bursts of generalised delta as well as runs of posterior one Hz delta activity which occasionally had a triphasic appearance. There were no periodic features in any of the three EEGs.

A trial of steroids in mid-October was stopped after four days because of a marked deterioration. She became incontinent of urine and virtually mute and was transferred to a hospice. Myoclonus developed, and she was blind and akinetic mute prior to her death in mid-November. An autopsy was not performed.

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