THE CLINICO-PATHOLOGICAL PHENOTYPE OF SPORADIC CREUTZFELDT-JAKOB DISEASE

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DECLARATION

This thesis is all my own original work except for those sections where the work of other members of the NCJDSU is acknowledged.

This work has not been submitted for any other degree or professional qualification. Nor has it been published elsewhere.

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ABSTRACT

Creutzfeldt-Jakob Disease (CJD) is a rare neurodegenerative disorder of the human central nervous system. It occurs in four main forms, defined essentially according to aetiology: sporadic, variant, iatrogenic and familial. All forms of the disease are characterised by the deposition of an abnormal cellular protein, the prion protein (PrP^{sc}), within the brain. Definitive diagnosis depends on identifying this along with other neuropathological changes such as spongiform degeneration and astrocytic gliosis.

To date variant CJD has followed a relatively stereotyped clinical course with fairly consistent pathological findings. However, various clinico-pathological phenotypes of sporadic CJD have been described. It is believed the disease phenotype is influenced by a number of factors including agent strain (PrP^{res} isotype being used as a surrogate marker), and genotype (particularly a polymorphism at codon 129 of the prion protein gene).

Data on 99 cases of sporadic CJD and 43 cases of variant CJD were analysed. The sporadic CJD cases were divided into 6 sub-groups, according to genotype at codon 129 (either methionine homozygous, valine homozygous or heterozygous) and PrP^{res} isotype (either type 1 or 2A). Some trends in clinico-pathological phenotype were found. Notably, the methionine homozygous cases with type 1 PrP^{res} isotype formed the majority of cases and followed a reasonably uniform disease course. The other groups tended to include atypical cases. These differences did not achieve statistical significance and there was considerable overlap amongst cases.

14-3-3 protein and the use of MR imaging were analysed. The results suggest that these investigations may improve the diagnostic classification of sporadic CJD.

The variant CJD cases followed a relatively consistent clinico-pathological course, consistent with a distinct aetiology and probably a distinct strain. The data do not support the hypothesis that different strains of the sporadic CJD agent cause distinct clinico-pathological phenotypes.

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LIST OF ABBREVIATIONS

Apo E	apolipoprotein E
ASN	aspartane
BSE	bovine spongiform encephalopathy
CJD	Creutzfeldt-Jakob disease
CNS	central nervous system
CSF	cerebrospinal fluid
CT	computed tomography
C-terminal	carboxy-terminal
CWD	chronic wasting disease of mule and elk
DNA	deoxyribonucleic acid
DY	drowsy
EDTA	ethylene diamine tetra-acetic acid
EEG	electroencephalogram
ELISA	enzyme linked immuno-assay
ESR	erythrocyte sedimentation rate
FFI	fatal familial insomnia
FLAIR	fluid attenuated inversion recovery
FSE	feline spongiform encephalopathy
GP	general practitioner
GPI	glycophosphatidyl inositol
GSS	Gerstmann-Sträussler-Scheinker syndrome
H&E	haematoxylin and eosin
HGH	human growth hormone
HY	hyper
ICD	international classification of diseases
Met	methionine
MRI	magnetic resonance imaging
MRM	mechanically recovered meat
NCJDSU	National CJD Surveillance Unit

NG	nasogastric
NIH	National Institute of Health
N-terminal	amino-terminal
ORF	open reading frame
PCR	polymerase chain reaction
PBS	phosphate buffered saline
Prion	proteinaceous infectious particle
PRNP	human PrP gene
PrP	prion protein
PrP^{C}	cellular form of PrP
PrP^{Sc}	abnormal pathogenic form of PrP^{C}
PrP^{res}	product of PrP^{Sc} after digestion with proteinase K
PrP^{27-30}	product of PrP^{Sc} after digestion with proteinase K
SAF	scrapie associated fibrils
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
Sinc	gene for scrapie incubation period
SFI	sporadic fatal insomnia
SOD	superoxide dismutase
TME	transmissible mink encephalopathy
TSE	transmissible spongiform encephalopathy
UK	United Kingdom
Val	valine
vCJD	variant Creutzfeldt-Jakob disease
WHO	World Health Organisation

CHAPTER 1: INTRODUCTION

The Transmissible Spongiform Encephalopathies (TSEs) are a group of rare neurodegenerative diseases that affect animals and man. They are characterised by the fact that they are transmissible, and by the neuropathological hallmarks of spongiform change and deposition of an insoluble form of a cellular protein, the prion protein (PrP) in the central nervous system. The precise aetiology is unknown but it has been hypothesised that despite a lack of nucleic acid, PrP is the causative agent.[1] Although to date this remains unproven, it remains the most widely accepted theory.

There are a variety of TSEs affecting a number of species, including scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and kuru and Creutzfeldt-Jakob disease (CJD) in humans. (See Table 1.1) The TSEs are unique in that they exist in sporadic or hereditary forms, and yet may be acquired, being transmissible under certain circumstances; e.g. by inoculation of diseased brain tissue peripherally or into the central nervous system of an experimental host. There is no evidence of an immune response however. Despite considerable research into all of the TSEs many details of the mechanisms of the disease process remain elusive.

The neuropathological hallmark of human TSEs is spongiform change within the brain and spinal cord along with variable neuronal loss and astrocytic gliosis. Spongiform change is not universal among the diseases and deposition of PrP and analysis of the human PrP gene (PRNP) on chromosome 20 are also important in confirmation of the diagnosis.[2]

Sporadic CJD is the commonest form of human prion disease, but it is still rare with an average worldwide incidence of approximately 1 per million population.[3] To date no cause has been found for the disease, despite epidemiological analysis in various

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Sporadic CJD is the commonest form of human prion disease, but it is still rare with an average worldwide incidence of approximately 1 per million population.[3] To date no cause has been found for the disease, despite epidemiological analysis in various

case-control studies.[4] In particular, no genetic, infectious or iatrogenic cause has been found.

Table 1.1: TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES.

Disease	Species	Described	Distribution	Aetiology
Animal Diseases				
Scrapie	Sheep, goats	18th century	World wide except Australia	Infectious, sporadic
Transmissible Mink Encephalopathy	Captive mink	1947	North America, parts Europe	Scrapie infected feed
Chronic Wasting Disease	Captive deer, elk	1967	North America	Unknown
Bovine Spongiform Encephalopathy	Cattle	1985	UK, parts of Europe	? scrapie, ? somatic mutation
TSE of captive wild ruminants	Captive bovidae	1986	UK	Feed contaminated with BSE
Feline Spongiform Encephalopathy	Domestic cat	1990	UK	Feed contaminated with BSE
Human Diseases				
Classical Creutzfeldt Jakob Disease	Humans	1920, 1921	World wide	Unknown
Gerstmann Sträussler Sheinker syndrome	Humans	1936	World wide	Somatic mutation in PRNP
Kuru	Humans	1957	Papua New Guinea	Acquired-cannibalism
Iatrogenic CJD	Humans	1974	Europe, North America	Contaminated instruments and hormones
Familial CJD	Humans	1989	World wide	Somatic mutation in PRNP
Fatal Familial Insomnia	Humans	1992	World wide	Somatic mutation in PRNP
Variant CJD	Humans	1996	UK, France, Ireland	BSE transmission to man
Sporadic Fatal Insomnia	Humans	1998	World wide	Sporadic

The disease occurs mainly in late middle age with a mean age at death of 65 years. It typically follows a very characteristic course of a rapidly progressive dementia with

myoclonus. Cerebellar and pyramidal signs are commonly seen, and the patient deteriorates quickly to a state of akinetic mutism and death. The median duration of illness is 4 months.[5]

Routine investigation of patients with possible sporadic CJD is often surprisingly normal, however analysis of the electroencephalogram (EEG) reveals the presence of characteristic generalised periodic triphasic complexes in 60-70% of cases.[6] More recently, cerebrospinal fluid (CSF) analysis for the presence of 14-3-3 protein has been shown to be a sensitive and specific marker for sporadic CJD in the appropriate clinical context.[7] In addition, it has been suggested that high signal in the basal ganglia on magnetic resonance imaging (MRI) may also be useful in aiding diagnosis.[8]

Atypical forms of sporadic CJD are well described although the majority of cases do have a fairly consistent clinical course. A small percentage of cases have a relatively longer duration of illness of 1-2 years or more.[9] In other forms presentation is predominantly cerebellar: the Brownell-Oppenheimer variant,[10] or with cortical blindness: the Heidenhain variant.[11] Panencephalic and thalamic types, characterised by distinct neuropathological changes have also been described.[5] Deposition of kuru-type amyloid plaques is sometimes present in cases of CJD.[12]

The reason for the differences in clinico-pathological phenotype in sporadic CJD and the other TSEs has been the subject of speculation over the years. The disease process and neuropathological changes seem to be influenced by a number of factors within both the infectious agent and the patient or host.

Analysis of codon 129 in human PRNP has shown that this is the site of a methionine/ valine polymorphism. It is known that 33% of Caucasian populations are methionine homozygous at this site but 71% of all cases of sporadic CJD carry this genotype,[13] suggesting it predisposes to the development of the disease. Recent

evidence suggests that heterozygosity or valine homozygosity at codon 129 is associated with atypical forms of sporadic[14] and familial disease.[15]

In addition, transmission studies of scrapie to goats have demonstrated different clinical features[16], and in transmission studies to mice of the same genotype (hence apparently removing the host influence on disease phenotype) different pathological changes have been shown.[17] It has been suggested that this is due to different strains of the prion agent. Recent studies of Transmissible Mink Encephalopathy (TME) have suggested that strain variation may be due to differences in composition or conformation of PrP.[18]

Prion diseases are associated with the deposition of an insoluble form of normal cellular PrP (PrP^{c}). This is termed PrP^{Sc} (from scrapie).[19] A protease resistant core prion protein (PrP^{res}) is the product of PrP^{Sc} after proteolysis. This product can be analysed by Western blotting. The molecular size of PrP^{res} and the ratio of glycoforms present can be used to classify the PrP^{res} present into protein isotypes.

Protein isotyping is currently regarded as a convenient surrogate for agent strain although the precise molecular basis of strain variation is uncertain. It has been proposed that PrP isotype therefore partially determines differences in clinico-pathological phenotype in sporadic CJD.[14]

Different groups have identified slightly different isotypes of PrP^{res},[14, 20] presumably related to differences in Western blotting technique. To date published information is available on a limited number of cases, but the clinical and pathological features of two isotypes: types 1 and 2, have been described in sporadic CJD. Type 2 has been subdivided into 2A and 2B and it seems that PrP^{res} type 2A is also associated with atypical forms of the disease.[14] Thus in sporadic CJD there are 6 possible combinations of PrP^{res} isotype and codon 129 genotype (See Table 1.2) and each is potentially associated with a different clinico-pathological phenotype.

At this time only one genotype and PrP^{res} isotype has been seen in variant CJD (vCJD) but this may well change with time. These cases have followed a relatively consistent and distinct clinical and pathological course. In addition iatrogenic CJD due to human growth hormone also has relatively consistent clinical and pathological findings. This supports the hypothesis that a different single strain is involved. All cases of variant CJD have been associated with a distinct protein isotype, type 2B. However, both protein isotypes have been seen in iatrogenic CJD

Table 1.2: GENOTYPE/ PROTEIN ISOTYPE COMBINATIONS

MM / PrP^{res} type 1	MV / PrP^{res} type 1	VV / PrP^{res} type 1
MM / PrP^{res} type 2	MV / PrP^{res} type 2	VV / PrP^{res} type 2

Recently, 300 cases of sporadic CJD from Europe and North America were analysed to look for clinical and pathological correlates within the 6 groups. Some trends were suggested. However, these data were amalgamated from various countries, many of which do not have a dedicated surveillance system. Much of the data may have been extracted retrospectively from case notes. In addition, the criteria for analysis of the EEG and MRI were not detailed and it was not clear if these were reviewed for the purposes of the study.

I have analysed 99 cases of sporadic CJD referred to the National CJD Surveillance Unit (NCJDSU) in whom the results of genotype and isotype are available. In as many as possible cases the patient or their relative has been interviewed and the patient has been examined during life. The EEG and MRI were reviewed and reclassified personally where possible. It is believed that these data were as full and complete as possible, bearing in mind the difficulties of surveillance of a rare disease. The cases were allocated to the 6 groups listed above and their clinical and

pathological features and the results of investigations were analysed. A further 43 cases of vCJD were reviewed and compared.

These cases were compared to previous publications in an attempt to classify and aid the diagnosis of atypical cases of sporadic CJD, and to assess the influence of protein isotype and genotype on the clinical and pathological phenotype of this disease.

HYPOTHESIS

Different phenotypes of sporadic CJD are related to different isotypes of PrP^{res} and codon 129 genotype. If PrP^{res} is a surrogate marker of CJD, this provides evidence of distinct strains of CJD.

- Sporadic CJD usually follows a relatively characteristic clinical and pathological phenotype.
- Atypical forms of the disease are recognised.
- This is partly due to the influence of codon 129 genotype.
- It may also be due to different strains of the agent.
- Protein isotype is a possible surrogate marker of strain.
- Analysis of cases of sporadic CJD from the NCJDSU was performed looking for trends related to protein isotype and codon 129 genotype.
- These data were compared with variant CJD cases in which it is believed there is good evidence that a different single strain is involved.

CHAPTER 2: HISTORICAL PERSPECTIVE

The first of the TSEs to be described was scrapie, a neurodegenerative disease of sheep that was characterised by spongiform change in the brain. The earliest reports of the disease are from the eighteenth century when it was (and still is) endemic in various European countries.[21] The aetiology of scrapie was unknown but in 1936 Cuillé and Chelle reported the successful transmission of the disease by inoculating spinal cord of scrapie-infected sheep into the brains of healthy sheep.[22] Despite the long incubation period following inoculation, this experiment provided evidence that scrapie might be an infectious disease. However, it was clear the agent responsible was unlike recognised microbiological vectors.

In 1957 Gajdusek and Zigas described another condition associated with spongiform change.[23] This disease was endemic in the Fore-speaking tribes of the eastern highlands of Papua New Guinea and was characterised by progressive cerebellar degeneration and tremor leading to death, usually in about one year. The disease was called "kuru" which means "trembling with fear" in the native Fore language.[24]

One of the interesting features of kuru was that it affected mainly the women and children of the tribe, an unusual epidemiological phenomenon. [23] There is good evidence that ritualistic endocannibalism was practised by the local tribes. The women and children may have been particularly exposed to the infectious agent in the preparation of bodies for consumption. (Some would have been the contaminated brains of victims of kuru.) It seemed that Kuru was transmitted by the oral route and/or by inoculation through scratches and breaks in the skin or the conjunctiva by rubbing the eyes. The incidence of disease decreased as the practice of cannibalism ceased.[25]

The first person to suggest a connection between scrapie and kuru was a vet in Cambridge named W.J. Hadlow. In 1959 he published a paper noting similarities in the epidemiology, clinical features and pathology of the two diseases, and suggested

that kuru might also be a transmissible disease.[26] Seven years later Gajdusek successfully transmitted kuru to chimpanzees by intracerebral inoculation. The resultant clinical syndrome occurred after an incubation period of 18-21 months and was similar to scrapie.[27]

Prior to this, in 1920, H.G. Creutzfeldt, a clinician trained in neuropathology, had described the case of a 22-year-old female who suffered a progressive dementia associated with spasticity and myoclonus.[28,29] Over the next 4 years A.M. Jakob, a neuropathologist described five further cases of dementia associated with extrapyramidal and pyramidal signs and symptoms.[30,31,32,33] Jakob coined the term "spastic pseudosclerosis". He noted clinical and neuropathological similarities with the condition described by Creutzfeldt.[34]

Various aetiologies were suggested for this collection of cases, including syphilis and alcohol abuse. In 1922, Speilmeyer, who was the dean of neuropathology at the same university as Creutzfeldt, first used the term Creutzfeldt-Jakob disease (CJD).[35] This eponym, encompassing various syndromes, many with spongiform change in the brain, was used until the clinical and pathological criteria of CJD were redefined in the 1960s.

Both Creutzfeldt and Jakob concluded they had described a neuropathological entity associated with many underlying disorders and that there was no unifying underlying disease. Jakob noted the presence of spongiform change in only one of his five cases.[33] Indeed at that time spongiform change was believed to be an artefact from preparation of brain tissue.[34] Subsequent analysis of the original clinical and pathological description by Creutzfeldt suggests that this case was not CJD. However re-examination of Jakobs' original neuropathological material has confirmed that at least two of his cases were CJD.[36]

In 1959 it was noted that the pathology of CJD and kuru was similar.[37] The successful transmission of CJD to chimpanzees in 1968 gave further support to a connection between these two diseases,[38] and a body of literature continued to accumulate. It seemed that kuru, CJD and scrapie were related, not only by their pathology, but also by the fact that they were transmissible. The transmitted disease developed in a host months to years following inoculation but produced a similar clinico-pathological syndrome. It was presumed that an infectious agent was involved, despite the absence of an immune or inflammatory response. The most popular hypothesis was that this was a "slow virus".[39]

The original transmission experiments were hampered by the long incubation period and a low rate of successful transmissions. In 1961 Chandler successfully transmitted goat scrapie to mice, producing a scrapie-like illness.[40,41] This provided a much easier laboratory model to work with. In particular the incubation period was shorter.

However, it was still not possible to transmit CJD to mice and experiments on this disease continued using primates as the host. Transmission studies on Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis and other subacute and chronic neurodegenerative diseases were attempted, as it seemed possible that they too were caused by a "slow virus", but there was generally little success.[42] Kuru, scrapie and CJD seemed to give more consistent results and these conditions became known as the transmissible spongiform encephalopathies (TSEs).

Isolation of the agent proved elusive. Nevertheless, several properties were established: There was a long incubation period between inoculation and the first signs of host disease.[22] The agent was highly resistant to heat,[43] formalin [43, 44] and ionising radiation.[45] It could pass through a dialysis membrane,[46] suggesting it was a very small particle. These properties were not recognised in any viruses or known infective agent.

Studies on the serial transmission of infected brain tissue between different species and through successive animals of the same species were attempted and several other properties of the TSEs became apparent: When scrapie was transmitted from one animal species to another there tended to be a long incubation period and atypical clinical signs and neuropathology in the recipient. In transmission between animals of the same species the incubation period shortened and the signs and pathology were fixed. In addition transmission to some species was impossible. Pattison proposed that this was indicative of a species barrier.[47]

In 1936 Gerstmann, Sträussler and Scheinker had described a family affected by a progressive cerebellar disease associated with dementia.[48] Neuropathological analysis demonstrated the accumulation of atypical amyloid plaques and gliosis. Over the years other families with a heterogenous clinico-pathological picture were described. This condition became known as Gerstmann Sträussler Scheinker Syndrome (GSS). Latterly a family with the typical picture of GSS were noted to have spongiform change in the brain.[49] This supported an association between GSS and CJD. A year later Masters et al. demonstrated the successful transmission of GSS to primates.[50]

Cases of CJD occurring within the same family had first been reported in 1952.[51] However, a family in whom several members suffered from a degenerative clinical syndrome had been described in 1924 by Kirschbaum. Jakob noted similarities with the cases described by him, and this may be the first description of familial CJD.[52] A review of the literature in 1979 cited 15% of cases were familial,[53] however it was not until 1989 that an insertion mutation on chromosome 20 was linked to a family with CJD.[54] More mutations within the same gene were identified over the next few years. Although two independent groups had transmitted familial CJD in 1973 and1974, it was difficult to reconcile the notion of an infectious agent with a condition that was hereditary in 15% of cases. [55,56]

Until this time, Kuru was the only human spongiform encephalopathy in which there was a probable identifiable exogenous source of infection, but in 1974 a 55-year-old woman developed CJD eighteen months after receiving a corneal graft. The graft donor was subsequently shown to have died of sporadic CJD.[57] In 1977 two patients developed CJD 16 and 20 months after stereotactic electroencephalographic depth recordings. The same electrodes had previously been used in a patient with CJD.[58] They were sterilised with 70% alcohol and formaldehyde vapour. The prion agent has subsequently been shown to be resistant to this process. Indeed one electrode successfully transmitted disease when implanted in the cortex of a chimpanzee.[59]

Other case reports followed of probable iatrogenic transmission of CJD following neurosurgery,[60] cadaveric dura mater graft transplantation,[61] and in recipients of pituitary-derived human growth hormone,[62,63,64] and human pituitary gonadotrophin.[65] Reports of case-to case transmission of CJD continued to be unusual but there was concern as the number of reports of CJD in recipients of human growth hormone increased.[66]

In these cases there was an identifiable source of infection. However, surveillance of CJD in many countries had established an incidence of approximately 1 case per million and despite some reports of geographical clustering,[67] no consistent risk factor was found for the majority of CJD cases.[68] About 85% appeared to occur sporadically.[3]

Clearly the transmissible agent was unlike any recognised vector of infection: It was associated with diseases occurring in acquired, sporadic or hereditary forms; There was an absence of inflammatory changes in the brain and cerebrospinal fluid, unlike any other infectious disease; There was a long incubation period and the majority of cases had no obvious common exposure to other infected individuals, through diet or by geography, with only rare reports of clustering in the human diseases. (Epidemics

of scrapie within herds of sheep were described in Europe throughout the late eighteenth and nineteenth century.[69])

These were unusual epidemiological properties for any infectious agent and it was hard to conceive of a route of person-to-person spread of the disease. As protein biochemistry techniques improved other properties of this highly resistant agent became evident. It was about the same size as a small RNA virus[70] with an apparent molecular mass of 100 000 kDa.[71] It seemed to be concentrated in the cell membrane of brain tissue.[72] No virus could be seen under an electron microscope, and the lack of an immune response seemed odd. In addition immune-suppressed mice developed scrapie in the same way as normal mice.[73]

Some of the information about the properties of the scrapie agent came from transmission studies, in which it was shown that different isolates of scrapie produced different reproducible clinical phenotypes when passaged into the same species. This was first demonstrated in goats in which two separate clinical syndromes, termed "nervous" and " scratching", were consistently found following intracerebral inoculation.[16] As well as clinical syndromes, differences in incubation period[74] and neuropathological lesion profile were also found.[17] This appeared to suggest there were different strains of the agent.

Over several years it was shown that the agent was inactivated by proteases but not by lipases, glycosidases and, most importantly, nucleases.[75] This implied that the agent was devoid of nucleic acid, a hypothesis that had been explored in 1967 by Alper.[76] An infectious agent that carried strain dependent properties, but apparently contained no DNA, contradicted all genetic principles.

In 1982 Prusiner reawakened the controversial theory that the agent consisted of only protein. He demonstrated that scrapie infectivity was associated with a protein

that was partially resistant to proteolytic degradation and had a molecular mass of 27-30 kDa.[19] He called the agent a prion (from "proteinaceous infectious agent").[1]

The protein was isolated and termed PrP, (for prion protein). Further analysis suggested that PrP was a structural component of the prion rather than a product of infection with a prion disease.[77] PrP was identified in the brains of patients with CJD and seemed to be concentrated in the amyloid plaques.[78] PrP antigens were also demonstrated in normal brain tissue in addition to CJD-affected brain, suggesting it was derived from host proteins.[79] Prusiner, extending a theory originally proposed by Griffith in 1967,[80] suggested that the scrapie agent was an abnormal isoform of a host protein, and might be self-replicating.[1]

The gene encoding PrP was identified[81] and soon located on the short arm of chromosome 20.[79] Human PrP was fully sequenced and shown to consist of 253 amino acids.[82] It was already known that familial CJD and GSS were inherited in an autosomal dominant pattern and in 1989 a 144-base-pair insertion was identified on PRNP.[54] Not long after, a missense mutation at codon 102 of PRNP was identified and linked to GSS.[83] Further support for the central role of PrP was provided when an analogous mutation in transgenic mice produced spontaneous neurodegeneration. This suggested that an abnormality within the structure of the protein caused spongiform degeneration, rather than a genetic mutation predisposing to infection from an unidentified agent.[84]

This hypothesis has become the most widely accepted. Further evidence has demonstrated that PrP is a ubiquitous protein.[85] Prusiners' group have shown that in disease the protein changes structure, increasing the insoluble β–pleated sheet content. This may set off a self-replicating chain reaction.[86] He hypothesised that in hereditary disease an underlying mutation predisposes to this change. In acquired disease the introduction of exogenous abnormal PrP provides a template for host PrP

to undergo change and in sporadic disease a random stochastic event or a somatic PRNP mutation causes change to occur.[1]

There has been some resistance to this hypothesis however. The exact nature of the agent and in particular the absence of DNA remains controversial.[87] Other hypotheses have been suggested, such as the virino hypothesis, in which the agent consists mainly of nucleic acid and uses host protein in replication.[88]

Over the years other diseases in animals have been included in the family of TSEs: TME has been recognised on mink farms since 1947. The clinical features and neuropathology are similar to the other TSEs but the source of TME remains unresolved. Scrapie has never been successfully transmitted to mink by the oral route. BSE has been transmitted both orally and intracerebrally but the clinical signs and pathology are distinct from recognised TME.[89] No other potential source has been identified.

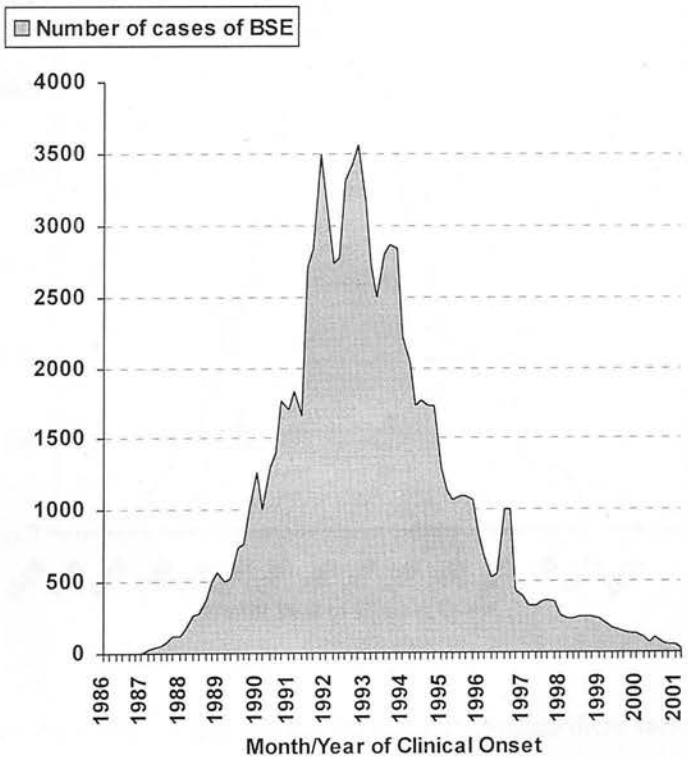
Chronic Wasting Disease (CWD) is a naturally occurring spongiform disease. It has only been reported in deer and elk from Colorado and Wyoming, and has mostly occurred in animals in captivity although there are reports of the disease in free-living animals.[90] The first case occurred in 1967 but the disease was not reported in the literature until many years later.[91] The clinical signs and neuropathology are akin to other TSEs. Like scrapie, the mechanism of transmission of the disease remains an enigma and attempts at eradication have failed.[90]

Interest in prion diseases escalated in the 1980s with the emergence of BSE. In 1986 two cases of a spongiform encephalopathy were identified in cattle in the UK.[92] Retrospective analysis suggested the first case occurred in 1985.[93] The disease reached epidemic proportions in the early 1990s by which time measures, such as the banning of ruminant-derived protein in animal feed in the UK, had been introduced to eradicate the disease. Up to 30 June 2001 there have been over 178,000 cases of

BSE in the UK.[94] There are now only a few cases each month in comparison to the epidemic of the early 1990s. (See Figure 2.1.)

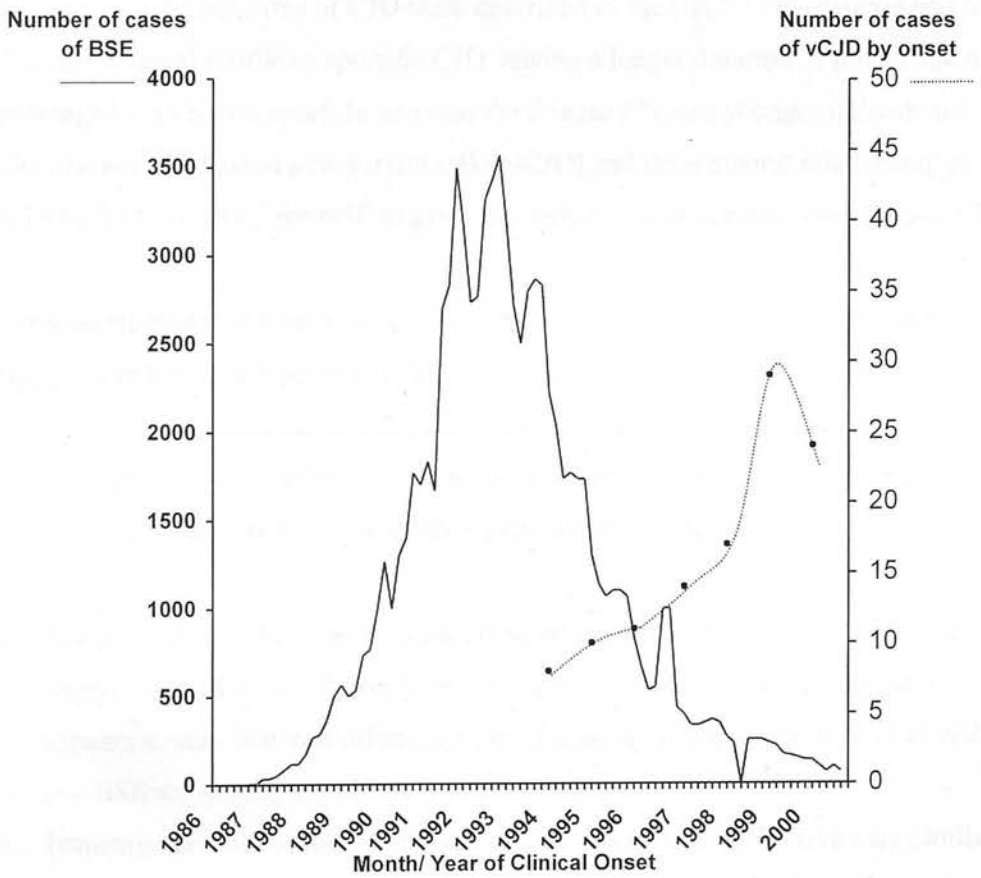
It was suggested that the disease derived from exposure of cattle to a scrapie-like agent. The most likely source was cattle feedstuffs containing ruminant-derived protein beginning in 1981/82. About that time changes in rendering processes reduced the temperature and shortened the timing of preparation of animal material. In addition fewer hydrocarbons for fat extraction were used.[95] There is compelling epidemiological evidence for this theory but the exact mechanism will probably never be established definitively.

Figure 2.1: NUMBER OF CASES PER YEAR OF BSE



It has also been suggested, most recently in the summation of the BSE Inquiry, that the disease was the result of a chance stochastic mutation in cattle PrP. Abnormal PrP was then propagated through the recycling of cattle in ruminant feed.[96] If the latter theory is correct then BSE might continue to occur long after the expected projections, which are based on current figures and assume that cases of BSE did not occur unnoticed before the epidemic.

Figure 2.2: BSE EXPOSURE AND INCIDENCE OF vCJD BY YEAR OF ONSET



Despite the evidence that scrapie did not transmit to humans there were fears that if scrapie had now passed to cattle then BSE might in turn pose a threat to the public.

In 1986 a captive nyala developed a spongiform encephalopathy.[97] A number of cases of TSE in a variety of captive wild ruminant species including gemsbok, Arabian oryx, greater kudu, eland and scimitar-horned oryx followed. A case of TSE was reported in a domestic cat in 1990, Feline Spongiform Encephalopathy (FSE).[98] The temporal relationship to the BSE crisis all pointed to a common origin of infection with BSE. In addition, BSE was successfully transmitted to mice in 1988, indicating it was another transmissible disease.[99]

In 1996 ten unusual cases of CJD were described in the UK.[100] This disease had a different clinical profile to sporadic CJD, having a longer duration of illness and a prolonged psychiatric onset. In addition the disease affected younger individuals. The disease was named new variant CJD (vCJD) and the temporal relationship to BSE fuelled concerns that BSE might have transmitted to humans. (See Figure 2.2.)

Subsequent evidence from transmission studies has given support to the causal relationship between BSE and vCJD:

1. Intracerebral inoculation of BSE contaminated material to macaques produced clinical, molecular and neuropathological disease characteristics similar to vCJD.[101]
2. The prion protein has two sites for potential sugar attachment, thus it may be unglycosylated, monoglycosylated, or diglycosylated. The glycoform ratio appears to vary between different types of disease. The glycoform ratio of vCJD and BSE are similar but different from sporadic CJD.[20,102]
3. Transmission studies to mice have shown that BSE and vCJD have very similar incubation periods and neuropathological lesion profiles. The same pattern is seen with FSE and experimentally induced BSE in sheep and goats. Notably, the profile is distinct from sporadic CJD and other spongiform encephalopathies.[103,104]

To 16/12/02 there have been 129 confirmed and probable cases of variant CJD in the UK and a further 6 cases in France[105,106] and one each in the Republic of Ireland, Italy, Canada and USA.[107] It is not clear how many people will develop the disease. Predictions are hindered by insufficient information about the dose required to cause disease, the number of people exposed, the susceptibility of those exposed and the likely incubation period. In addition all cases to date have been homozygous for methionine at codon 129 and it is not known if cases will emerge in valine homozygotes and heterozygotes.

The number of cases was originally estimated to be increasing at a rate of 33% per year since 1994 when the disease was first reported.[108] However, more recent estimates have shown the rate to be approximately 16% and it has even been suggested the epidemic may have reached a peak. (NCJDSU website). Based on mathematical modelling of these figures and an average incubation period of 20 years a prediction of an upper limit of about 136,000 cases has been made.[109] However, these figures are based on many unknown parameters and recent models have given predictions ranging from an optimistic 205[110] to a more pessimistic "few thousand".[111]

This chapter covers the following topics:

- Description of the recognition of a group of diseases with a common pathogenesis known as the TSEs.
- Development of the "protein only hypothesis".
- Emergence of BSE and related conditions.
- Description of vCJD and predictions about the future number of cases.

CHAPTER 3: HUMAN SPONGIFORM ENCEPHALOPATHIES

3.1: BACKGROUND

Despite its rarity, CJD has become a well-recognised disease in recent years due to extensive media attention. Historically various syndromes have been described in the medical literature which in retrospect may well be sporadic CJD. A number of aetiologies for the condition have been proposed during that time.

In 1954 Nevin and Jones published a description of two cases of a rapidly fatal illness occurring in late adult life associated with myoclonus and recurrent sharp-wave discharges on EEG. Spongiform changes were found in the brain at autopsy.[112] They remarked on similarities between their two cases and five others described in the literature and suggested a vascular aetiology, distinct from hypertension. Although they included three cases described by Heidenhain, they noted he differentiated his three cases from "Jakob-Creutzfeldt's syndrome".

Nevin et al. reviewed 23 cases in 1960. He described the various onsets of a subacute encephalopathy, including progressive visual failure and a cerebellar onset. He noted that mutism was common and myoclonus was often present. The disease progressed steadily with dementia and paralysis. He commented on the great variation in the course and duration of the disease. The EEG showed progressive slowing with the appearance of sharp wave complexes.[113]

In 1967 Nevin went on to comment on the diversity of conditions given the blanket-term Creutzfeldt-Jakob disease and proposed the use of the term "syndrome". Noting the original descriptions by Creutzfeldt and Jakob, he proposed that this syndrome was distinct from subacute spongiform encephalopathy.[52] However, following the successful transmission of sporadic CJD in 1968, [38] serial transmission experiments had also been successful,[114] inducing spongiform change in the experimental animal.[115] Transmission experiments were used to show that subacute spongiform

encephalopathy and CJD were the same disease.[55] Spongiform change, neuronal loss and gliosis were subsequently identified as the pathological hallmarks of CJD and the TSEs.[116]

3.2: SPORADIC CJD

Although it is rare, the clinical and pathological features of sporadic CJD are well described. Systematic epidemiological studies have been undertaken in various countries,[68,117,118,119,120] and European collaborative surveillance has been in place since 1993, giving scope for a rare disease to be studied in greater detail.

The eponymous title sporadic Creutzfeldt- Jakob disease is now used to describe a rapidly progressive dementia associated with neurological decline including typically myoclonus, cerebellar and pyramidal signs leading to a state of akinetic mutism and death, usually within 4 months. The EEG characteristically shows generalised periodic triphasic complexes. Confirmation of the diagnosis is dependent on the neuropathological triad of spongiform change, neuronal loss and gliosis.[121]

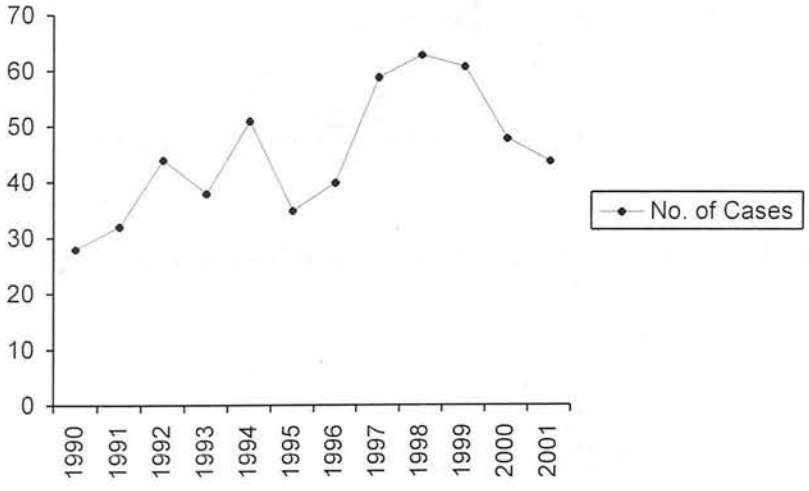
A: EPIDEMIOLOGY

The incidence of CJD approximates to 1 case per million. Sporadic CJD accounts for about 85% of all human spongiform encephalopathies.[122] Because the disease is of short duration, incidence and mortality rate are inter-changeable. In 1999-2000 mortality rates from sporadic CJD in England, Scotland, Wales and Northern Ireland were, respectively, 0.63, 0.86, 1.00 and 0.46/million/year.[123] These rates were comparable to those observed in Europe and elsewhere in the world. Regional variation in the observed mortality rate occurs but to date the variation has not achieved statistical significance when adjusted for sex and age, and no evidence of space-time clustering has been identified.[123] (See Figure 3.1)

The reported mortality rate may be influenced by a number of factors including the number of cases in which necropsy is performed.[122] The average number of cases per

annum of sporadic CJD has been consistently higher since 1990. This is believed to be because of increased case ascertainment since the BSE crisis although it is not possible to exclude an underlying rise in actual incidence of the disease. A similar phenomenon has been observed in other European countries.[123] In addition there has been an increase in the number of young cases, and atypical cases reported.[117,122,123,124] Retrospective reviews of deaths in England from 1979-96 found no new cases of likely sporadic or variant CJD, suggesting that this increase in young cases is likely to be genuine and cases are not being missed.[125,126]

Figure 3.1: ANNUAL MORTALITY RATE OF SPORADIC CJD IN UK: 1990-2001



*data from later years incomplete

The increased mortality rate has been noted in all age groups but the greatest increase has been in the over 75-age group. [123] The disease becomes more common with age, age specific incidence rate peaking in the 70-79 years age group.[123] This has changed from earlier studies which had suggested a peak mortality in the 60-64[127] and 65-69 age group.[128] The reason is believed to be improved case-

ascertainment but may be due to a cohort effect. It may be that autopsy rates have also increased in the elderly. (See Figures 3.2 and 3.3)

Figure 3.2: AGE SPECIFIC INCIDENCE OF SPORADIC CJD IN UK: 1970-89

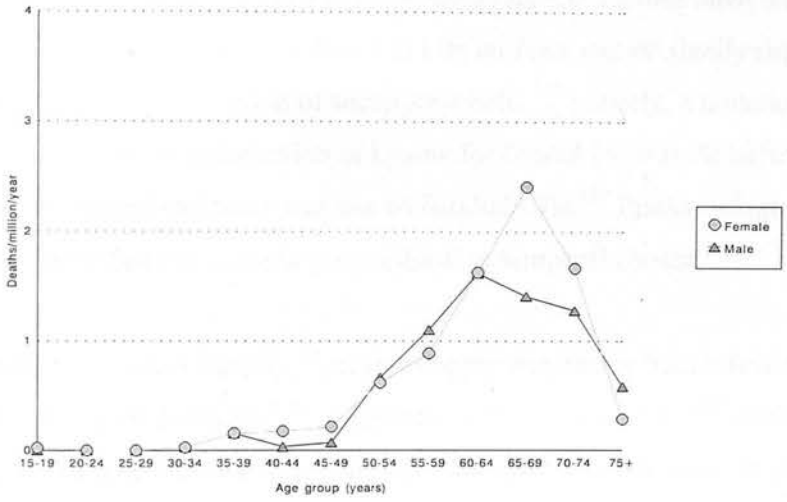
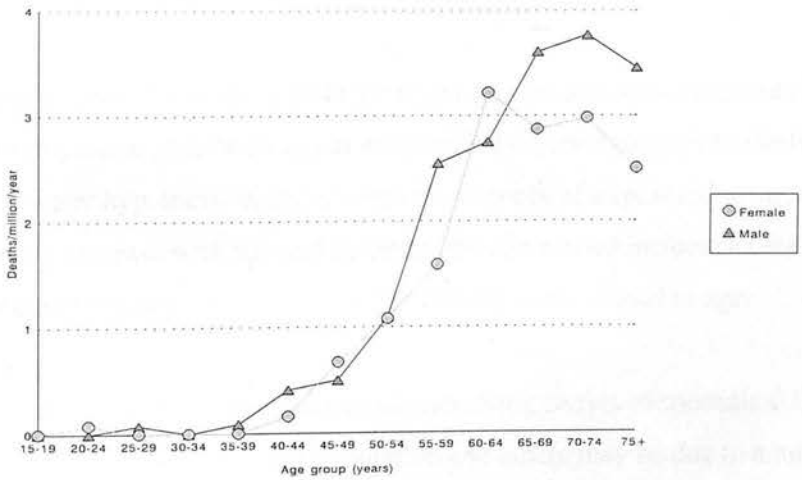


Figure 3.3: AGE SPECIFIC INCIDENCE OF SPORADIC CJD IN UK: 1995-2000



During the period 1990 to date in the UK the median age at onset is 65 years (range 15-94) and age at death is 66 years (range 20-95). The median duration of illness is 4 months (range 1-74). Most studies have shown an over-all excess of female cases (male/female ratio 1:1.4, not corrected for age [122])

The cause of sporadic CJD is unknown. Geographical clusters have been described over the years.[67] An apparent cluster in Libyan Jews was originally suggested to be due to the local consumption of sheep's eyeballs.[129] Latterly, a mutation at codon 200, resulting in the substitution of Lysine for Glutamine was identified, showing that the increased incidence was due to familial CJD.[130] Epidemiological analysis has not identified a consistent geographical or temporal cluster.[4]

In addition, previous surgery,[53] previous upper respiratory tract infection,[131] diet,[132] a family history of dementia,[133, 134] occupation[128] and even stress[135] have been proposed as potential risk factors for sporadic CJD over the years. In particular, exposure to scrapie, dietary or otherwise, seemed less likely to be a risk factor when in 1979 it was shown that there was no relation between the worldwide distribution of scrapie and of sporadic CJD.[53] Case-control studies have failed to identify a common and consistent environmental risk factor for the development of the disease and the cause of sporadic CJD remains unknown.[4,136]

It is not known if sporadic CJD is the result of a chance stochastic mutation within the prion protein gene or an as yet unidentified external source of infection. Clearly if the former hypothesis is correct then the chances of a spontaneous mutation occurring increase with age and in theory the age-related incidence data should not show exponential growth but should be directly proportional to age.

It is possible that there are a variety of underlying causes of sporadic CJD. Some cases may be due to a stochastic mutation and others may be due to a number of different acquired causes that a case-control study might not be able to establish or

exclude. The increasing incidence with age may be explained by an increasing susceptibility to an unidentified exogenous factor.

B: CLINICAL FEATURES

Classically, sporadic CJD is described as a rapidly progressive dementia associated with multifocal neurological dysfunction and myoclonus. The patient deteriorates rapidly to a state of akinetic mutism and death within a period of months.[55]

A prodrome has been noted in about one third of cases.[5,137] Symptoms such as depression, disturbances of sleeping and eating patterns and weight loss, usually beginning a few weeks before the onset of neurological dysfunction have been described. A similar frequency of symptoms has been seen in controls and their presence may not always be attributable to CJD.[3]

In one study the onset was abrupt in about 5%, suggesting a diagnosis of stroke,[138] but progressive neurological signs and symptoms ensued, suggesting an alternative diagnosis. In a further study an abrupt onset of focal symptoms or signs was noted in 20% of cases but other features at onset made the diagnosis of stroke unlikely.[5]

The presenting symptoms of sporadic CJD are usually of cognitive decline or ataxia. Rare presentations are well recognised however, including an onset with visual symptoms, progressing to cortical blindness, usually associated with visual hallucinations: the Heidenhain variant. This term was coined by Meyer et al. who described a case of CJD associated with cortical blindness and pronounced occipital pathology. [139] They attributed the original description to Heidenhain, who in 1929 had described 3 cases of rapidly progressive dementia charaterised by visual failure leading to cortical blindness in the early stages of the disease.[11] The Heidenhain variant of sporadic CJD encompasses cases who present with visual failure before or with dementia.

A pure cerebellar presentation has also been described, known as the Brownell-Oppenheimer variant.[10] Brownell and Oppenheimer described 10 cases with an onset of ataxia before going on to develop dementia and other features of CJD.[10] A later review of cases presenting with ataxia, including the 10 described by Brownell and Oppenheimer, gave an average duration of illness of 8 months (range 2-18), slightly longer than might be expected. Most of the cases were noted to have a typical EEG but other case reports of this variant have emphasised the EEG is not always diagnostic.[140] Pathologically, the cerebellum was particularly affected and two cases had Kuru plaques. Further analysis suggested that 10% of cases of CJD presented with a pure cerebellar syndrome.[141]

Table 3.1: CLINICAL CHARACTERISTICS OF SPORADIC CJD: SYMPTOMS AT PRESENTATION

	England & Wales 1970-1979 [137] (n=124) %	France 1968-1977 [127] (n=124) %	USA 1963-1993 [121] (n=232) %
Dementia	21	29	48
Ataxia	19	29	33
Behavioural Disturbance	18	30	29
Dizziness	11	8	13
Visual	9	17	19
Involuntary Movements	5	1	2
Dysphasia	5	N/A	4
Sensory	4	2	N/A
Headache	3	10	N/A

It has been suggested that the presence of cerebellar or visual disturbance early in the clinical picture may point towards a diagnosis of CJD rather than another dementia

such as Alzheimer's disease.[142] Other cases may have a particularly focal onset with, for example, progressive aphasia.[143]

The frequency of the most common symptoms and signs at presentation, which were determined in various large epidemiological studies, are listed in Table 3.1 The French and UK studies include a small number of familial cases. It is not possible to separate these cases from the data as a whole.

Table 3.2: CLINICAL CHARACTERISTICS OF SPORADIC CJD: SIGNS DURING COURSE OF ILLNESS

	England & Wales 1970-1979 [137] (n=124) %	France 1968-1977 [127] (n=124) %	USA 1963-1993 [121] (n=144) %
Dementia	100	100	100
Myoclonus	82	84	78
Pyramidal	79	44	62
Dysphasia	62	N/A	N/A
Cerebellar	42	56	71
Akinetic Mutism	39	N/A	N/A
Primitive Reflexes	30	N/A	N/A
Cortical Blindness	13	N/A	N/A
Extrapyramidal	3	60	56
Lower Motor Neurone	3	12	12
Seizures	9	9	19

The disease follows a relentless course. The presence of various symptoms and signs and the timing of them can vary from patient to patient. Dementia develops in all and myoclonus is present in over 80%.[5] Pyramidal and cerebellar signs are common. Most cases deteriorate to develop primitive reflexes, paratonic rigidity and

dysphasia. A proportion develop cortical blindness. The terminal stage is often of akinetic mutism. Seizures are unusual, reported in 10%. (See Table 3.2)

75%-80% of cases of sporadic CJD have been reported to have a characteristic periodic EEG.[5,137] The typical clinical features of the disease, together with the EEG have been used to establish diagnostic criteria for sporadic CJD.[5,53] These have been altered slightly over the years, more recently to include CSF analysis of 14-3-3 protein, but have proved remarkably consistent and reliable for the majority of cases.[144] Recently, high signal in the basal ganglia on T2-weighted Magnetic Resonance Imaging (MRI) has been reported in 79% of sporadic CJD cases.[8] This investigation may also prove to be useful in aiding diagnosis.

C: ATYPICAL CASES

Although the majority of sporadic CJD cases follow a relatively characteristic course, atypical cases are well described. Early epidemiological studies used differing clinical and pathological criteria to sub-classify the disease. An early review of 38 cases attempted to rationalise previous classifications and used duration of illness as well as clinical and pathological criteria to generate three sub-types of CJD.[131] It was noted that cases with a longer duration of illness (1-2 years) were on average 10 years younger than the more acute groups. A typical EEG was regarded as a feature of cases of short duration.

One of the hallmarks of sporadic CJD is the short duration of illness and so cases of long duration have attracted added attention. The number of cases with a prolonged duration varies in several studies from 5%[5] to 24% in Japan where a higher prevalence of panencephalopathic cases occurs.[145] Brown et al. reported that no particular "form" of illness could be correlated with the variables of age at onset or duration of illness. He found that the youngest patients tended to have the longest illness,[5] although Masters et al. did not find such an association.[50]

Brown et al. reported their findings in 33 cases with long duration CJD, i.e. an illness of 2 years or more,[9] amounting to 9% of cases of CJD seen by this group. (Although these were a selected group of cases and this figure is higher than would be expected when studying cases of sporadic CJD as a whole.) A number of patients had a family history of CJD and, with the benefit of genetic analysis, might now have an identifiable mutation. Otherwise, the cases tended to be younger. There was a lower frequency of myoclonus, but no other significant clinical differences from classical sporadic CJD. Less than half of the cases had a periodic EEG, suggesting this investigation is less helpful when the disease is of long duration, potentially making diagnosis more difficult. However, it is possible that the EEG was not repeated in these cases because CJD was not considered a likely a diagnosis.

Young cases of sporadic CJD have attracted attention in the medical literature because the disease occurs typically in the elderly or late middle age. A case of a 19-year-old girl was reported in 1985. The course of the disease was typical for sporadic CJD and duration of illness was 4 months. An EEG showed 1-2 cycle per second pseudoperiodic sharp wave spike activity.[146] At that time only one other case of sporadic CJD had been reported in the literature in an adolescent.[147] Other cases in young people had occurred in familial and iatrogenic disease.[53,58] Another case had been reported in a 26-year-old woman,[148] and three cases have been reported in Poland of disease duration 3 to 10 months. The EEG was not typical in any of the cases but all three had changes of spongiform encephalopathy at autopsy.[149]

Several reports of CJD in young people in the UK were subsequently shown to be cases of vCJD. [150,151,152] Since its description in the UK in 1996 there have been over 100 cases of vCJD. A young person with features of CJD in the UK may be suspected of having variant rather than sporadic CJD, because, in this age group vCJD is relatively more frequent. (Although there are of course other important differences between the conditions.) However cases of sporadic CJD still occur in

young people, and the most common diagnosis in the differential of vCJD is sporadic CJD.[153]

It has already been suggested that the clinical presentation of CJD is important. Isolated case reports have commented on various patients with atypical presentations, including alien hand occurring as the presenting sign,[154,155] Wernicke-Korsakoff syndrome[156] and acute psychosis.[157]

Sensory symptoms in sporadic CJD are unusual. Various reviews have reported sensory symptoms in 3%[119] to 6%[120,121] at onset. Some of the cases described by Brownell and Oppenheimer were also noted to have vague sensory symptoms at the onset.[10] A further 11%[121] to 16%[119] developed sensory symptoms at some stage during the illness. In the study by Lundberg et al. the sensory symptoms included paraesthesias, itching or pain and were more common in younger patients.[119] Peripheral neuropathy has been described in sporadic CJD.[158]

Many of the original reports of sporadic CJD included an amyotrophic variant i.e. dementia with motor neurone disease.[137] These cases are no longer classified as prion diseases because transmission studies have been unsuccessful.[159] Indeed, some of these early reports may not in retrospect have been sporadic CJD and it is now widely accepted that motor neurone disease can be associated with a severe dementia in some cases.[160] Lower motor neurone signs are rare, noted in 6% of the NIH series,[121] but amyotrophy can occasionally be a prominent feature of prion disease.[161]

Other variants of sporadic CJD include the thalamic variant. The first case was probably described in 1939.[162] Since then other cases of a thalamic variant of sporadic CJD have been reported.[163,164] Recently, the phenotype has been described as sporadic fatal insomnia (i.e. fatal familial insomnia with no recognised PRNP mutation).[165] The clinico-pathological phenotype is characterised by dysautonomia, dementia and ataxia. Sleep disturbances are common. Cases tend to be of longer

duration and do not have a typical EEG. At autopsy there is invariably thalamic and olivary neuronal loss and astrogliosis.

There are also many reports of a panencephalopathic variant in the literature. These cases are usually of long duration with a disproportionately large involvement of spongiosis, neuronal loss and gliosis in the white matter of the cortex and brainstem.[166,167] The panencephalopathic variant has been reported frequently in Japan, and it has been stated that as many as 38% of Japanese cases are of this type.[168]

D: INVESTIGATIONS

A definite diagnosis of CJD depends on neuropathological findings. However, brain biopsy is usually undesirable in life and a diagnosis of suspected CJD is often reached by exclusion; most investigations, including Computed Tomography (CT) imaging of the head and routine CSF analysis are usually normal or negative, thus excluding structural disease or other conditions such as cerebrovascular or inflammatory disease. There are no specific diagnostic blood tests but underlying toxic or metabolic problems can be excluded. Liver function tests have been reported as abnormal in 40% of cases,[137] but this may reflect the general condition of the patient or be due to other factors such as drug treatments. Other biochemical and haematological parameters are usually normal.

i): ELECTROENCEPHALOGRAM (EEG)

The EEG has been the mainstay in the clinical diagnosis of sporadic CJD until recently. Nevin and Jones first described periodic complexes in association with spongiform encephalopathies in 1954.[112] A classical EEG in sporadic CJD is reported to show slowing of background rhythm with stereotyped periodic complexes which are often asymmetrical. (See Figure 3.4) Serial EEG recordings have recorded the presence of typical periodic sharp-wave complexes in 75% to 94% of cases, often developing as the disease progresses.[137,169] The EEG has been found

to be typical in about two thirds of cases at the NCJDSU. Until recently the EEG was the single criterion that differentiated probable from possible cases in the agreed diagnostic criteria.

Figure 3.4: A TYPICAL EEG IN SPORADIC CJD

The absence of periodic complexes was felt to be evidence against a diagnosis of CJD.[170] However, more recently, a negative EEG has been shown to have a negative predictive value of only 30%.[171] Part of the difficulty is that there have been few attempts to devise objective criteria for a "typical" EEG in sporadic CJD and there are numerous case reports in the medical literature of conditions in which a "CJD-like" EEG has been recorded. Some of these causes, such as metabolic disorders, can be distinguished on medical grounds. Others, such as Alzheimer's disease can

occasionally give rise to confusion, despite stricter evaluation of the EEG.[144] (The criteria used in this study are set out in chapter 7: Methods.)

In addition, the EEG may become "typical" only as the disease progresses and repeat EEG recordings are not always undertaken. It is possible that the EEG is less likely to be repeated if the first record is not suggestive of CJD and the diagnosis is not considered, i.e. the case is atypical from the outset. There are not many other conditions in which serial EEG recordings are helpful. Thus, there may be an under-reporting of typical EEGs in atypical cases of sporadic CJD.

Two recent studies have reported a sensitivity of 66% and 75% and specificity of 74% and 86% respectively. [171,172] Steinhoffs' study defined periodic sharp wave complexes as "being mono- or multi-phasic, occurring in a strictly periodic fashion at an interval between 500 and 2000 milliseconds". However it also included lateralised and localised complexes that would not be included as typical in this study. This may account for the higher sensitivity and specificity seen in his study. The final stages of the disease are characterised by generalised attenuation of the EEG.[169] A normal EEG does not absolutely exclude a diagnosis of sporadic CJD, but is very unusual; however, there are reports of a normal EEG even in the terminal stages of the disease.[173]

ii): CSF ANALYSIS

Routine analysis of CSF is usually normal in sporadic CJD. It is generally accepted that inflammatory cells are not seen in the CSF but a moderately elevated protein, (a rather non-specific result), occurs in some cases.[174] Three series report that CSF pleocytosis was never seen.[121,175,176]

A few case reports have commented on a raised CSF white cell count and protein. In one study 23% (13/57) of cases had an abnormal CSF. Of these, the protein was raised all 13 cases (52-150 mg/dl), and 6 cases (11%) had a pleocytosis (10 to

303/mm^3). No further details are given.[120] This is a Japanese study and it is well recognised that the clinical features and pathology of CJD in Japan are rather different from that seen in Caucasians. There are little, if any, data given on the clinical features of other cases with abnormal CSF in the literature, e.g. in most cases it is not clear if a raised protein is associated with a raised red cell count or if serial LPs were performed.

As a general rule, abnormal CSF results, in particular a pleocytosis, should make one consider an alternative diagnosis to CJD. Oligoclonal bands in CSF but not in serum have also been reported but are not commonly seen.[177]

In recent years, analysis of various brain-specific proteins has become a useful and reliable tool in the diagnosis of CJD. These are normal brain proteins that may be released into the CSF in disease states. Hence, they are not specifically related to the underlying disease mechanism of CJD or to PrP.

Initially four abnormal proteins were identified by 2-dimensional gel electrophoresis.[178] Two of these proteins were found pre-mortem in the CSF of two cases of CJD in whom brain biopsy had been unhelpful.[179] A simpler assay for a protein whose amino acid sequence matched these 2 proteins was subsequently developed. This protein was named 14-3-3.[180] Initial evaluation of the 14-3-3 immunoassay by the German surveillance system has reported a sensitivity of 94% and specificity of 93.3%.[7]

As might be expected, the specificity of this test has fallen as it has become more widely used. False positives have been reported in hypoxic brain damage, meningoencephalitis, metastatic brain disease, Alzheimer's disease, fronto-temporal dementia, stroke, herpes simplex encephalitis, Rett's syndrome, subarachnoid haemorrhage, paraneoplastic syndrome and acute transverse myelitis.[7,176,180,181,182,183,] Some of these cases would not meet WHO criteria for "possible" CJD however, and

it is arguable whether or not 14-3-3 was an appropriate investigation in the context.[184] False negative results have also occurred.[183,185]

A more recent analysis has re-evaluated the sensitivity and specificity of 14-3-3 analysis to be 94% and 84% respectively. The specificity of CSF analysis was greater than that of the EEG (74%).[171] Based on these results either a positive 14-3-3 or typical EEG are now used in the diagnostic criteria for sporadic CJD, (sensitivity 97%, specificity 65%)

.

14-3-3 protein is the most specific of the brain-specific proteins in the diagnosis of sporadic CJD. However, other proteins have also been detected in the CSF and may be used: S-100, a product of astrocytic breakdown has been detected at significantly raised concentration.[186] Sensitivity and specificity has been found to be 84.2% and 90.6% respectively at a cut-off level of 8 ng/mL. [187] S-100 can also be detected in serum and it has been suggested there is potential for serum analysis of this protein.[188]

In addition neurone-specific enolase (NSE) has been detected and has a reported sensitivity of 80% and specificity of 92% at a cut-off level of 35 ng/mL.[189] Tau-protein can be detected by ELISA in the CSF of CJD patients at a higher level than other dementias, including Alzheimer's disease, and may also be a useful discriminator.[190]

iii): CEREBRAL IMAGING

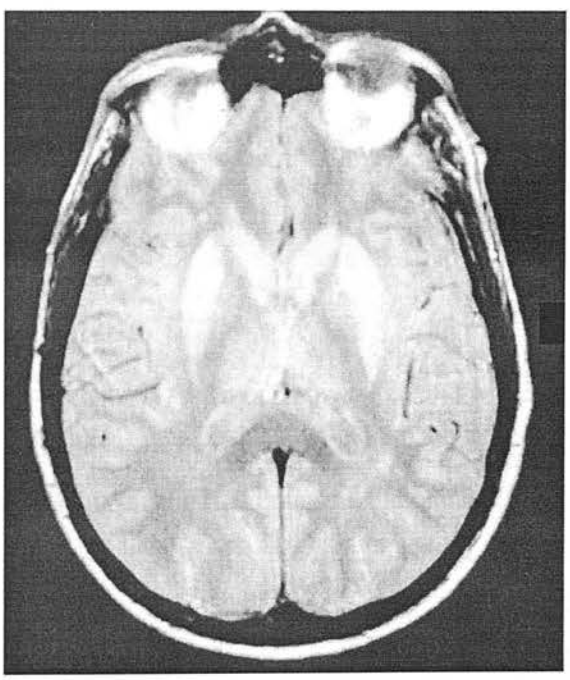
The cerebral CT imaging in CJD is usually normal, even in advanced disease, with severe dementia, cerebellar ataxia, cortical blindness and myoclonus. There are case reports of atrophy but no other abnormality.[191]

The advent of MRI did not initially provide more information other than isolated case reports: Serial imaging noted the development of cerebral atrophy and non-

specific white matter changes in 2 cases.[192] Severe cerebellar atrophy on MRI has been reported in a case of panencephalopathic CJD of 17 months duration.[193] Another panencephalopathic case was noted to show periventricular hyperintensity.[194] T2-weighted, Fluid Attenuated Inversion Recovery (FLAIR) and diffusion-weighted MRI have been reported to show high signal intensity changes throughout the cerebral cortex in sporadic CJD.[195,196,197,198]

A review of MRIs in 1992 reported scattered high signal in 4 of 12 cases (33%). 2 cases (17%), of duration 6 and 10 months, showed mild to moderate atrophy. 5 (42%) cases were normal and 1 had movement artefact.[199]

Figure 3.5: MRI OF SPORADIC CJD SHOWING HIGH SIGNAL ON T2-WEIGHTED IMAGING IN THE BASAL GANGLIA



Milton et al. reported high signal in the basal ganglia in a case with pronounced extrapyramidal features in 1991.[200] There have been other reports of high signal in

the striatum and thalamus on T2-weighted images, some linking MRI changes to areas of more pronounced status spongiosis and gliosis in these areas. [201, 202, 203,204, 205, 206] These changes have also been noted on T-weighted, FLAIR and diffusion-weighted images.[207,208]

The presence of high signal in the putamen and basal ganglia on T2-weighted imaging was evaluated by the German surveillance system in 29 cases of sporadic CJD. The abnormality was found in 79% of cases.[8] (See Figure 3.5) Further evaluation of these cases reported a sensitivity of 67% and specificity of 93% for sporadic CJD.[209] An Italian review of 31 cases found the changes in 100% of their cases.[210] It has been suggested that the MRI changes should also be used in the diagnostic criteria for sporadic CJD.[209] These early results suggest it may yet prove to be useful in aiding the diagnosis of this disease, however, the use of MRI in sporadic CJD needs further evaluation.

Correlation of the MRI changes with the clinical picture has been examined in an isolated review. 10 of 15 cases with extrapyramidal involvement in the illness had basal ganglia changes on MRI. It was noted that patients without basal ganglia changes had a longer duration of illness.[211]

D: DIAGNOSTIC CRITERIA

Masters et al. devised criteria for the diagnosis of CJD in 1979.[53] These have been somewhat modified and current criteria use the presence of 14-3-3 protein in addition or instead of a typical EEG to aid the diagnosis. (See Chapter 7: Methods) To be considered a possible case of sporadic CJD, there should be a rapidly progressive dementia of less than 2 years duration. In addition there should be 2 of the following clinical signs: myoclonus, pyramidal, cerebellar and extrapyramidal signs, cortical blindness and akinetic mutism.

To be considered a "probable" case, either the EEG should be positive or 14-3-3 protein should be positive and the disease should be of less than 2 years duration. Currently a diagnosis of possible CJD carries about a 50% chance of being correct. However, a probable diagnosis is approximately 95% specific.[212]

F: PATHOLOGY

The definitive diagnosis of CJD rests on the neuropathological examination of central nervous system (CNS) tissue. This is usually done at autopsy although cerebral biopsy is sometimes done during life. The classical changes of spongiform change, usually accompanied by neuronal loss and gliosis, are the main diagnostic features. More recently techniques for PrP staining have been developed and improved, aiding diagnosis. The neuropathological diagnostic criteria have been published in a consensus document.[213]

Macroscopically the brain often appears normal although there may be focal or generalised cortical atrophy.[213] The presence of spongiform change, neuronal loss and astrocytosis is highly variable. Severe cortical damage may be characterised by the lack of any specific feature and is termed "status spongiosis". Spongiform change is usually distributed in varying severity in the grey matter, sometimes confined only to a few focal areas. (See Figure 3.6a)

The degree of gliosis increases as the disease progresses and spongiform change becomes less obvious.[116] However, an analysis of 50 cases with pronounced pathological cerebellar changes found no correlation with more marked clinical cerebellar symptoms and signs. Nor was there a relationship between the degree of cerebellar atrophy and the duration of disease.[214]

Amyloid kuru plaques are seen in 10-15% of sporadic CJD cases. The typical kuru plaque consists of a central eosinophilic disc with radiating amyloid fibrils and often a pale halo. They do not always require immunostaining for identification and are

normally present in the cerebellum, particularly the granular layer.[215] (See Figure 3.6c) In some cases the plaques have been noted in association with a cerebellar onset.[216] There are reports in the medical literature of atypical cases of CJD associated with kuru plaques[217] and a review of case reports in 1988 suggested that the plaques tend to be associated with a younger age at onset and a longer duration of illness.[216] However, 5% of Browns NIH series[121] had kuru-type plaques and their presence bore no relation to the duration of illness.

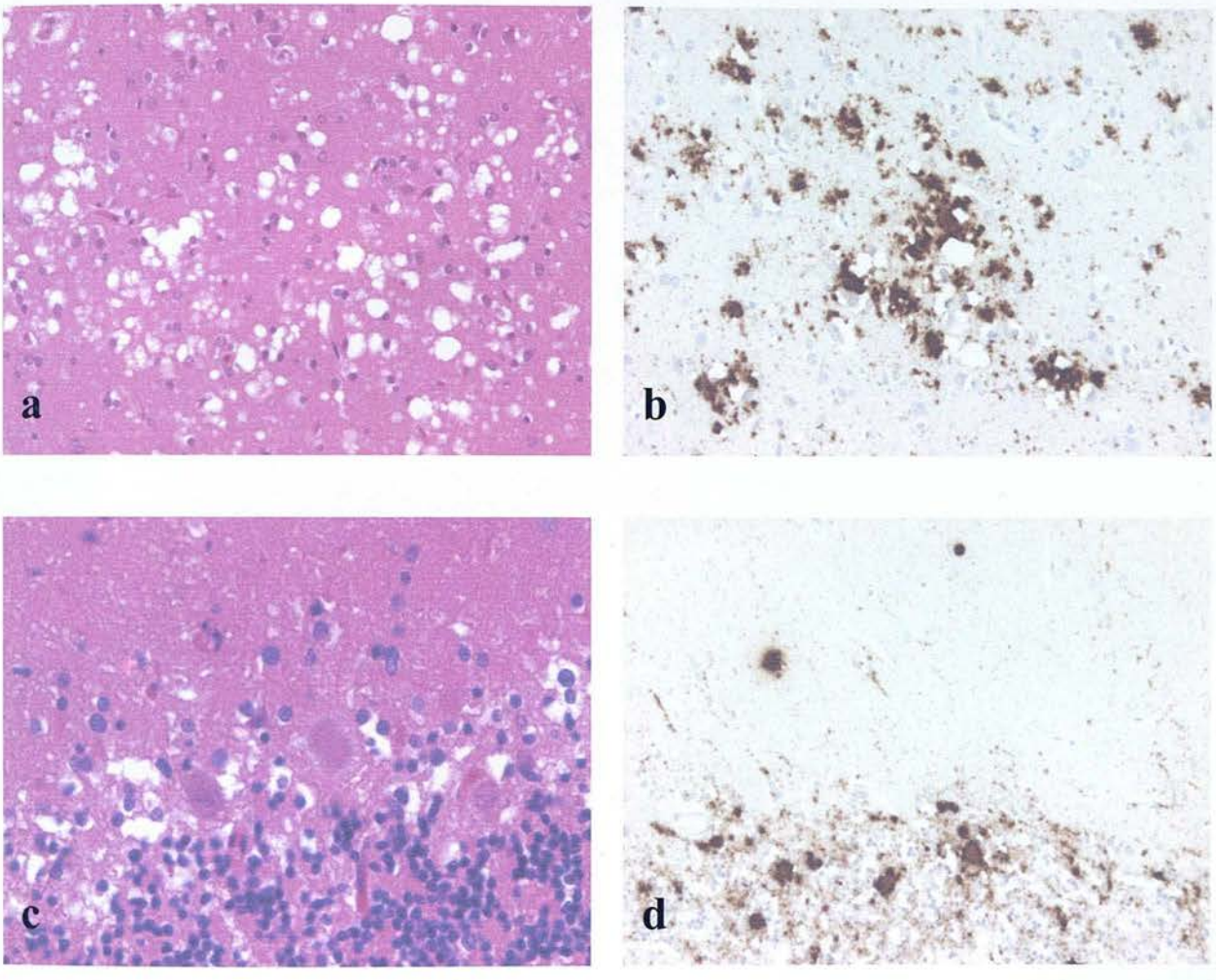
The panencephalopathic variant may also show spongiform changes in the white matter.[213] These cases seem to be of two types depending on the presence or absence of kuru-type plaques, those with plaques being of longer duration.[167]

The identification of PrP either by western blotting or by immunocytochemistry aids diagnosis. As it is not possible to distinguish between normal and disease-associated PrP, pre-treatment with proteinase K is required prior to western blot.[20] Various treatments including autoclaving at 121°C, immersion in 96% formic acid and then 4M guanidine thiocyanate are required prior to immunocytochemistry for PrP.[215]

PrP may be distributed in three patterns, often overlapping: plaque, diffuse synaptic and patchy/ perivacuolar. The PrP deposits tend to be more finely distributed in the cortex (See Figure 3.6b) however in the cerebellar cortex the distribution is more prominent, particularly in the granular layer.[213] (See Figure 3.6d)

PrP has been found in the muscle of a patient with inclusion body myositis (although there was no evidence of CJD or prion disease).[218] The significance of this is unclear. It is interesting that transgenic mice expressing a high degree of PrP were found to have a necrotising myopathy with accumulation of the transgene PrP within the muscle fibres.[219]

Figure 3.6: PATHOLOGICAL CHANGES IN SPORADIC CJD



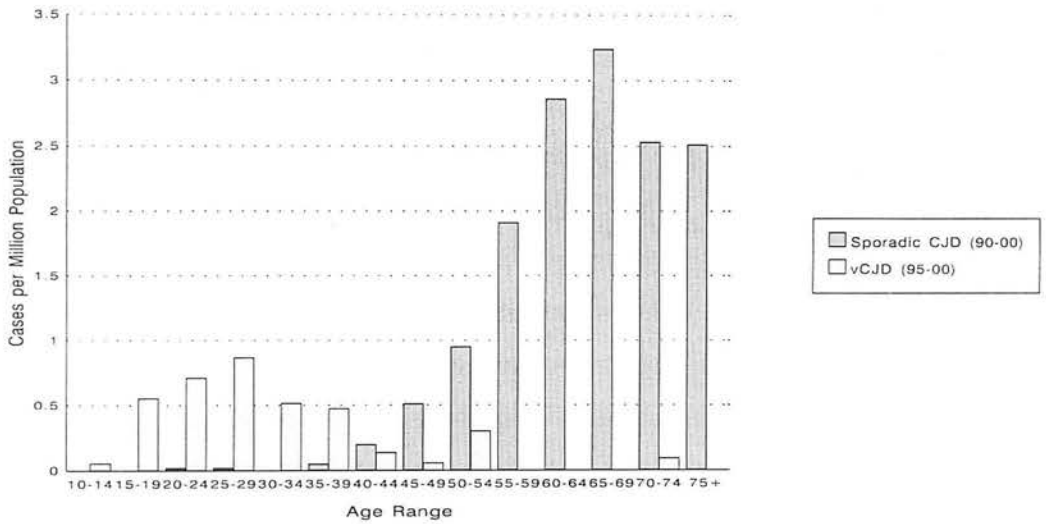
a) Spongiform change (x20)
b) PrP deposition in the cerebral cortex (x20)
c) Kuru plaque within the cerebellum (x40)
d) PrP deposition in the cerebellum-sporadic (x20)

PrP immunolabelling with KG9

3.3: VARIANT CJD

The clinico-pathological phenotype of vCJD has remained fairly constant since it was first described in 1996.[100] The disease is relatively distinct from sporadic CJD, both in the clinical and pathological features, (although there are also similarities between the two conditions). It tends to affect younger individuals and has a longer duration of illness.

Figure 3.7: VARIANT AND SPORADIC CJD; AGE SPECIFIC INCIDENCE AT ONSET



To date there have been 129 cases of definite or probable vCJD within the UK. A further 6 cases have occurred in France and 1 each in Ireland, Italy, Canada and USA [102,103,104] The male to female ratio is 1.1:1. The median age at onset is 26 years (range 12-74), and at death is 28 years (range 14-74). One of the distinguishing features of variant from sporadic CJD is that it tends to affect younger individuals. (See Figure 3.7) However, recently a case has been reported in a 74-year-old man.[220]

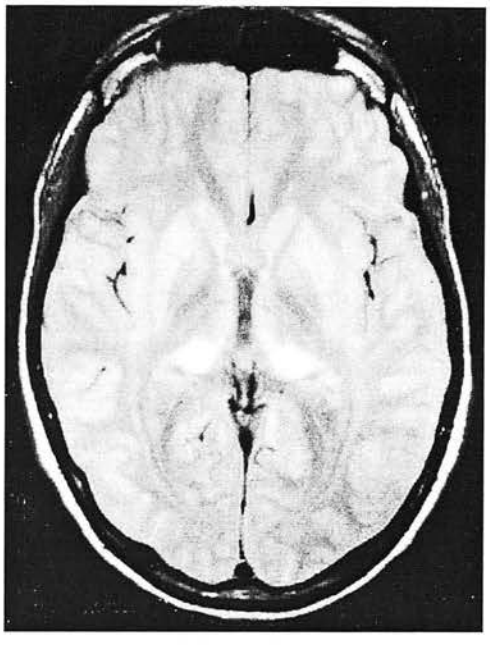
The clinical and pathological features of the disease were similar to other cases of vCJD however this case is 20 years older than any other identified case of vCJD. There was no unusual identifiable exposure to the BSE agent. Clearly a case of vCJD within this age group has important epidemiological implications.

Typically, the disease onset is characterised by early psychiatric symptoms, in particular depression, anxiety and withdrawal. These symptoms are often present for many months prior to developing other neurological signs. More frank psychotic symptoms have been present in a minority of cases.[221, 222] Sensory symptoms are a prominent feature in about a third of cases from the onset and developed at some stage in nearly two-thirds of cases. These are often non-specific including limb pain, dysaesthesia and numbness or tingling.[223] After a median period of 6 months patients go on to develop ataxia and cognitive impairment. Involuntary movements, often dystonic or choreiform in nature, are usually present.[163] Patients deteriorate to a state of akinetic mutism and death, similar to the terminal stages of sporadic CJD.

Diagnosis of vCJD was initially difficult. The EEG is non-specifically abnormal in most cases; none have shown the typical periodic complexes of sporadic CJD.[224] Routine CSF analysis is normal in most cases and 14-3-3 protein is positive in about 50% of cases. In combination with a raised level of tau protein this test may be more sensitive but as CSF tau is relatively non-specific there is still no reliable CSF test in the diagnosis of vCJD.[225]

As with sporadic CJD, routine CT scanning is unhelpful. However, MRI scanning in vCJD has proved to be very useful. Many of the cases have exhibited high signal on T2 or proton density weighted imaging in the pulvinar area of the thalamus. These changes can be asymmetrical and are present bilaterally. High signal changes are also seen in the medial thalamus and periaqueductal grey matter.[226] (See Figure 3.8) Of the 101 available scans at the NCJDSU the pulvinar sign has been present in 92% of cases of vCJD. FLAIR MRI may be the best way of visualising these findings.[227]

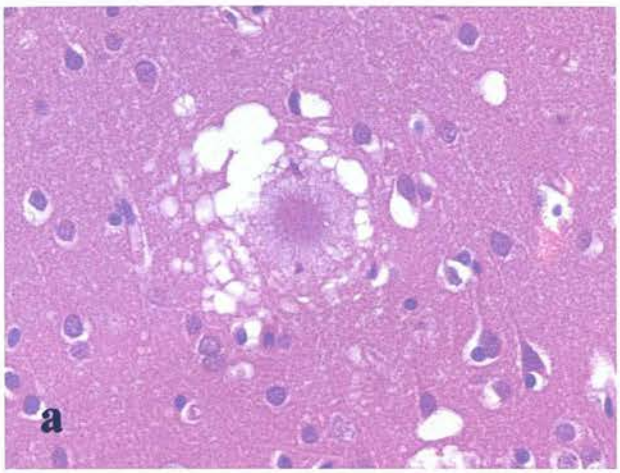
Figure 3.8: HIGH SIGNAL IN THALAMUS ON T2-WEIGHTED IMAGING IN vCJD

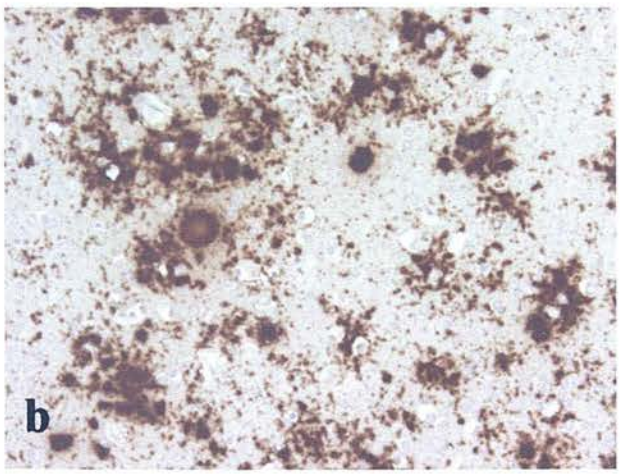


The clinical features together with a positive scan have been used to develop diagnostic criteria for vCJD. (See Appendix D) These have been validated and the diagnosis of probable vCJD has a sensitivity of 92% and specificity of 100% to date. (Personal communication, NCJDSU)

Prion protein has also been identified in the lymphatic tissue of the appendix and tonsils in cases of vCJD, distinguishing the condition from other forms of CJD.[228,229] Because of these findings, tonsil biopsy has been suggested as a useful diagnostic

Figure 3.9: PATHOLOGICAL CHANGES IN VARIANT CJD





a) Florid plaque within cerebral cortex (x40)
b) PrP deposition within the cerebral cortex (x40)

PrP immunolabelling with KG9

tool.[230] This is an invasive procedure and in the presence of a positive MRI scan is probably not necessary.[231] However, in the case of a negative MRI scan, the identification of PrP in a tonsil biopsy would also make a case with the appropriate clinical setting "probable vCJD".

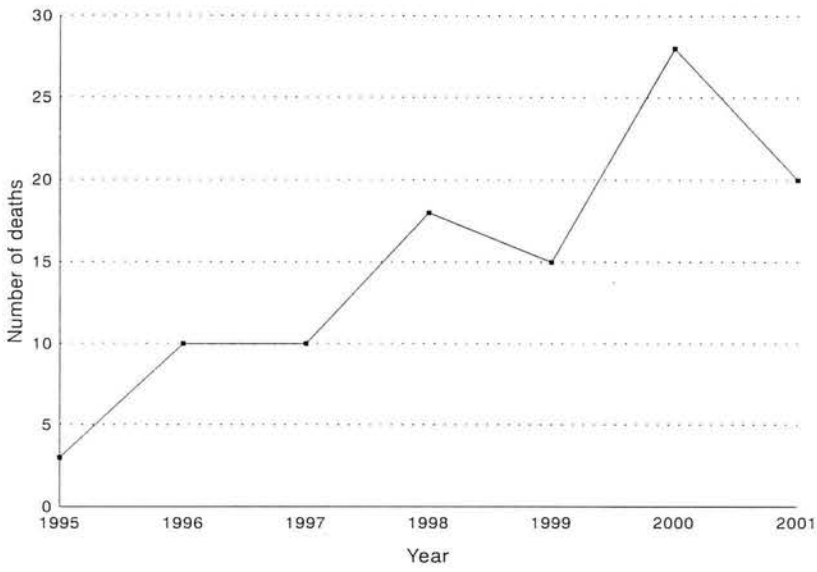
Confirmation of diagnosis still rests upon neuropathology. Spongiform change, in the absence of plaques, is focally distributed throughout the cerebral cortex, particularly in the occipital and inferior frontal regions. Extensive spongiform change is seen in the caudate nucleus and putamen. Marked astrocytosis and neuronal loss is present in the posterior thalamic nucleus and midbrain. PrP is deposited in the form of florid plaques, particularly in the cerebral and cerebellar cortex.[232] These plaques are distinct from the kuru-type plaques seen in sporadic CJD. (See Figure 3.9a) PrP staining is widespread throughout the brain, seen particularly in the occipital cortex and cerebellar cortex. (See Figure 3.9b)

There are a number of unknown variables that may influence the future number of cases of vCJD. These include the number of people who have been exposed to infectious material, the susceptibility of individuals, the dose of infectious material required to develop disease and the incubation period between exposure and first signs of disease.[233] As stated previously, estimates of the predicted number of cases vary considerably and recent analyses have suggested from 205 to a " few thousand" cases are possible.[110,111] The number of cases of vCJD continues to increase. Analyses of short-term trends currently indicate an approximate doubling in the number of cases of vCJD in the UK every 3 years.[108] However, despite early concerns of an increase in the number of deaths from the condition, an exponential rise in case has not been seen to date.[234] (See Figure 3.10)

In addition there have been concerns that cases of vCJD could have been missed, either because an individual has died and the disease has been unrecognised, or because cases have died from other causes prior to a diagnosis of vCJD being made.

Look back studies of case records and neuropathological material have failed to identify missed cases of vCJD.[125,126]

Figure 3.10: DEATHS FROM vCJD PER YEAR



* data from later years likely to be incomplete

A further cause for concern is the possibility of secondary transmission of vCJD, either by blood products or contaminated surgical instruments. It is conceivable that apparently healthy individuals are silently incubating the BSE agent and pose a potential risk of secondary spread of vCJD. The magnitude of this risk remains unknown. Look back studies of sporadic CJD have not identified any cases that could be attributed to the administration of blood or blood products,[235] however, transmission of CJD has been demonstrated using intracerebral inoculation of blood or buffy coat in experimental rodent models. [236]

Of course the risk from vCJD may be different from that of sporadic CJD. Theoretically it could be higher because PrP is found in tonsillar tissue in vCJD[237] and it has been shown that the buffy coat probably has the highest degree of infectivity.[236] Laboratory models have shown that at least 5 to 7 times the amount of infectious agent would be required to transmit disease by the intravenous rather than the intracerebral route, and that there is probably little significant plasma infectivity before the onset of symptomatic disease.[238] In addition, retrospective study of prion-protein accumulation in tonsil and appendix tissues from surgically resected samples from the (presumed) healthy UK population did not identify PrP in any of over 3000 cases.[239]

Never the less, measures have been taken within the UK to reduce the chance of potential secondary transmission. These include universal leucocyte depletion of blood products, limiting the use of fractionated plasma products and donor selection, deferring donors who have a family history of CJD and those who have a history of corneal or dura mater grafting, neurosurgical intervention or human pituitary-derived hormone exposure.

It is assumed, although unproven, that vCJD transmitted to humans through the oral route. The likely source of infection would be tissue high in BSE infectivity; i.e. brain, spinal cord, dorsal root ganglia and retina. This would not normally be consumed, but bovine vertebral column has been used in the production of mechanically recovered meat (MRM) in New Zealand and it is not unreasonable to assume that similar practices were used in the UK.[240] It is believed that MRM was used in the production of various foods such as beef burgers, pies and sausages.

Recently a potential cluster of cases has been described in the Leicestershire area.[123] Analysis of butchering practice in this area suggested that unusual methods were used that may have exposed individuals to meat contaminated with brain material. However these data are not yet published.[241] (Indeed, it is not yet established if the

butchering methods used were peculiar to the Leicester area only.) Other reports have suggested clustering close to rendering plants. [242] However analysis has shown the distribution of these cases to be due to chance. [243]

A geographical variation of cases of vCJD has been demonstrated however; the disease has occurred more frequently in the North of the UK compared to the South. The reason for this is not clear. Regional variation in diet has been suggested as a cause but it has not to date been possible to determine whether or not this is the case.[244]

Recently, treatment of CJD has been suggested based on studies of acridine and phenothiazine derivatives. These substances were shown to partially inhibit the formation of PrP^{sc} *in vitro*.[245,246] Despite initial enthusiastic press reports of substantial clinical improvement in one case of vCJD[247], it remains to be seen if these products will prove to be effective in a clinical setting.

3.4: IATROGENIC CJD

Human prion disease can also be acquired through a variety of external routes. The first instance of possible iatrogenic transmission was in the recipient of a corneal graft in 1974 who developed CJD. The donor had also died from CJD.[57] Three years later, it was suggested that infection by contaminated stereotactic intracerebral EEG needles caused two further cases of CJD.[58] In retrospect it is possible that three of the cases described by Nevin and Jones may have been transmitted by neurosurgical instruments.[60] Epidemiological studies have reported increased rates of head surgery in cases of sporadic CJD over the years,[53, 248] possibly related to cross-contamination by neurosurgical instruments. However, review of case-control studies has not found a consistent risk factor, including previous surgery.[4]

More recently iatrogenic transmission by contaminated human growth hormone [64] and dura mater grafts have been described.[249] The latter have mostly occurred in

Japan. Recently published figures counted 139 growth hormone cases (more than half occurring in France), and 114 dura mater cases.[250] Four recipients of human pituitary gonadotrophin have developed CJD in Australia.[65]

The clinical features of human pituitary growth hormone (HGH) related CJD are relatively distinct from sporadic CJD. In some cases there is a prodrome of sleep disturbance and personality change. The majority present with a progressive cerebellar syndrome and in many, dementia is not a prominent feature, at least until late in the disease.[251] Disease duration tends to be longer than in sporadic CJD, in 34 HGH recipients in France the median duration was17 +/- 9 months.[252] It has also been reported that dura-mater cases usually have a cerebellar presentation, irrespective of the site of the graft.[250] In iatrogenic CJD due to central inoculation the clinical features are similar to classical CJD. The clinical picture is dominated by a dementing illness.[59]

Periodic complexes typical of sporadic CJD were seen in only 2/34 (6%) of a French review.[252] It has been suggested that a typical EEG occurs more frequently than this.[250] A positive 14-3-3 protein result has been reported in 100% of cases in a recent French study of HGH cases, however the result is often negative in the first months of illness and becomes positive some months into the disease progression.[253] Some cases have been reported to show MRI changes in the putamen.[250]

The pathology of iatrogenic CJD is similar to sporadic disease, exhibiting the triad of spongiform change, astrocytosis and neuronal loss. The distribution varies from case to case. There is prominent cerebellar involvement in HGH cases, with marked cerebellar atrophy, extensive neuronal loss, widespread spongiform change and PrP plaque formation.[254]

There is a diffuse pattern of staining of PrP in the granular layer of the cerebellum on immunocytochemistry.[2] However, atypical cases associated with plaque-like deposits have been described.[255]

3.5: FAMILIAL CJD

Prion diseases are inherited in 5-15% of cases.[256] There are now 22 mutations identified in PRNP that are associated with disease. These include 13 point mutations, a stop codon mutation at codon 145, and 8 insertion mutations (consisting of 1,2 and 4-9 repeats of 24 base pairs located between codons 51 and 91).[257] Deletions and polymorphisms are also recognised, although these are not necessarily associated with disease. The best known is the codon 129 polymorphism but 6 other polymorphisms, some silent, have been described.[257] There is also a silent deletion of one 24-base pair repeat found in 1-2.5% of the population.[258]

The diseases are classified as Gerstmann-Sträussler-Scheinker disease (GSS), associated with a point mutation at codon 102 of PRNP, fatal familial insomnia (FFI), associated with a D178N mutation encoupled with methionine at codon 129 on the mutated allele, and familial CJD with which several mutations of PRNP are recognised. (See Table 3.3)

The pattern of inheritance is invariably autosomal dominant. The degree of penetrance of most of the mutations is not known although it is generally believed to be high. However, the codon 200 mutation has been shown to have incomplete penetrance and elderly unaffected carriers have been identified.[259, 260] Some of the disease mutations show a trend towards anticipation.[261, 262]

In general, compared to sporadic disease, the age at onset is often younger and the disease duration often longer in familial CJD. [263] The clinical syndromes are variable but the codon 200 mutation is characteristically reported to show a disease phenotype similar to sporadic CJD.[6,264] There is also variation in clinical

presentation both within and between families;[265] pedigrees of GSS have been identified in which some family members present with the GSS phenotype and others with a phenotype similar to sporadic CJD.[266]

Diagnosis of familial CJD rests on identifying the genetic mutation on PRNP within an appropriate clinical setting. The EEG often does not show periodic sharp waves,[267] indeed these changes have only been described in a single case of GSS,[268] although the EEG may be positive in up to 75% of codon 200 mutations.[257] 14-3-3 analysis may be helpful in about 50% of cases.[6] In general cases with a disease phenotype similar to sporadic CJD are more likely to have a typical EEG or positive 14-3-3 protein. Oligoclonal bands have been reported in a case of FFI.[269] There is very little information on the use of MRI. In some cases imaging has shown a degree of atrophy and non-specific white matter changes.[270,271]

Table 3.3: CLINICAL FEATURES IN FAMILIAL PRION DISEASE [6]

MUTATION	CLINICAL FEATURES
Codon 178 (valine at codon 129) Codon 180 Codon 200* Codon 208 Codon 210 Codon 232	Similar to sporadic CJD
Codon 102* (GSS) Codon 117 Codon198 (Indiana kindred) Codon 212 Codon 217	Slowly progressive ataxia, pyramidal signs and dementia. Some cases similar to sporadic CJD
Codon 178 (methionine at codon 129) (FFI)	Dysautonomia, insomnia, progressive dementia
Codon 105	Progressive spastic paraparesis, dementia
Insert mutations*	Variable-features of sporadic CJD or GSS
Codon 145	Slowly progressive dementia
Codon 183	Personality change, dementia, parkinsonism

* Atypical phenotypes described

As with all human prion diseases, confirmation of the diagnosis rests on neuropathological examination. Familial CJD can only be diagnosed if the characteristic pathology is present along with a recognised mutation of PRNP in an appropriate clinical setting. A wide variation in the degree of spongiform change has been reported and there are case reports of a recognised mutation of PRNP, in association with dementia, but with no characteristic pathological abnormalities.[272] Cases of FFI have also been reported with no spongiform change where the pathology is predominantly of thalamic gliosis.[273]

GSS is characterised by the presence of multi-centric amyloid plaques in the brain, particularly the cerebellum. Spongiform change is also present in the CNS and neurofibrillary tangles are present within the cerebral cortex in the codon 198 mutation. The pathology is again characteristically variable.[254]

3.6: KURU

The clinical features of kuru are well described, although it is now almost extinct, and as far as we know affected only one defined population. Study of the condition has provided the most extensive knowledge of an acquired prion disease. In most cases the duration of illness was 3-6 months and rarely over a year.[24] In some of the later cases an average duration of illness of 16 months was found [274] and cases of a duration up to 2 years have been noted.[24] The age at onset varied from 5 years to over 60.[275] The disease occurred more commonly in females.[24]

Most of the cases of kuru followed a fairly uniform clinical course. [25, 274] The clinical features were of a progressive ataxia and tremor of the trunk, head and limbs. The tremor was exaggerated during voluntary motor activity or fatigue and settled at rest. Pyramidal tract signs and sensory symptoms were generally not a feature in the original description. However in later descriptions extensor plantar responses were noted in some patients. In some cases, the disease was preceded by a history of joint pains. [24]

Dementia did not appear to be an early feature but it did appear after some months, often with inappropriate euphoria. In other cases a prominent frowning was noted and hallucinations and aggression have been seen. The patients deteriorated to a state of immobility and were often left to die at this stage. Mutism, rigidity and choreoathetoid movements often developed and a convergent strabismus was noted in nearly all patients.[24]

The results of blood and CSF testing were normal although, the Erythrocyte Sedimentation Rate (ESR) was raised in some cases.[24] Autopsy findings were of a widespread neuronal degeneration, affecting particularly the cerebellum and extrapyramidal system. Spongiform change was present in a variable distribution in the cerebral cortex, basal ganglia, thalamus and cerebellum.[254] The most striking abnormality was the presence of amyloid plaques, occurring in about 70% of cases, particularly in the cerebellum.[37]

This chapter covers the following topics:

- Discussion of the possible aetiology and the epidemiology of sporadic CJD.
- Review of the typical clinical and pathological features and investigation of sporadic CJD.
- Review of atypical cases of sporadic CJD.
- Review of the clinical and pathological features and investigation of variant CJD.
- Review of the clinical and pathological features and investigation of iatrogenic and familial CJD and of kuru.

CHAPTER 4: MOLECULAR BIOLOGY OF PRION DISEASES

4.1: PRION PROTEIN

Although there is still debate over the nature of the causative agent in prion diseases,[276] understanding of the underlying molecular biology, biochemistry and disease mechanisms has improved.

PrP is a ubiquitous protein, present in leukocytes, heart, skeletal muscle, lung, intestine, spleen and other organs.[85] It is concentrated particularly in the central nervous system, especially in neurones.[277] The normal cellular form of PrP is usually termed PrP^{C}. Prion diseases are associated with the deposition of an abnormal form of PrP^{C},[278] often termed PrP^{Sc} (from scrapie). Whilst PrP^{C} is rapidly digested by proteinase K, PrP^{Sc} is largely protease-resistant. Only the amino-terminus (N-terminus) is cleaved to yield a protein of 27-30 kDa,[77] which may be denoted as PrP^{res} or $PrP^{27\text{-}30}$. PrP^{C} and PrP^{Sc} have the same primary structure but differ in several other respects, in particular the tertiary structure: PrP^{C} is predominantly composed of α-helix and PrP^{Sc} has a high β-pleated sheet content.[86]

In order to understand the pathogenesis of prion diseases it is important to understand the mechanism of PrP^{Sc} production from PrP^{C}. There has been an increased understanding of the structure and cell biology of both forms of PrP over the years, and there is now a greater knowledge of some of the aspects of this transformation.

Of greatest importance to the pathogenesis of prion diseases is the fact that transgenic mice which are devoid of PRNP ($PrP^{0/0}$) are resistant to scrapie.[279] It is also known that normal PrP^{C} must be expressed in a cell for the toxic effects of PrP^{Sc} to take effect.[280] Whether the neurodegeneration seen in prion diseases is related to the toxic effects of PrP^{Sc} or the loss of PrP^{C}, or indeed a combination of both is not known.

Several experimental approaches are used:

- structural analysis of PrP isoforms by spectroscopy
- transgenic mice carrying modified or ablated PrP genes
- *in vitro* reconstitution of PrP^{Sc} from PrP peptides and purified proteins
- cell culture, metabolic labelling and subcellular fractionation of PrP

4.2: PrP^{C}: STRUCTURE AND FUNCTION

PrP^{C} is a normal host encoded protein. The entire open reading frame (ORF) of PRNP is encoded on the short arm of chromosome 20.[79] PRNP is highly conserved across mammalian species suggesting an important role maintained throughout evolution.[281] The polypeptide product consists of 253 amino acids. There are several distinct domains including a 22-amino acid N-terminal signal peptide, a series of 5 proline and glycine rich octapeptide repeats, a central hydrophobic region and a Carboxy-terminal (C-terminal). The protein is synthesised in the rough endoplasmic reticulum and transits the Golgi on its way to the cell surface.[282]

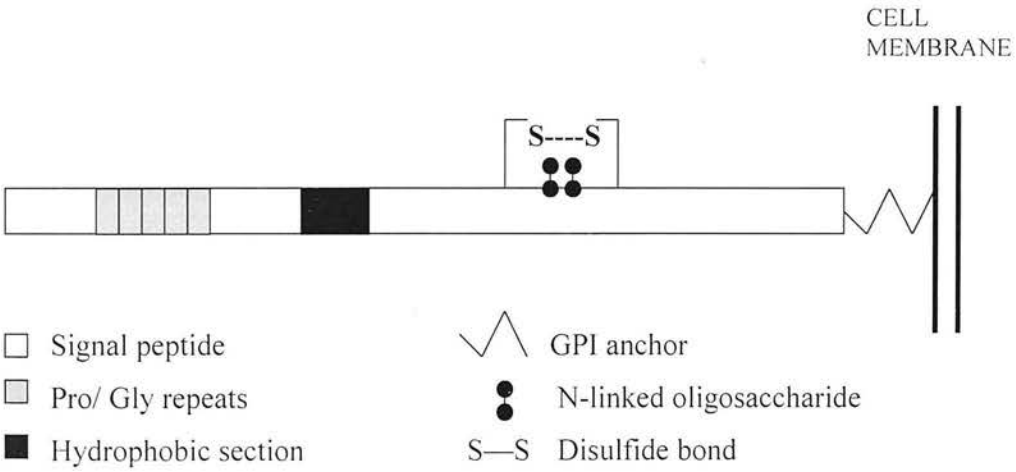
Several post-translational modifications occur during biosynthesis of PrP^{C} including cleavage of the N-terminal signal peptide and addition of a glycophosphatidyl inositol (GPI) moiety at the C-terminal. This anchors the protein to the cell membrane.[283] (See Figure 4.1) There are 2 possible sites for *N*-glycosylation,[82] situated within a loop formed by a disulfide bond between two cysteine residues at codons 179 and 214. In PrP^{C} and PrP^{Sc} both sites can potentially be glycosylated.[284]

Available evidence suggests that the oligosaccharide chains and GPI anchors of PrP^{C} and PrP^{Sc} do not differ, although complete structures have only been worked out for PrP^{Sc}.[282] *In vitro* cell culture studies in which the glycosylation sites of PrP^{Sc} and PrP^{C} were mutated showed that the PrP^{C} molecule exhibited a limited number of the biochemical properties of PrP^{Sc}. The authors concluded that PrP^{C} has an intrinsic

tendency to adopt some PrP^{Sc}-like features during its normal conformational maturation but that the N-linked glycan chains protect against this.[285]

Nuclear Magnetic Resonance spectroscopy analysis of recombinant PrP^{C} has shown that the molecule consists of a long, flexible N-terminal tail, 3 α-helices and 2 small, anti-parallel β-strands that flank the first α-helix.[286,287,288] The flexible N-terminal appears to be the site of binding of copper ions.[289,290]

Figure 4.1: STRUCTURE OF PrP (Adapted from DA Harris[282])



PrP^{C} is predominantly localised to synaptic membranes.[291,292] It has been suggested that the protein is necessary for normal synaptic function,[293] but the definite function of PrP^{C} is still unknown. Localisation on the cell surface might suggest a role in cell adhesion and recognition, ligand uptake, or transmembrane signalling.[282]

In vitro studies have suggested the protein may be involved in the metabolism of copper[294] and that copper may stimulate endocytosis of PrP^{C} from the cell surface. PrP^{C} appears to influence the activity of superoxide dismutase (SOD) in the cell. This enzyme has a role in neuronal resistance to oxidative stress and PrP^{C}, by

binding copper, may be fundamental to this process. Copper binds to the octapeptide repeat region towards the N-terminal of PrP. This appears to confer increased stability on the random coil structure of the amino terminal.[290] Cells expressing amino-terminally truncated PrP, and hence missing the copper ion, are SOD deficient and have an increased sensitivity to oxidative stress.[295] PrP^C has been found to affect glutamate uptake by astrocytes. This may be excitotoxic to neurones or may exacerbate neuronal degeneration.[296]

Studies on mice with a disrupted or absent PRNP were devised to try and establish the function of PrP^C. In one study $PrP^{0/0}$ mice lines functioned and appeared phenotypically normal.[297] However in other similar studies neurophysiological abnormalities and alterations in circadian rhythm and sleep pattern were seen[293,298] It is not known how significant these findings are as a $PrP^{0/0}$ mouse model is one of congenital deficiency rather than of acquired deficiency in later life. In addition, other investigators could not reproduce these findings.[299]

However, an adult deficiency model has been created using bigenic mice expressing inducible transgenes. These animals can be rendered PrP deficient in a controlled fashion by the administration of doxycycline. In one study these PrP deficient mice remained healthy for over 1.5 years.[300] However, a third line of mice in another study developed ataxia and loss of cerebellar Purkinje cells.[301] It has been suggested that a further protein, designated doppel, which is encoded for by a gene located 16Kb downstream of PRNP, may have a role in provoking neurodegeneration. In the $PrP^{0/0}$ mice that developed ataxia and Purkinje cell degeneration doppel was upregulated in CNS cells, but this did not occur in the ataxia-free mice.[302]

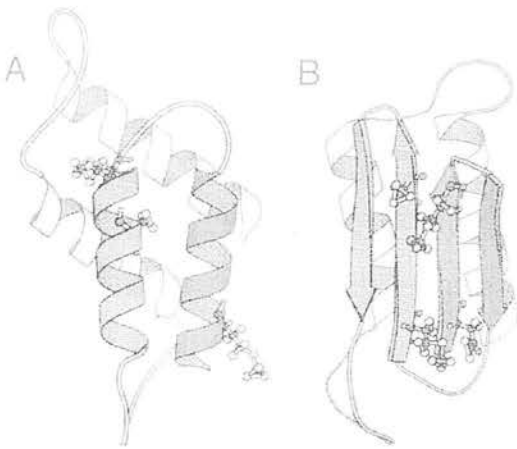
In contrast to deficiency models, overexpression of PrP^C in transgenic mice resulted in a spontaneous neurodegenerative disease, including the development of truncal ataxia, hindlimb paralysis and tremors.[219] The mice developed a necrotising

myopathy in skeletal muscle. It has been suggested however that this was because the hamster PrP used may be more toxic to mouse muscle than native mouse PrP.[303]

4.3: PrP^{Sc}: STRUCTURE AND FUNCTION

PrP^{Sc} is the major isoform of PrP^{C} deposited in prion diseases. It is disease related and the dominant view is that PrP^{Sc} is in fact the transmissible agent, or a component of it.[1]

Figure 4.2: MODEL FOR TERTIARY STRUCTURE OF PrP^{C} AND PrP^{Sc}
From Prusiner et al. [304]



A: PrP^{C} predominantly α-helix structure.

B: PrP^{res} predominantly β-sheet structure.

PrP^{Sc} has the same primary structure as PrP^{C}, being transcribed from the same host gene. Mass spectrometry and Edman sequencing have supported this. In addition no modifications been identified in the GPI anchor or the 2 oligosaccharides linked to the molecule.[305] Thus any difference in the 2 molecules seems to be due to a post-translational conformational change: PrP^{C} has been shown to have a high α-helix

content (42%) and little in the way of β-pleated sheet content (3%), in contrast PrP^{Sc} has a high β-sheet content (43%) and an α-helix content of 30%.[86]

The structure of PrP^{Sc} is not yet fully established, although it is the N-terminal end and possibly part of one α-helix that may transform into β-sheet.[306] (See figure 4.2) The importance of the amino-terminal region in PrP^{Sc} is still a matter of some debate.[307] However study of prion strains in TME has suggested that the drowsy and hyper strains have consistently different resistance to proteolysis and that this is related to the site of N-terminal proteinase K digestion.[18] (See Chapter 5: Strain Variation)

The increased β-sheet content of PrP^{Sc} confers different physicochemical properties on the protein. The structure is relatively protease resistant and it has a tendency to form amyloid structures, and sometimes amyloid plaques in the affected tissue. (See Table 4.1)

Table 4.1: DIFFERENCES BETWEEN PrP^{C} AND PrP^{Sc}

PrP^{C}	**PrP^{Sc}**
Mostly α-helix structure	Significant β-sheet structure
Soluble in detergents	Tends to aggregate in detergents
Relatively protease sensitive	Relatively protease resistant
Normal cellular turnover	Forms amyloid structures

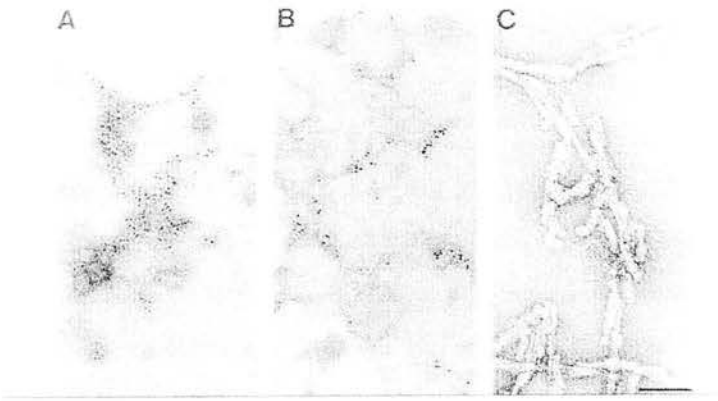
Amyloidosis occurs in a number of diseases, including Alzheimer's disease and CJD. In these conditions fibrillar proteins with a β-sheet conformation are deposited extracellularly. The protein deposited in each disease differs and in prion disease the amyloid fibrils (SAF) have a characteristic morphology observed exclusively in these conditions.[308] Like other amyloidogenic proteins, PrP^{Sc} has a tendency to

aggregate into rod shaped amyloids after treatment with proteinase K *in vitro* and it seems that PrP^{Sc} forms part of the protein deposit seen in prion diseases.[309] (See Figure 4.3)

Both PrP^{C} and PrP^{Sc} undergo post-translational cleavage, removing a portion of the N terminus. The site is distinct in each conformer. In PrP^{Sc} cleavage occurs within the region attacked by proteinase K to yield PrP^{27-30}. It has been suggested that this may be important in the pathogenicity of PrP^{Sc}.[310]

As stated previously it is unclear whether the pathogenesis in prion diseases is due to the expression of PrP^{Sc}, the loss of PrP^{C} or a combination of both. Various studies have set out to establish the role PrP^{Sc} has to play in the neurodegeneration of CJD.

Figure 4.3: ELECTRON MICROGRAPHS OF NEGATIVELY STAINED AND IMMUNOGOLD-LABELED PRION PROTEINS[311]



A: PrP^{C}, B: PrP^{Sc}: Neither PrP^{C} nor PrP^{Sc} forms recognizable, ordered polymers. C: Prion rods composed of PrP^{27-30} were negatively stained. The prion rods are indistinguishable from many purified amyloids.

In vitro experiments with various neuronally derived cell lines have shown that only some cells appear to be susceptible to infection with scrapie prions. Surprisingly, although the infected cells continuously produce low levels of PrP^{Sc}, they display no obvious cytopathology in the majority of cases.[282] One study, in which the cells were derived from hypothalamic neurons (GT1 cells), demonstrated apoptosis in a subpopulation.[312]

Other *in vitro* studies have used a synthetic peptide homologous with residues 106-126 of human PRNP. This region seems to be required for the conversion of PrP^{C} to PrP^{Sc},[313] and to have a key role in conformational conversion.[314] Some studies with this peptide induced apoptosis and astro-gliosis in primary rat hippocampal neurones.[315,316,317] However, a more recent study failed to confirm these findings.[318] This peptide has a tendency to form amyloid fibrils but it forms different secondary structures in different pH environments. Further *in vitro* experiments showed that neurotoxic effects occurred in the absence of amyloid fibrils using a wild type sequence, suggesting it is not the presence of amyloid that induces neurodegeneration.[319] It has also been demonstrated that microglia were required for $PrP^{106-126}$ to have neurotoxic effects.[320]

However, a separate *in vitro* study has shown that it is residues 118-135 of PrP that are important in the formation of amyloid fibrils, inducing liposome fusion, hence destabilising the cell membrane.[321] It is clear that the exact mechanism of neurodegeneration in prion disease is still not understood. There are considerable difficulties in extrapolating the results of *in vitro* studies to the *in vivo* situation, in which the physico-chemical environment of the cell and other unidentified factors may have a significant influence that may not be reproducible in an *in vitro* model.

4.4: CONVERSION OF PrP^{C} TO PrP^{Sc}

How cells take up PrP^{Sc} during the initial stages of infection is still not clear.[282] In addition, the mechanism for conversion from PrP^{C} to PrP^{Sc} remains theoretical. The

event apparently takes place post-translation.[322] It would require a large amount of kinetic or thermodynamic energy for the protein to spontaneously convert from one stable conformation to another.[323] It has been suggested that stochastic fluctuations occur in the structure of PrP^{C} so that at times it partially unfolds. This unfolded isoform could revert to the α-helix structure of PrP^{C} or it could by random chance form the β-sheet structure of PrP^{Sc}.[324] Studies of transgenic mice suggest that PrP^{C} and PrP^{Sc} form a complex during conversion.[325, 326] Additional molecules of PrP^{Sc} would then be created in an autocatalytic reaction.[282]

In infectious forms of the disease PrP^{Sc} might act as a template for the transformation of PrP^{C}.[327] Again this might become more likely in the presence of a less stable intermediate conformer. In hereditary disease PrP mutations would destabilise the structure of PrP^{C}, increasing the chance of conversion to PrP^{Sc} or its intermediate.[324] Sporadic CJD might occur if there is a chance conformational change of PrP^{C} or if a somatic mutation occurs in PRNP.[323]

It has been proposed that this transformation may require a molecular chaperone, provisionally designated protein X.[328] A potential site for protein X has been proposed at the C-terminal end of PrP[329] although protein X itself has not been identified and it is not even established if the molecule is a protein or not.

Models of familial CJD using transgenic mice have been devised using PrP molecules carrying an insertional mutation. These were found to induce a neurodegenerative disorder characterised by ataxia, with changes of cerebellar atrophy, gliosis and PrP deposition seen neuropathologically. The mutant PrP^{Sc} molecules were seen to be mildly protease resistant and have a degree of detergent insolubility.[330] This was proposed as supportive evidence of the presumed spontaneous conversion of PrP^{C} to PrP^{Sc} in cases with a PRNP mutation.

Understanding the kinetics of the transformation of PrP^{C} to PrP^{Sc} might also lead to a greater understanding of the mechanism of conversion. One study showed that PrP^{Sc} in infected cell line models appeared to be stable for as long as 24 to 48 hours whereas PrP^{C} had a cellular turnover of 4 to 6 hours.[322] Only a minority of PrP^{C} molecules were converted to PrP^{Sc}. The remainder were degraded, presumably by pathways similar to those seen in uninfected cells.[331] The kinetics of PrP^{Sc} formation in sporadic CJD are more difficult to comprehend if these figures are correct. It must take an exceptionally long time for sporadic CJD to become established and for the disease process to become clinically evident from a somatic mutation or chance transformation of PrP^{C} into one PrP^{Sc} molecule.

There is more than one model for the formation of PrP^{Sc} and there is still considerable debate over the details of conversion. Crucial to the understanding of this process is evidence of the physical state of PrP^{Sc} within the cell. *In vitro* experiments have created conditions where human PrP could switch between a soluble form (presumed PrP^{C}) to a partially proteinase K resistant form (presumed PrP^{Sc}), [332] and PrP^{C} has been converted to PK-resistant PrP^{Sc} *in vitro* when incubated with pre-exiting PrP^{Sc} in a cell-free environment. This might suggest a direct interaction between PrP^{C} and PrP^{Sc} is sufficient for propagation of PrP^{Sc}.[333] *In vitro* experiments have successfully converted recombinant PrP^{C} to PrP^{Sc} but bioassays of such conversion products have not shown any detectable infectivity.[334]

The areas of PrP^{C} important in conversion to PrP^{Sc} are also not established. Some work has shown that the C-terminal end of the molecule is necessary for transformation.[335] The degree of glycosylation may also be important, as blockade of glycosylation seems to cause the protein to misfold in some experiments.[285] However, in common with many of the *in vitro* studies, the results are often conflicting. In one experiment using recombinant PrP corresponding to human residues 91-231 reduction of the disulphide bond between Cys^{179} and Cys^{214} resulted in α-helix conformational change to β-sheet,[336] however in other studies the

disulphide bond was required for PrP^{res} production.[335] Evidently further research is required to fully establish the nature of conversion of PrP^{C} to PrP^{Sc}.

4.5: DETERMINANTS OF RATE OF ACCUMULATION OF PrP^{Sc} AND REGIONAL DISTRIBUTION

It is clear that many aspects of the pathogenesis of prion diseases remain unresolved. It is still not known if the presence of PrP^{Sc} correlates with histopathological changes seen in the brain and how the disease process spreads to involve different brain regions. The influence of the structure of PrP^{C} within different brain regions on the final structure of PrP^{Sc} is also unknown. Other as yet unidentified factors such as the biochemical milieu within different cell types may also be involved.

The distribution of PrP^{res} within the brain of cases with a codon 178 mutation was studied to try and gain some understanding of the mechanisms involved. This is an interesting mutation because two distinct clinico-pathological phenotypes are recognised depending on the methionine/ valine polymorphism at codon 129: FFI, characterised by untreatable insomnia, dysautonomia and marked atrophy of the thalamic nuclei, is linked to the presence of methionine at codon 129 and familial CJD[178] has a phenotype similar to sporadic CJD and is linked to a valine at codon 129.[15] The influence of the codon 129 genotype on the clinical features and pathology will be discussed later. However, the amount of PrP^{res} was analysed in different regions of the brain in the two phenotypes. In this study the pathological process at the onset of the illness and the topographic progression seemed to be the same regardless of codon 129 genotype. [337]

Impaired neuronal function seemed to be a result of PrP^{res} accumulation (or other unidentified factors) in the absence of significant pathology and the rate of PrP^{res} accumulation was dependent on disease duration but varied in different regions of the brain.[337] Some areas such as the white matter seemed to have little if any PrP^{res} deposition. It appears that despite the ubiquitous presence of the codon 178

mutation, PrP^{res} deposition selectively starts in subcortical areas and only later spreads to the cerebral cortex.[338] In addition it seems that the overall amount and rate of accumulation of PrP^{res} present in different familial CJD mutations and in iatrogenic and sporadic CJD varies, suggesting a basis for the phenotypic variability of human prion diseases.[339]

- PrP^{C} is a ubiquitous cellular protein that binds copper and seems to be involved in the function of SOD.
- PrP^{Sc} is a post-translationally modified form of PrP^{C}.
- PrP^{Sc} is disease associated. It has a high β-pleated sheet content and is relatively protease resistant.
- The conversion of PrP^{C} to PrP^{Sc} is poorly understood but appears to be important in all forms of CJD, whether genetic, infectious or sporadic.

CHAPTER 5: STRAIN VARIATION

5.1: SCRAPIE STRAINS

The relevance of the conformation and structure of PrP becomes more important when considering strains of prion disease and the species barrier. It was recognised early on that different isolates of scrapie produced reproducible distinct clinical phenotypes in the same species in transmission studies. This was first demonstrated in goats in which two clinical syndromes, termed "nervous" and "scratching", were consistently found following intracerebral inoculation.[16] These differences were believed to be due to different strains of the prion agent. However, the existence of strain was difficult to reconcile with the absence of DNA.[340,341]

Studies of strain variation have also looked at human TSEs. Strains of CJD were first described when sporadic CJD and Kuru were transmitted into a variety of primates, cats and rodents. The host range, incubation time, duration of illness and type of clinical disease varied amongst isolates. This work is slightly confusing in that 112 different strains were apparently isolated, however, this was an indication of the number of different CJD and kuru patients used.[342]

Some properties seemed to be transmissible from host to recipient. E.g. kuru plaques in a patient with a panencephalopathic variant of CJD also produced plaques in mice when the disease was transmitted to them.[343] Transmission studies of iatrogenic (HGH) CJD to one hamster demonstrated a predilection for the cerebellum. It was proposed that this might represent a particular strain of CJD with a tropism for the cerebellum because HGH iatrogenic CJD also appeared to particularly affect the cerebellum.[344]

A number of strain defining properties have been used over the years in transmission studies, predominantly in mice. These include clinical manifestations such as differences in incubation period[72] and weight gain, possibly related to targeting of

the hypothalamus in disease.[345] Other strain dependent properties of PrP^{Sc} included resistance to decontamination and susceptibility to thermal inactivation.[346, 347] Other parameters are neuropathological, such as neuronal vacuolation[17] and amyloid plaque deposition.[348] Deposition of PrP^{Sc} also appears to be region-specific according to the strain inoculated.[349,350,351] From these markers a lesion profile can be constructed which is remarkably consistent and specific to different strains of scrapie.[17]

A large number of phenotypically distinct strains of scrapie have now been identified in transmission studies. Some of these have emerged from serial passaging of scrapie-infected material through one species and then back to the original or into another host. Some of the properties of a particular strain (e.g. degree of vacuolation and amyloid plaque deposition) became permanently altered when passaged into another host and then back into the original host through serial passage.[352] The literature can at times be confusing and some apparently new strains have turned out to be "re-isolation of the same strain".[353]

5.2: INCUBATION PERIOD

The host genotype also has a strong influence on the incubation period. If the same bank of mice were inoculated with the Chandler scrapie isolate two alleles were identified termed s7 and p7, denoting short and prolonged incubation periods respectively. A mouse gene was proposed as the mechanism for this, denoted *Sinc* (for "scrapie incubation period"). The length of incubation was consistently short or long depending on which of two alleles were present in the mouse host,[354] and on whether the mouse was homozygous or heterozygous for the allele. However, no major differences were seen in the lesion profile, although it has been suggested that the *sinc* gene has some influence on this.[355]

The *sinc* gene was co-dominant and the incubation period in s7/p7 heterozygotes was intermediate between the short and long incubation period for a particular strain.

However, the chromosome location of *sinc* took some time to determine. It was originally tentatively associated with another gene termed *prn-p* on mouse chromosome 2.[356, 357] This raised the question of whether the 2 genes were congruent, [358] but it was not until 1998 that it was proven that *sinc* was part of mouse *PRNP*. The incubation period is controlled by dimorphisms at codon 108 and /or 189 of mouse PRNP.[359]

5.3: TITRE AND ROUTE OF INOCULUM

Other factors influence the incubation period: It has been shown that there is an inverse relationship with the titre of inoculum.[360] Experiments with the route of inoculation do not always reproduce the same result however. In some studies the route of infection, whether oral or intracerebral, did not seem to affect the incubation period, or the number of successful transmissions.[361] In another study the brain was inoculated at various sites and this did not appear to affect the strain dependent distribution of PrP^{Sc},[362] but earlier studies had shown that amyloid plaque deposition depended on the site of inoculation, irrespective of strain.[363]

In fact, the kinetics of accumulation of PrP^{Sc} has been shown to be dependent on the infecting strain of agent, the mouse genotype and the route of infection,[364] and it can be difficult to isolate the host genotype influence on disease characteristics from the properties of different scrapie strains. As well as incubation period, the lesion profile[353] and PrP staining pattern[348] are both influenced to some extent by host genotype and are not just markers of strain. This fact has been used to support the protein only prion hypothesis, and it has been suggested that almost all the aspects of pathogenesis seen in prion diseases can be explained by an interaction between donor and host prion protein.[365]

5.4: HOST–SPECIES BARRIER

A further characteristic of the TSEs is the existence of the host-species barrier. Transmission of scrapie-infected material from one species to another is usually

accompanied by a prolongation of the incubation period in the first passage and incomplete penetrance of the disease. This was first described as a "species barrier" by Pattison[47] but a possible species barrier was demonstrated as early as 1936 in Cuillé and Chelles' original study. Inoculation of sheep had a low transmission rate, however 3 years later they transmitted scrapie to goats with 100% success.[366]

Subsequent passage in the same species occurs with a higher number of successful transmissions and shortened incubation times. Pattison also showed that the incubation period was shorter if the donor and host animal were of the same species. If transmission was into a different species, there tended to be atypical clinical signs and histopathology on first passage in the host.[47]

The species barrier can be overcome by using transgenic mice. If mice are passaged with hamster PrP^{Sc} they do not develop disease. However, if transgenic mice expressing Syrian hamster PRNP are passaged with the same hamster PrP^{Sc} they are highly susceptible to disease. The lesion profile produced is characteristic of hamster scrapie rather than mouse scrapie.[367] This gives further support to the hypothesis that PrP^{Sc} acts as a template for the transformation of PrP^{C}.

Similarly, transgenic mice expressing human PRNP were more susceptible to PrP^{Sc} from a case of sporadic CJD than from mouse PrP^{Sc} This may be because human PrP differs from mouse PrP in 28 amino acids. The more homologous the host and infective PRNP, the easier it is to overcome the species barrier.[368] A cell-free model for PrP^{C} conversion to PrP^{Sc} also shows species-specificity; the species barrier corresponding to differences in amino acid residues.[369]

5.5: MOLECULAR BASIS FOR STRAIN VARIATION

A: *IN VITRO* STUDIES OF N-TERMINAL CLEAVAGE

Strain variation has also been demonstrated in TME.[370] Similar to the original studies on scrapie, a "hyper" (HY) and "drowsy" (DY) strain of TME was identified by the

third passage.[371] These characteristics were independent of the host genotype and were not due to changes in the amino acid sequence. It was shown that DY PrP^{TME} was more sensitive to protease digestion than HY PrP^{TME}. Proteolytic treatment resulted in a 1 to 2kDa difference in migration on polyacrylamide gels.[18]

The proteolytic changes were later shown to correspond to cleavages at distinct N-terminal sites. It was proposed that this was consistent with different conformations of the two strains. If less of the N-terminal was bonded into a β-sheet conformation, a larger piece could be cleaved by proteinase K. The two strains also appeared to localise to different areas of the brain.[372] Conversion of PrP^{C} into the two distinct strains of PrP^{TME} has also been performed *in vitro*, suggesting the conformation of infecting PrP^{TME} determined the structure of the newly formed PrP^{TME}. This experiment was sensitive to chemical conditions however and cannot allow for possible differences in the chemical and physiological conditions within the brain.[373]

Similar results were demonstrated when extracts from the brain of a patient with FFI was transmitted to transgenic mice with human PrP. This material contained PrP^{res} with a molecular mass of 19kDA after deglycosylation. This was compared with extracts from patients with sporadic CJD and with familial CJD^{200}. The PrP^{res} in these cases had a molecular mass of 21kDa. When the two different PrP^{res} products were transmitted to the transgenic mice, PrP^{res} of two distinct molecular weights was produced: The PrP^{res} produced in the FFI case was of 19 kDa and that produced in the sporadic and familial CJD^{200} cases was of 21 kDa. In addition there were corresponding differences in the incubation period and in the neuropathological distribution of disease.[374] This appears to add weight to the theory that the conformation of the prion PrP^{res} acts as a template for conversion of host PrP^{C}.

B: *IN VITRO* STUDIES OF β-SHEET CONFORMATION

A further study was performed using an immunoassay that is sensitive to different conformations of PrP^{Sc}. The assay was developed for transgenic Syrian Hamster expressing human PrP. This assay used monoclonal antibodies directed against the N-terminus of PrP. Different antibodies had different affinities for the α-helical and β-sheet conformations, comparing denatured to native PrP^{Sc}. Eight different conformations of PrP^{Sc} were identified, believed to be indicative of prion strains of different conformation.[375] Another study analysed PrP^{res} from hamsters infected with three TSE strains by infrared spectroscopy. Differences in the amount of β-sheet between two of the strains were found.[376]

Recently, β-sheet breaker peptides have been used in *in vitro* experiments as a potential therapeutic agent for CJD. Variable results were obtained with PrP^{Sc} purified from different species and different strains, again supporting a difference in conformation or aggregation in distinct prion strains.[377]

Further work with transgenic mice has shown that changes in prion strain characteristics depend on the sequences of PrP encoded by the host during multiple serial transmissions. The strain characteristics of the ancestral source did not seem to be important following serial passage. In this study two different strains were serially passaged through a transgenic mouse host to yield identical strains with respect to incubation time and pathology, implying prion strain diversity is restricted to a finite number of conformations of PrP^{Sc}.[378]

C: PrP ISOTYPES IN CJD

Following the identification of two isotypes of TME corresponding to the hyper and drowsy strains, the human TSEs were studied to look for a molecular basis for strain variation. When brain tissue from cases of CJD are treated with proteinase K and analysed by western blotting techniques two isotypes are identified of 19kDa and

21kDa mobility.[14] The same isotypes have now been identified in all subtypes of CJD, whether infective, hereditary or sporadic. They have also been seen in kuru.[379] It is believed that these isotypes are surrogate markers of strains of CJD.

The number and classification of the protein isotypes differ slightly between centres and will be discussed later but differences are likely to be due to alternative methodology and purification techniques.[14,20,380]

Analysis has shown the isotypes differ in the site of N-terminal truncation: It is known from studies on human neuroblastoma cells that PrP^{Sc} seems to be a longer molecule than PrP^{C} and the N-terminal section seems to be the site of cleavage by proteinase K.[310] Protein electrophoresis has a low resolution and provides limited information on the precise size and possible variety of the PrP^{Sc} fragments resistant to proteases. The N-terminal sequence has been analysed in more detail using mass spectrometry. Two primary sites of cleavage by proteinase K have been identified: In type 1 PrP^{Sc} the site is at residue G82 and in type 2 it is at residue S97. This would be consistent with molecular masses of 21kDa and 19kDa respectively as seen on protein electrophoresis.[381]

This may not be the same *in vivo*. The site of cleavage may be affected by the quaternary structure of PrP^{C}.[20] Alternatively, an associated molecule, which is not attached covalently to PrP, may protect the cleavage site of the N-terminal. Possibilities for this molecule include sulphated proteoglycans,[382] Protein X[328] or other exogenous factors.[383] The conformation of types 1 and 2 also seems to be dependent on metal ions, possibly zinc or copper, presumably by exposing a new proteolytic cleavage site, although these findings have not been demonstrated in all isotypes.[384]

D: GLYCOSYLATION

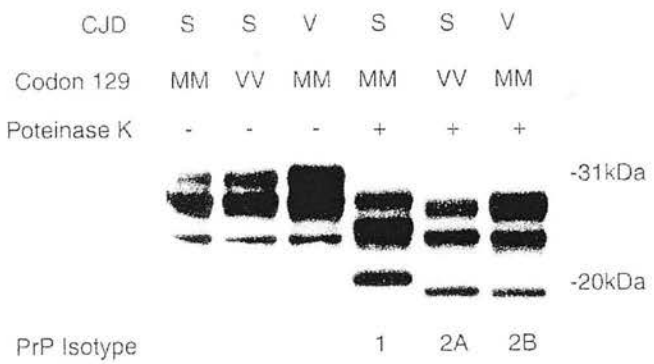
It is known that glycosylation is an important modification in any protein and can affect many of its' properties, including increasing the stability of the molecule against proteolytic degradation.[385] There are two potential sites for the attachment of a glycan to the prion protein.[82] It appears that either none, one or both sites can be glycosylated on both PrP^{C} and PrP^{Sc}.[284] The degree of glycosylation is likely to depend to a certain extent on the chemical environment within different cell types and the relationship of the degree of glycosylation to prion strain is not clear. As with other aspects of the study of prion protein there is no definite conclusion at this stage on the importance of glycosylation in determining prion strains.

If PrP^{Sc} is analysed by Western blotting techniques following treatment with proteinase K two different isotypes are identified of 19 and 21kDa, as shown previously. In addition differences are seen in the amount of unglycosylated, monoglycosylated and diglycosylated PrP produced. This is termed a glycoform ratio and, like the weight of the fragment sizes, this is maintained on passage in transgenic mice expressing human PrP.[20]

It was shown in transgenic mice that deletion of one or other of the oligosaccharides altered PrP^{C} trafficking and prevented infection with two prion strains. In addition the pattern of PrP^{Sc} deposition was altered. It was proposed that glycosylation modified the conformation of PrP^{C}, affecting the affinity of PrP^{C} for a particular conformer of PrP^{Sc}.[386] Further transgenic mouse studies showed that brain regions in Syrian hamsters synthesised different glycoform ratios of PrP^{C}, suggesting that PrP^{Sc} accumulation and vacuolation pattern are governed by neuron-specific differences in PrP^{C} glycosylation. This may influence the targeting of specific brain regions by different PrP^{Sc} strains.[387] The carbohydrate groups may influence the interaction of PrP^{C} and PrP^{Sc} through protein recognition events or through modulation of the structure, dynamics and stability of PrP.[388]

At this stage it is not clear to what extent the glycoform ratio is strain specific and to what extent it is determined by the regional affinity of the host neuronal population. As PrP glycosylation occurs before conversion to PrP^{Sc}, particular PrP^{Sc} glycoforms may replicate most favourably in neuronal populations with a similar PrP glycoform expressed on the cell surface.[389] Hence the glycosylation profile may simply be a reflection of the neuronal cell type, rather than the result of targeting of a specific area of the brain by a particular strain of scrapie. Glycosylation of PrP^{C} did not influence species specificity in the cell free model.[369] In addition other groups have suggested that the glycoform ratio is not maintained on serial passage and may change on the first or subsequent passage in transmission studies.[390]

Figure 5.1: SDS-PAGE OF PrP^{res} FROM VARIANT AND SPORADIC CJD



Because of this, the importance of glycosylation is not fully established. Although it seems likely that PrP conformation would be the primary determinant of strain type, the glycoform ratio has also been used as a marker of strain. Notably western

blotting has shown that the mobility of the type 2 isotype seen in sporadic, familial and iatrogenic CJD is indistinguishable from variant CJD. The only difference is the glycosylation pattern; vCJD has a higher proportion of diglycosylated PrP^{res}.[381] This has been denoted type 2B (in contrast to type 2A seen in other forms of CJD.) (See Figure 5.1) Recently type 1B (i.e. 21kDa mobility but with a high proportion of diglycosylated PrP^{res}) has been seen in a familial case of CJD.[380]

The method used by the NCJDSU recognises two mobilities of PrP^{res} on SDS-PAGE; type 1 of 21 kDa and type 2 of 19 kDa. In addition, each isotype is further characterised by the glycoform ratio. Type 2 is further classified as A or B depending on this ratio. Type 2B has to date only been seen in vCJD. (High proportion of diglycosylated PrP^{res}.)

E: INFLUENCE OF GENOTYPE

It has been shown already that it is often difficult to differentiate the influence of strain from the influence of the host, in particular the host genotype, on disease phenotype in prion diseases. In human prion diseases a common polymorphism at codon 129 of *PRNP* is important in the clinico-pathological phenotype of the disease. Either methionine or valine can be expressed on each allele. Methionine homozygosity has been shown to predispose to the development of sporadic CJD.[13] Heterozygotes and valine homozygotes tend to have an atypical clinical and pathological phenotype of disease. (See Chapter 6: Previous studies on the clinico-pathological phenotype of CJD)

In hereditary CJD a mutation at codon 178 can be associated with two different disease phenotypes; FFI is linked to methionine at codon 129 but familial CJD^{178} is linked to valine at codon 129. In addition if the overall codon 129 genotype is homozygous (i.e. the affected and unaffected alleles have the same amino acid at codon 129), the clinical course is shorter than if the patient is heterozygous.[15]

Western blot of the electrophoretic mobility of the two diseases demonstrated a mobility of 21kDa in fCJD[178] and of 19kDa in FFI.[391] In addition the FFI isotype had less unglycosylated PrP^{res}. In a human neuroblastoma cell model, the FFI PrP^{res} had less of the unglycosylated form, making it less stable and more easily degraded.[392] These findings implied that the differences did not come from an abnormality of the primary structure of the protein i.e. the genetic mutation found in FFI/ fCJD[178], but were related to the polymorphism at codon 129.[391] It has been shown that residues 129 and 178 of *PRNP* are connected by a hydrogen bond, suggesting an interaction which might influence the site of cleavage. Alternatively, the codon 129 polymorphism may interact with another molecule, not yet identified.[393]

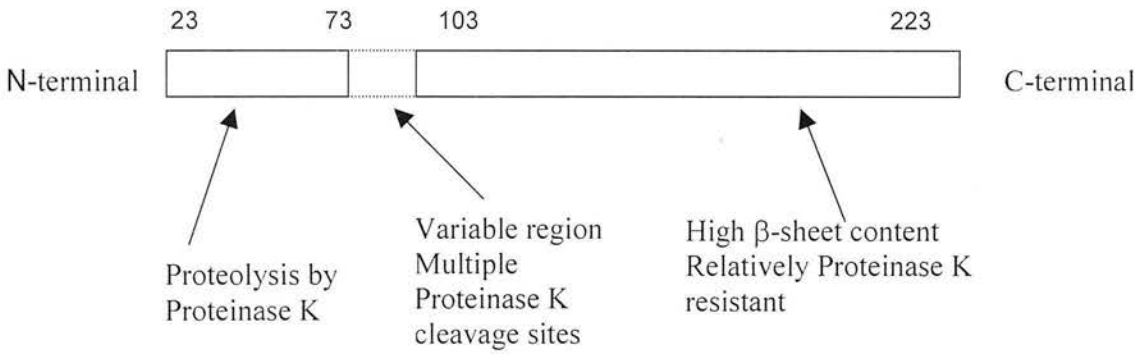
Studies have shown that the codon 129 polymorphism also has a role in influencing the proteinase degradation site of PrP^{res}: Type 1A protein isotype tended to be seen with methionine at codon 129, whereas type 2A tended to occur with valine, suggesting the amino acid at codon 129 influenced the site of cleavage by proteinase K.[380] The role that the degree of glycosylation had to play in this is not clear.

Recently, protein sequencing was used to identify the proteinase K cleavage site of PrP^{Sc}. As shown previously, two primary cleavage sites are seen at residues 82 and 97 for type 1 and type 2 respectively. In addition, there are a number of secondary cleavages around these two sites, suggesting that PrP^{res} has a ragged terminus. Surprisingly, irrespective of whether the disease was infectious, sporadic or familial these sites seemed to be influenced by the genotype at codon 129.[381] The N-terminal was less heterogenous in the familial cases however, possibly because the disease is derived from only one mutant allele rather than both alleles.

Current thinking is that there are three main structural regions on the PrP^{Sc} molecule: an N-terminal region from residue 23 to residue 73 that is proteolysed by proteinase-K. This is probably unstructured as in PrP^{C}; a C-terminal region from residues 103-231 that probably has a high β-sheet content and is highly proteinase-K resistant; and

a variable region from residues 74 –102. This region is subject to conformational changes at various sites. This seems to be regulated by the codon 129 polymorphism. The presence of methionine or valine influences the size of the region and may also influence the amount of β-sheet transformation in PrP^{Sc}.[381] (See Figure 5.2)

Figure 5.2: STRUCTURAL VARIATION OF HUMAN PrP^{Sc} RELATED TO PROTEINASE K CLEAVAGE SITE



F: BSE AND vCJD

The use of transmission studies and western blotting techniques in demonstrating strain variation has been extended to studies on BSE and related prion diseases such as vCJD, FSE and the exotic ungulate encephalopathies: Brain extracts from BSE in eight unrelated cattle sources have been successfully transmitted by intracerebral inoculation to mice. A similar incubation period and lesion profile was produced from each source, suggesting each cow was infected with the same strain of agent.[394] Analogous incubation periods and lesion profiles were seen when TSEs in domestic cats, a greater kudu and a nyala were transmitted to mice, indicating these species were infected with the same strain of BSE.[394,395]

Transmission studies to mice of vCJD and BSE have also produced very similar results, suggesting the same strain of agent is involved in the two diseases.[104]

Comparable results between vCJD and BSE have been produced in transgenic mice expressing bovine PrP.[103] In addition, when analysed by Western Blot techniques, the glycoform ratio in BSE and vCJD were alike but distinct from other TSEs.[20]

However, there is still some dispute over the mobility of vCJD. Parchi et al. found vCJD to have type 2 mobility with a different glycosylation pattern, termed type 2B.[14] Collinge and colleagues have found a slightly different mobility which they have termed type 4 but with a similar ratio of glycosylation.[20] These differences in mobility may be due to technical differences in the process used by the two centres.[381,396]

G: ISOTYPE HETEROGENEITY

As shown previously, the glycoform ratio can vary between different areas of the brain in a neuroblastoma cell model.[387] Piccardo et al. analysed a number of cases of GSS with the same PRNP mutation (P102L) and demonstrated heterogeneity of the glycoform ratio between cases. In addition, in one case, differences were found in glycoform ratio between different regions of the same subject.[397]

However, there has also been one isolated case in the literature where both isotypes were found in the same brain.[398] Parchi et al also found both isotypes in the cerebral cortex of a minority of patients.[399] They surmised this may have been due to technical difficulties or that PrP^{res} forms different conformations in different regions of the brain. In the Puoti study the PrP^{res} isotype appeared to be predictable depending on the histopathology of the region. This might imply that various PrP^{res} types have evolved simultaneously in the brain or that regional differences in the PrP^{C} form may contribute to variability in PrP^{res}.[400] Clearly further analysis of the regional distribution of each isotype is required.

Finally, different centres have proposed slight differences in the mobility of type 1 and 2 prion protein.[14,380] This is likely to be due to differences in methodology,

perhaps due to the chelation of heavy metals in the preparation of PrP^{res}. However, a different classification of PrP isotype has been proposed, involving several more mobilities of PrP^{res} and involving greater analysis of the glycoform ratio.[20] Clearly, these differences need to be resolved in order to try an establish a uniform classification of PrP^{res} isotype, and to fully evaluate its application in the clinico-pathological phenotype of prion diseases.

- Transmissions studies of scrapie in mice suggest that strains of the prion agent exist.
- A molecular basis for this was identified in TME, in which 2 protein isotypes, related to different clinical phenotypes were identified.
- The same 2 isotypes have been identified in CJD and it has been suggested these relate to strains of the CJD agent.
- The importance of glycosylation of the prion protein is not clear.
- A molecular basis for the differences seen due to codon 129 genotype has also been suggested.
- BSE and vCJD have the same protein isotype, which is different from other prion diseases, suggesting these conditions are due to a distinct single strain.

CHAPTER 6: PREVIOUS STUDIES ON THE CLINICO-PATHOLOGICAL PHENOTYPE OF CJD

The diverse clinical and pathological features of sporadic CJD might indicate that different strains of the agent exist. However it is difficult to separate the influence of host genotype from the possible influence of strain on the clinico-pathological phenotype of the disease. As can be seen from the previous chapters a molecular basis has been suggested for the influence of both strain and host genotype. However, this is still speculative. Other reasons for the differences in phenotype cannot be excluded. One potential influence on the clinical characteristics is the site in the brain where the pathogenic process begins. This might influence the symptoms at onset and the subsequent progression of disease.

Over the years a number of studies have analysed in detail the influence of the host genotype on the clinico-pathological phenotype of CJD. More recently, following the discovery of two different protein isotypes in CJD, which are believed to be a surrogate marker of strain, studies have set out to analyse trends in clinical and pathological features related to PrP^{res} isotype also.

6.1: INFLUENCE OF GENOTYPE

Codon 129 of PRNP is the site of a well-known polymorphism. Adenosine or guanine can be expressed, resulting in the expression of either methionine (Met) or valine (Val).[401] This was initially reported as a "double-allele" mutation in 1989 [402] but in fact either allele is expressed with sufficient frequency to be a polymorphism; in a normal population of 106 individuals 51% were heterozygotes, 37% were Met homozygotes and 12% were Val homozygotes. [403]

The importance of this polymorphism became evident when it was reported that 4/7 iatrogenic cases were Val homozygous at this site.[403] (In the original description of the polymorphism both iatrogenic cases were also Val homozygous.[402]) A later

analysis of 22 definite sporadic CJD cases showed that 16 (73%) were Met homozygous at codon 129 and 5 (23%) were Val homozygotes. Overall 22 cases (95%) were homozygous at codon 129.[404] 49% of the control population were homozygotes.[403] This seemed to suggest that homozygosity at codon 129 predisposed in some way to the underlying disease process. Further studies suggested that Val homozygotes and heterozygotes may have a longer duration of illness and atypical clinico-pathological phenotype.

Nine papers have been published discussing the influence of codon 129 genotype on the clinico-pathological phenotype of a series of sporadic CJD cases in a Caucasian population. Each has analysed some or all of several variables, including the percentage ratio of each genotype, age at onset, duration of illness, and clinical and pathological features. There may well be overlap of cases between studies. In the following section some of the features discussed in each study and in iatrogenic and familial cases are described. I have amalgamated the data from each study, placed at the end of each section. The distribution of codon 129 within the normal population is considered first.

The studies are:

1. Palmer MS et al.[404] 22 definite Caucasian cases of sporadic CJD.
2. Windl O et al.[405] 58 definite and probable cases referred to NCJDSU
3. De Silva et al.[406] 29 cases of definite sporadic CJD referred to NCJDSU. NB subset of Windls' study therefore pathology only considered.
4. Laplanche J-L et al.[407] 41 definite and probable cases referred to French surveillance group.
5. Salvatore M et al.[408] 31 definite and probable cases referred to the Italian surveillance group. Stated "Caucasian".
6. Schulz-Schaeffer WJ et al.[409] 47 definite cases of sporadic CJD.
7. Miyazono M et al.[410] 21 Japanese patients.

8. Hauw J-J et al[411] 70 patients referred to French surveillance system with definite pathology and PrP immunohistochemistry.
9. Tranchant C et al.[412] 14 deaths from sporadic CJD in a French hospital.

A: NORMAL POPULATION

The distribution of the codon 129 polymorphism within the normal population appears to be very different from that of sporadic CJD. However, only a few studies have been done and each has involved a relatively small number of cases. The source of controls is not identified in every study and it is conceivable that there may be a bias in some of the control populations used. Nevertheless, the resultant ratios have been broadly similar in many of the published studies; suggesting the codon 129 distribution does not vary greatly within the general population.

In Table 6.1 all published studies that have used a control population have been amalgamated to give an overall ratio for the codon 129 genotype within the normal population. Assuming these figures are representative, the overall incidence of each genotype is:

	Mean (%)	Median (%)	Range (%)
MM	42	42	35-50
MV	48	49	40-54
VV	10	10	0-15

However, many of these studies use a Caucasian control population. It is of note that figures for the Japanese population are different. They do not report the distribution of each genotype but instead give a Met: Val frequency of 0.958: 0.042.[413] In comparison the UK allele frequency is 0.675: 0.325.[414] A Val at codon 129 is seen as a point mutation rather than a polymorphism in Japan because it occurs so rarely. Collinge et al comment that they have found a similar frequency of Val expression to

the Japanese in a Thai population.[415] This would suggest that the distribution of genotype within the Asian population is different from Caucasians.

Table 6.1: AMALGAMATION OF CONTROL POPULATIONS

REFERENCE	SOURCE	N=	MM	%	MV	%	VV	%
Salvatore et al.[408]	Not stated	186	84	**45**	75	**40**	27	**15**
Lampe et al.[416]	Not stated	57	23	**40**	29	**51**	5	**9**
Medori et al.[417]	Not stated	20	9	**43**	8	**42**	3	**15**
Zimmermann et al.[418]	Blood donors	300	129	**43**	146	**49**	25	**8**
Collinge et al.[403]	Not stated Caucasian	106	39	**37**	54	**51**	13	**12**
Deslys et al.[419]	Not stated	69	25	**36**	37	**54**	7	**10**
Laplanche et al.[407]	Blood donors	92	38	**41**	45	**49**	9	**10**
Brown et al.[420]	Hypopituitary patients	24	12	**50**	12	**50**	0	**0**
Brown et al.[66]	Not stated	86	33	**38**	44	**51**	9	**11**
Schulz-Schaeffer et al.[409]	Not stated	74	31	**42**	33	**45**	10	**13**
Labauge et al.[421]	Not stated	45	19	**42**	22	**49**	4	**9**
Gabizon et al.[422] **	Healthy Libyan Jews	66	25	**38**	33	**50**	8	**12**
	TOTAL	1125	467	**42**	538	**48**	120	**10**

**NB numbers don't add up in original paper.

B: SUSCEPTIBILITY

This raises the question of how consistent the frequency of each allele is in other populations and does this influence the incidence of CJD in these populations: The incidence of sporadic CJD is roughly similar throughout the UK. Any differences seen do not achieve significance and are likely to be due to chance.[123] The reported

incidence of sporadic CJD varies slightly throughout countries in Europe (See Table 6.2)[122] but this depends on several factors including case ascertainment; methods used vary from country to country, as do post mortem rates. For example in Austria, where it is rare for autopsy not to be performed, annual incidence rates of CJD of up to 1.5 cases per million per annum have been reported.[423]

Table 6.2: MORTALITY RATE OF CJD IN EUROPE: 1993-95
(Excluding iatrogenic CJD)[122]

COUNTRY	Cases per million per year
France	0.84
Germany	0.55
Italy	0.56
Netherlands	0.81
Slovakia	0.62
UK	0.77

Alternatively, there may be a genuine difference between the incidences of sporadic CJD in different countries due to variation in the allele frequency at codon 129. An analysis of cases in Crete found a relatively high incidence of sporadic CJD and a higher rate of Methionine homozygotes in the background population.[424] This would require further study but other relatively isolated populations with a low influx of "foreign genes" might also differ from the quoted "normal" population rates.

Another possibility, albeit unlikely, is that Val homozygotes and heterozygotes die at a younger age. As sporadic CJD is in general a disease of the elderly, the excess of Met homozygotes might be representative of the elderly population as a whole. Similarly, the excess of Val homozygotes in the young may again be representative of this population. There is no age-stratified study of the normal population distribution of the codon 129 genotype with which to reject this unlikely hypothesis.

As far as we know the codon 129 genotype is not associated with ill health in any other form but it has recently been shown that there is an increased incidence of met homozygosity in cases of inclusion-body myositis.[413]

The reported data on the increased number of Met homozygotes in cases of sporadic CJD and the increased incidence of Val homozygosity in iatrogenic CJD prompted further investigation of the importance of codon 129: A study of cases referred to the French Surveillance study between 1992 and 1993 showed over-representation of Met homozygosity in the 41 definite and probable sporadic cases but did not show an excess of Val homozygotes. Two studies of 31 definite and probable sporadic CJD patients in Italy,[408] and 58 definite and probable sporadic CJD cases in the UK,[405] identified a similar excess of Met homozygotes. A German study of 47 cases of definite sporadic CJD showed an excess of both Val and Met homozygotes.[409]

Amongst iatrogenic cases the trend is slightly different: 102/128 cases (80%) were homozygous at codon 129. However, although Met homozygotes made up the majority of dura mater cases, (74% of 43 cases), a greater proportion of HGH related cases were Val homozygotes (32% of 82 cases). The reasons for this are unclear.[250]

The largest study of codon 129 in a population of sporadic CJD cases comes from European collaborative surveillance.[13] Many of the cases included in this population will have been used in the individual studies cited above. They reported an overall frequency of each genotype in 748 cases of sporadic CJD, although they did not state if the figures included probable cases, or if only autopsy confirmed cases were included. Nevertheless, the excess of homozygotes is readily apparent:

MM	70%
MV	13%
VV	16%

However, when the genotypes were analysed by 10-year age bands, the Val homozygotes were significantly more frequent in the under 50-age band and the Met homozygotes show a positive linear relationship with increasing age. Heterozygotes also seemed to be more frequent in the under 50-age group but this result did not achieve significance.

There are several possible reasons for this. There has been an increase in the number of young atypical cases of sporadic CJD identified since vCJD was described. Val homozygotes tend to be young and atypical and so there may be a bias in the surveillance system. In addition 14-3-3 protein has been identified as a sensitive and specific tool in the diagnosis of sporadic CJD in recent years.[171] This test seems to be helpful in Val homozygotes, who often have a negative EEG,[124] and so cases who might previously have been missed may now be identified. Alternatively, older sporadic cases who are Val homozygous may have an atypical phenotype and could be missed in the current surveillance systems.

All cases of vCJD to date have been Met homozygotes and it is not yet known if other genotypes will develop the disease.[123] Kuru is the only other orally transmitted TSE and so information on the genotype in this condition might give some indication of the susceptibility of other genotypes to vCJD. 92 cases were examined in a retrospective study. Met homozygotes were over represented in the younger age groups and heterozygotes were over represented in the older age groups.[425]

Other factors may influence these data however. Different age groups may have been exposed to different types of cannibalism, and hence a different amount or route of the infectious agent. Both these factors have been shown in transmission studies to influence the incubation period and the chance of inoculation.[41, 426] Therefore this may be a cohort effect rather than an indication of varying susceptibility between genotypes.

Table 6.3: GENOTYPE DISTRIBUTION

Study No	MM %	MV %	VV %
1	73	4	23
2	83	9	9
4*	71	19	10
5	81	16	3
6	66	17	17
8**	56	16	29
9	71	7	21

* 7 cases in this study were excluded because they were "possible" sporadic CJD. 2 were MM and 5 were MV. It is possible these were cases of CJD but atypical.
** This study has a larger number of Val homozygotes and heterozygotes. There may be a post mortem bias towards atypical cases.

Eight of the studies quoted the percentage of each genotype. These results are set out in table 6.3. The overall median value of Met homozygotes was 71% compared to 42% in the general population, Val homozygotes constituted 17%, and only 16% were heterozygotes in comparison to 10% and 49% in the general population respectively. These figures are similar to the amalgamated data for Europe.[13]

	Median %	Range %
MM	71	56-83
MV	16	4-19
VV	17	3-29

C: AGE OF ONSET AND DURATION OF DISEASE

Codon 129 also influences the age of onset and duration of disease: This was first demonstrated within a family carrying the 144 base-pair insertion. Those dying under the age of 50 carried Met on the affected allele but those dying over the age of 50 carried Val.[427] This was statistically significant. A similar relationship between codon 129 and the age at onset was also identified in the same family although only a small number of cases could be analysed.[262]

Because hereditary CJD is rare, within the setting of a rare disease, it is not always possible to identify the degree to which codon 129 influences the clinico-pathological phenotype compared to the natural variation that is seen in many of the mutations. Often case reports comparing the influence of codon 129 are of one or two individuals within the same family.

Analysis showed that heterozygosity at codon 129 delayed the age of onset of the codon 178 familial CJD group, and prolonged disease duration of both familial CJD[178] and FFI phenotypes.[15,428] Although analysis of other kindreds with the same mutation did not support these findings.[414,429] In familial CJD[200] the genotype at codon 129 does not influence age at onset or disease duration.[419] However, heterozygotes had a later age at onset of disease in the Indiana kindred of GSS,[430] and a longer duration of illness with later onset in a family with the codon 187 mutation.[431]

Any analysis of the age at onset in HGH iatrogenic cases is hindered by the lack of data on date of exposure and dose required to develop disease. Both these factors may influence the incubation period in transmission studies.[41,426] This in turn may influence the age at onset in iatrogenic cases and it is difficult to separate the influence of codon 129 genotype from the incubation period, (as well as other unidentified factors).

There seems to be some evidence that heterozygosity at codon 129 prolongs the incubation period of HGH recipients.[432] This evidence has been disputed however because of the difficulties of estimating the point of infection in a treatment which could have continued for some years.[250] The incubation period for dura mater grafts is measured in months rather than years.[59] However, there are insufficient data in the literature to comment on the influence of codon 129 genotype on the age at onset and duration of illness.

In kuru a shorter duration of illness was associated with homozygosity.[425]

A larger number of cases can be compared in sporadic CJD. The case reports mentioned above often report disease duration and age at onset, occasionally with conflicting results.

A French study showed disease duration was shorter in Met homozygotes than in heterozygotes.[407] Patients with at least one Val allele (i.e. heterozygotes and Val homozygotes) were found to have a long clinical duration in a Japanese review.[410] twelve cases of sporadic CJD who were Val homozygous were reviewed by the UK surveillance system. The age at onset and duration of illness was in keeping with many unselected series of CJD cases.[433] However, in another study heterozygotes were associated with an older age at onset.[411]

Table 6.4: AGE AT ONSET AND DURATION OF ILLNESS

Study	Median Age at Onset (years)			Median Duration Illness (months)		
No	MM	MV	VV	MM	MV	VV
2	65	65	65	3	3	3
4	66	65	58.5	3	8.8	6
5	60.9*	63.4*	55*	6.4	7.6	6.7
6	68.7*	66.3*	58*	?	?	?
7**	?	-54-		?	-33-	
8	68.4*	70.9*	59.6*	3.7*	8.9*	11.5*
9	67	66	65	7	12	9

*Mean value given not median
**Heterozygotes and Val homozygotes grouped together. Not possible to separate data.

Amalgamation of data for Age of Onset:
7 studies analysed the median age of onset. (See Table 6.4) The trend was for Val homozygotes to develop disease at a younger age although there was considerable overlap between all 3 genotypes.

Age Onset (years)		Median	Range
	MM	66.5	60.9-68.7
	MV	65	54-70.9
	VV	58.5	54-65

Amalgamation of data for duration of illness:

7 studies analysed the median duration of illness. (See Table 6.4) Both heterozygotes and Val homozygotes tended to have a longer duration of illness, again all 3 genotypes overlapped with cases of long and short duration in each group.

Duration Illness (months)		Median	Range
	MM	3.7	3-7
	MV	8.8	3-33
	VV	7	3-33

D: CLINICAL FEATURES AND INVESTIGATION RESULTS

Perhaps the most compelling illustration of the influence of codon 129 on disease phenotype comes from the study of fatal familial insomnia (FFI) and familial CJD[178]. These are two different phenotypes of disease attributable to the same guanine to adenine mutation at codon 178. One illness, (familial CJD[178]), first described in a Finnish family, is similar clinically and pathologically to sporadic CJD.[15] The other, (FFI), is characterised by progressive insomnia, dysautonomia and motor signs. Pathologically there is marked atrophy of the thalamic nuclei and spongiform change is often scanty.[434] Further investigation showed that the codon 178 mutation is encoupled with Met at codon 129 on the mutated allele in FFI, and with Val in familial CJD.[15]

There are also isolated examples of codon 129 influencing the disease phenotype of some other mutations. Although there are few cases to compare, Val homozygosity

associated with a 193 base-pair insertion seems to be associated with a clinical picture of hypokinesia, rigidity and dementia,[435] but Met homozygotes seemed to have prominent psychiatric and cerebellar symptoms.[436] An atypical clinical and pathological phenotype was seen in a case of GSS with a codon 102 mutation,[437] and a familial CJD[117] case, both of whom were Val homozygotes.[438] Analysis of GSS due to a codon 102 mutation within a Sicilian family found that although there was a remarkably diverse clinical picture, this was unrelated to the codon 129 polymorphism.[439]

In iatrogenic CJD codon 129 does not appear to affect the disease phenotype although only a small number of patients have been reviewed.[406]

Influences on the clinical features of sporadic CJD have been described in case reports and studies: The UK study of Val homozygotes found that many presented with ataxia at onset. The subsequent clinical course was similar to the recognised pattern for sporadic CJD. However, typical EEG findings were absent in all the cases in whom an EEG was available.[433] Val homozygosity has also been associated with an atypical clinical phenotype in isolated case reports.[440,441]

There is also a report of the absence of a typical EEG and a negative 14-3-3 result, despite a fairly typical clinical course, in a heterozygote with sporadic CJD.[442] A French review of 14 definite sporadic cases identified typical clinical findings in the homozygotes. However, they identified one heterozygote who presented with dementia and ataxia, had a duration of illness of 12 months, and amyloid plaques in the cerebellum.[412]

Data on Clinical Onset:

Analysis of the clinical onset suggested that Met homozygotes presented mainly with dementia and the other genotypes often had an ataxic presentation. (See Table 6.5) It

was not always possible to differentiate symptoms and signs at onset from those that developed during the course of the illness.

Table 6.4: CLINICAL ONSET AND EEG RESULTS

Study	Clinical Onset			EEG Typical		
No	MM	MV	VV	MM	MV	VV
7**	10/13 dementia	5/7 ataxia		13/13	1/7	
9	6/10 dementia 2/10 visual	1/1 Dementia + ataxia	3/3 ataxia	9/10	0/1	0/3

** 7 cases chosen because of Val "mutation" at codon 129. Therefore it was not possible to give a frequency for each allele. 6 cases were heterozygotes and 1 case was Val homozygous. The properties of the isolated Val homozygotes were not differentiated.

Data on EEG results:

A typical EEG was seen in most of the Met homozygote cases but was only reported in one other case. (See Table 6.5) The exact parameters for a typical EEG varied between studies and were not always stated

E: NEUROPATHOLOGY

Codon 129 also seems to influence the neuropathological findings of sporadic CJD: Analysis of 8 cases with Val on one or both alleles found they had kuru-type amyloid plaques in the cerebellum, not seen in Met homozygotes.[420] This was demonstrated in 4 further studies in the UK, Germany and Japan.[406,409,410,443] The UK study also demonstrated amyloid plaques in two of three Met homozygotes.[406] Amyloid plaques have been associated with CJD with a long clinical course.[145] In familial CJD Val homozygosity was associated with amyloid plaque deposition in familial CJD[200], [444,445] and familial CJD[117].[446]

In Kuru, some studies have shown a correlation with pathology; plaques being associated with the Met allele, but this has not been demonstrated in all studies.[447]

Quantatative analysis of the pattern of pathology in sporadic CJD showed that codon 129 had some influence on the distribution of spongiform change. Val homozygotes showed greater involvement of the deep grey matter, particularly in terms of PrP deposition and astrocytosis,[433] whereas an intermediate pattern was seen in heterozygotes. Met homozygotes tended to have predominantly cortical pathology.[448] Concentration of spongiform change in the occipital cortex particularly of Met homozygotes has also been demonstrated.[14] The same group showed plaque-like deposits in the cerebellum in the heterozygotes and Val homozygotes.[399]

Analysis of 70 French cases, providing the largest published review of the neuropathological changes, supported these findings. However, like Parchi who only found kuru-type plaques to be a feature of heterozygotes, only one Val homozygote was identified with kuru-type plaques in this series and the plaques were mainly seen in heterozygotes.[411] The plaques identified on PrP immunocytochemistry in Val homozygotes were analysed in a UK study. These were morphologically distinct from kuru-type plaques and have been described as "plaque-like".[433]

Amalgamation of data on pathology:
5 studies analysed pathological data: Plaques were only seen in heterozygotes and Val homozygotes except for one study, in which two Met homozygotes also had plaques. In study 9 the amyloid plaques were differentiated as "kuru-type" in the heterozygotes and "plaque-like" in the Val homozygotes. Study 3 commented that the Val homozygotes tended to have spongiform change in the deep grey nuclei, whereas study 8 noted spongiform change in the occipital lobe of the Met homozygotes. (See Table 6.6)

Table 6.6: PATHOLOGY

Study No	Plaques			Areas of spongiform change		
	MM	MV	VV	MM	MV	VV
3	2/20	2/4	3/3			Deep grey nuclei
6	0/31	3/8	6/8			
7**	?	7/7				
8	0/39	6/11	1/20	occipital		
9	0/10	1/1 Kuru type	3/3Plaque-like			

F: OTHER POLYMORPHISMS

Other polymorphisms have been described: In the Japanese population a polymorphism at codon 219 was reported in which glutamic acid or lysine could be expressed. In cases of GSS, heterozygotes at this site were found to have a different clinico-pathological phenotype from glutamic acid homozygotes.[449] Subsequent analysis has shown that all cases of sporadic CJD are glutamate homozygotes at codon 219, but that 88% of a control population are heterozygous, suggesting that heterozygosity has a protective effect against sporadic CJD.[450] An analysis of 100 Italian controls and 104 cases of sporadic CJD, familial CJD and GSS and members of their families did not identify this polymorphism in any of the cases.[451] This suggests there may be an ethnic difference in this gene frequency (as there seems to be with codon 129 also). The numbers are small and this needs further analysis.

There are also three polymorphisms, occurring at codons 117, 124 and 161 that do not result in amino acid substitutions.[452,453]

G: CODON 129 IN OTHER DISEASES AND OTHER GENETIC FACTORS

Recently the relevance of codon 129 to Alzheimer's diseases has been analysed. The E2 variant of apolipoprotein E (Apo E) is known to have a neuroprotective effect in

dementia. It seems to delay the onset of disease in various dementing conditions, including sporadic CJD.[443] It is possible that the Apo E4 variant, in conjunction with Val at codon 129, may be associated with amyloid plaque formation in sporadic CJD.[443]

One small study has also shown that Met homozygosity is associated with inclusion body myositis.[413] The importance of this is not clear. Prion protein has been identified in inclusion bodies [218] but there are no features of this condition that resemble other prion diseases.

6.2: INFLUENCE OF ISOTYPE AND GENOTYPE

It seems likely however that the genotype of the patient is not the only influence on disease phenotype. Sporadic CJD does not neatly subdivide into 3 distinct clinico-pathological phenotypes. As discussed previously, strain dependent properties, akin to those seen in studies of scrapie, have also been demonstrated in sporadic CJD.[454,455] The differential distribution of PrP^{CJD} in iatrogenic CJD (human growth hormone) compared with sporadic cases of CJD led to the suggestion that a different strain was responsible for the iatrogenic disease, preferentially selected by a peripheral route of inoculation.[344]

A: STUDIES ON ISOTYPE AND GENOTYPE

In a study of the clinico-pathological phenotype of sporadic CJD, Parchi et al. reported that two isotypes of PrP^{res} were detectable with Western Blotting techniques[14] These were termed types 1 and 2. The isotypes differed in their electrophoretic mobility and in the ratio of glycosylation. The differences were consistent within the one individual, both in different sampling areas of the same brain, and in cases who had had a prior brain biopsy, temporally between early and late disease. When analysed along with the codon 129 genotype, there seemed to be consistent clinical and pathological features peculiar to each isotype.[399] It was suggested that the isotypes were surrogate markers of strains of CJD and further

study of these isotypes has suggested a structural basis for this (See Chapter 5: Strain Variation)

Parchi's initial study of 19 cases found that all Val homozygotes and heterozygotes displayed type 2 PrP^{res} (VV-2, MV-2 respectively), but Met homozygotes could be type 1 or 2 (MM-1 and MM-2 respectively). The MM-1 group was the most common and displayed the typical features of sporadic CJD. The other groups were smaller and tended towards an atypical course of disease, with a longer duration, atypical EEGs, and differences in histopathology and PrP^{res} accumulation.[14]

The original work was extended to 300 cases of sporadic CJD. In this study, type 1 PrP^{res} was also found in Val homozygotes and heterozygotes.[399] Thus, six potential groups of sporadic CJD were identified, dependent on host genotype and PrP isotype:

MM-1	**MV-1**	**VV-1**
MM-2	**MV-2**	**VV-2**

The majority of cases were in the MM-1 group, and the disease phenotype was that of classically described sporadic CJD. Heterozygotes and Val homozygotes tended to have a type 2 mobility. The cases that were not MM-1 tended to be atypical, either in disease duration or characteristics, investigation or pathology.

Parchi et al. proposed that there were clinical and pathological features attributable to each group, suggesting that sporadic CJD could be classified according to genotype and protein isotype. They identified two phenotypes within the MM-2 group, a thalamic form, analogous to sporadic familial insomnia and a cortical form, characterised by prominent dementia and cortical pathology.[399]

The picture is less clear cut however as Western blotting of cases by Cardone et al. has suggested two protein isotypes but of different molecular weight to those isolated by Parchi et al.[380] Collinge et al have identified three isotypes of sporadic and iatrogenic CJD, differing in electrophoretic mobility and glycosylation pattern.[20] These isotypes are grouped as follows:

Type 1: Sporadic CJD/ Met homozygotes
Type 2: Any genotype of sporadic CJD and
Iatrogenic CJD/ Met homozygotes
Type 3: Iatrogenic CJD by peripheral route and
Iatrogenic CJD/ heterozygotes or Val homozygotes.

Types 2 and 3 remain unchanged when passaged in transgenic mice expressing human PrP encoding valine at codon 129, but type 1 converts to type 2 on a similar passage.[20]

The number of protein isotypes is still being debated. Parchi et al. have reported that the Collinge group types 1 and 2 match the Parchi type 1 and the Collinge type 3 matched the Parchi type 2.[379] To date Parchi et al. and other groups have only identified two PrP^{res} isotypes on electrophoresis, despite analysing a case of kuru and cases of vCJD. The differences may be in methods used for Western blotting, e.g. copper binding may affect the mobility of different isotypes, but Collinge et al. have suggested that as techniques improve, further isotypes may be identified.[396]

No matter which technique is used, further work will hopefully resolve some of the discrepancies in the results. It is known that glycosylation of PrP^{res} is a co- and post-translational event.[379] The importance of glycosylation as a marker of strain is unresolved, but recently a different glycosylation pattern of type 1 PrP^{res} has been identified in some cases of familial CJD[102] and CJD[200]; type 1B. This pattern has not been reported in sporadic CJD.[380] Transmission studies of each isotype may support

the differences found using Western blotting but the studies, by their nature, are slow.

All cases of vCJD to date have shown a similar clinico-pathological phenotype. All those that have been tested have been Met homozygous at codon 129. (Personal communication, NCJDSU) There is concern that vCJD cases who are heterozygotes or Val homozygotes are being missed. It is possible that the disease phenotype in either of these genotypes is different, although it seems unlikely that a young person would die of an undiagnosed neurodegenerative condition and not have an autopsy. Two retrospective reviews of deaths in the UK have been performed, and it is believed unlikely cases of CJD, in particular vCJD, have been missed.[125,126]

Only one isotype of variant CJD has been identified. All cases to date have had a type 2 mobility and a distinct glycosylation pattern with a predominance of the unglycosylated form. This pattern has been reproducible and is distinct from that seen in other forms of CJD.[20,381] The pattern is maintained on passage through mice and is similar to that of BSE.[20] This would support the use of protein isotype as a marker of strain in CJD.

Isolated cases reports of young people with atypical sporadic CJD are emerging. Most recently a case was reported of a 27-year-old man with sporadic CJD, whose genotype was Val homozygous.[441] He is included in the VV-1 group in this study. The definitive test in such cases will be transmission studies, but this takes up to 2 years. Until then the diagnosis rests on the neuropathological findings and PrP isotyping.

There are fewer data on isotypic variation in familial and iatrogenic CJD: Details of only five cases of iatrogenic CJD with isotyping results have been published. Of these two were MM-1 and three were VV-2. There was no obvious clinical distinction between the two groups.[381] There are studies of the isotype in some

familial cases.[444] These are again isolated reports and the numbers are too few to comment on trends.

B: AMALGAMATION OF DATA

Various papers have discussed the influence of codon 129 on the clinical and pathological phenotype of all forms of CJD but each has only looked at a small number of patients. More recently the PrP isotype data of 300 cases has been analysed but again the numbers in some subsets are very small. A meta-analysis of all the papers discussing sporadic CJD might give a more meaningful result. Furthermore, discrepancies between the different studies might be illustrated:

Having described 19 cases of sporadic CJD in 1996[14] Parchi went on to discuss 300 cases of sporadic CJD which he divided into 7 groups (there were 2 subsets in the MM-2 group).[399] Isolated case reports[440,442] and two further reviews[412,456] of cases with both genetic and isotype data have been subsequently published. I have combined the data from all of these reports and studies. It is possible that some of the cases reported by Parchi et al. are duplicated in some of the other reviews as they amalgamated 300 European and North American cases, but I could not extract these cases from the data as a whole. (One case report of a VV-1 case was excluded because it was believed to be one of Parchi's 300 cases.[441])

The following studies were amalgamated:

1. Parchi et al.[399] Review of 300 cases of definite sporadic CJD from Europe and USA.
2. Zerr et al.[455] Review of 108 cases of definite sporadic CJD in whom full clinical, genetic and neuropathological data was available.
3. Tranchant et al.[412] 14 cases of sporadic CJD who died in the hospital at which the authors are based.
4. Isolated report of a case who is MV-2[442]
5. Isolated report of a case who is VV-1[440]

It is difficult to perform direct comparisons of the data, partly because there may well be duplication of cases, but also because the data are presented in different ways. Eg. Zerr et al. have commented on symptoms and signs at any point in the illness but Parchi et al. have divided the data into symptoms at onset and symptoms during the illness. It is also not clear how the data has been extracted. It is impossible to avoid a degree of observer variation and in some cases the data may be retrospective. Nevertheless, some trends are apparent (See Table 6.7)

A: Age at Onset and Duration of Illness

Parchi et al. found the VV-2 and MV-2 groups to be significantly younger at onset than the MM-1group. The VV-1 and MM-2 (thalamic) groups tended to be younger but this was not significant. Zerr et al. showed the VV-1 group were significantly younger but there was no significant difference between the other groups. In both the Parchi and Zerr studies the MM-1, MV-1 and VV-2 groups had a shorter duration of illness but, as with the genotype data, the variables overlapped considerably between each group.

B: Symptoms and Signs

Clinically, the Heidenhain variant, with visual symptoms at onset leading to cortical blindness, was peculiar to the MM-1 group. The VV-2 group tended to present with ataxia and cognitive problems were delayed, if present at all. Parchi found cerebellar signs to be common in the MV-2 group but this was not a feature of the Zerr cases. However an isolated MV-2 case in the literature also had prominent cerebellar features, though not at onset. This case was also atypical because of the presence of a peripheral neuropathy and she later developed the syndrome of inappropriate antidiuretic hormone secretion.[442]

Table 6.7: META-ANALYSIS OF CASES WITH ISOTYPE AND GENOTYPE DATA

Study No.	No. cases	%	Age onset yr	Duration mths	% Onset dementia	% Onset ataxia	% Onset visual	% EEG typical
MM-1								
1	203	68	65.5*	3.9*	70	33	26	46
2	70	65	68 **	5.2**	77#	19#	41#	80
3	10	71	67**	7**	70	0	20	90
MM-2								
1~	12	4	58.3*	15.7*	83	33	0	0
2	3	3	63	14	67	33	0	33
MV-1								
1	8	3	62.1*	4.9*	50	75	12	71.4
2	8	7	63	3.4	75	25	0	75
3	1	7	66	12	100	100	0	0
MV-2								
1	27	9	59.4*	17.1*	74	81	0	7.7
2	10	9	62	17.4	50	20	1	30
4 ?	1		68	29	100	0	0	0
VV-1								
1	3	1	39.3*	15.3*	100	0	0	0
2	2	2	27	25.5	100	0	0	0
5	1		49	15	100	100	0	0
VV-2								
1	47	16	61.3*	6.5*	27	100	0	7.1
2	15	14	62	7.5	33	47	20	0
3	3	21	65	9	0	100	0	0

~ Divided into 2 groups by original authors and joined together for the purposes of this analysis. (See later)

? May be included in Zerr figures.

* Not stated if mean or median.

**Median

Not clear if symptoms at onset.

Parchi et al. divided the MM-2 group into two subsets, a thalamic and cortical variant. The thalamic variant would be regarded as sporadic fatal insomnia, characterised by progressive insomnia and psychomotor agitation at night, the absence of a typical EEG and prominent thalamic and olivary atrophy. In the cortical variant dementia is prominent and the duration of illness is longer, but again the EEG does not show the typical periodic sharp waves. The pathology is characterised by prominent confluent vacuoles with relative sparing of the cerebellum. These two subsets have not been described elsewhere.

C: Investigations

The EEG is an important aid to the diagnosis of sporadic CJD. Parchi et al. found a typical EEG in 80% of the MM-1 and 71.4% of the MV-1 groups. Although they do not clarify if all cases has repeated EEGs. It is possible that in some cases the EEG was only performed once and if it was not suggestive of CJD it was not repeated. Zerr et al. specifically set out to study the results of EEG, CSF analysis and MRI scanning. (See table 6.8).

The numbers in each group are small except for the MM-1 group. However, they too found that the MM-1 and MV-1 groups tended to be the only cases with a positive EEG.

They note that analysis of 14-3-3 protein and the MRI may aid in the diagnosis when the EEG is negative. The majority of cases in each group, apart from the MV-2s, had a positive 14-3-3 result. The MRI also seemed to be positive in some cases in whom the EEG was negative. In particular, the MV-2 group seemed to have a negative EEG and 14-3-3 result but the MRI was positive in 8/9 cases. The isolated MV-2 case in the literature also had a negative CSF and EEG but positive MRI. However, this case may be included in the above analysis by Zerr et al.

Table 6.8: RESULTS OF INVESTIGATIONS IN STUDY BY ZERR et al.[455]

(n=108)

	PSWC in EEG (n)	14-3-3 Protein in CSF (n)	Positive MRI (n)
MM-1	(56/70) 80%	(67/70) 96%	(13/19) 68%
MM-2	(1/3) 33%	(3/3) 100%	(0/2) 0%
MV-1	(6/8) 75%	(8/8) 100%	(2/2) 100%
MV-2	(0/10) 0%	(3/10) 30%	(8/9) 89%
VV-1	(0/2) 0%	(1/1) 100%	(0/1) 0%
VV-2	(0/15) 0%	(15/15) 100%	(7/10) 70%

The isolated VV-1 case reported in the literature had a negative EEG and MRI and there is no comment made on CSF analysis. Zerr et al. found the only positive invesitigation in their VV-1 case was 14-3-3 protein. Three VV-2 cases reported by Tranchant had negative EEGs but there is no information about CSF or MRI analysis. Finally Kovacs et al. have reported 12 VV-2 cases. These are included in my analysis and so are not discussed further here.[433]

D: Pathology

Parchi et al. also noted specific pathological features in each group: The MM-1 and MV-1 groups showed the "classical" changes of sporadic CJD, with variable spongiform degeneration. The occipital lobes were most affected in 47% of cases. PrP staining tended to be "synaptic".

The VV-1 group tended to show severe spongiform change in the cerebral cortex and striatum, with relative sparing of the brainstem nuclei and cerebellum. PrP staining was in a faint synaptic pattern.

The MM-2 group was characterised by two phenotypes. The cortical group showed large confluent vacuoles with perivacuolar PrP staining in all cortical layers. There was little change in the cerebellum. The thalamic group had prominent atrophy of the thalamus and inferior olive with no spongiform change in these areas. 50% did not demonstrate PrP staining and it was extremely sparse in the other cases.

The MV-2 and VV-2 groups had prominent change in the limbic and subcortical grey matter with relative sparing of the cortex. Plaque-like deposits were present in the VV-2 group. Some areas showed coarser PrP staining as well as a prominent perineuronal pattern. In the MV-2 group the pattern was similar but there were definite kuru-type plaques well as plaque-like deposits.

Zerr et al. did not comment on the pathological changes in their series. Tranchant et al. found similar changes to Parchi et al. in their MM-1 and VV-2 cases. The single MV-1 case they had showed kuru-type plaques in the cerebellum unlike Parchi who only identified these changes in MV-2 cases. The pathological changes in the VV-1 case reported by Hillier et al.[440] were similar to Parchi's cases.

- Methionine homozygosity at codon 129 predisposes to sporadic and variant CJD.
- Valine homozygotes and heterozygotes at codon 129 tend to be of younger age at onset and the disease tends towards a longer duration.
- Genotype at codon 129 influences the pathological findings.
- Protein isotype also appears to influence the disease phenotype.
- Isotype 2 tends to be associated with an atypical form of the disease.
- There are 6 potential groups, related to genotype and isotype. There is some evidence that these groups can be distinguished by their clinical and pathological features.

CHAPTER 7: METHODS

7.1: BACKGROUND

The National CJD Surveillance Unit was established in the UK in 1990 in response to the recommendations of the Southwood Inquiry.[457] The unit has a responsibility for monitoring all cases of CJD throughout the UK. (Most cases seen are sporadic or variant CJD but the incidence and detail of iatrogenic and familial cases are also monitored.) Prior to this CJD had been studied in England and Wales retrospectively between 1970-1979[128,137] and prospectively from 1980-1984.[134,458] Further retrospective analysis has been performed in the intervening years until 1990.[117] Hence there is information available on most cases of CJD in the UK from the past 20 years.

The UK CJD Database consists of data from several sources:

- 1970-79: retrospective records based primarily on death certificate notification (England and Wales)
- 1980-84: prospective ascertainment of cases (England and Wales)
- 1985-April 1990: retrospective records (England, Scotland, Wales and Northern Ireland)
- May 1990-present: prospective ascertainment of cases (England, Scotland, Wales, Northern Ireland)

The NCJDSU relies on the referral of possible cases by physicians throughout the UK, Notifications are normally made by neurologists, but referrals are also received from psychiatrists, geriatricians and other specialties, including neurophysiologists.[459] In addition, cases identified at post mortem are referred by neuropathologists or other pathologists. Every six months a review is made of death certificates coded under the rubrics 046.1 and 331.5 in the 9th ICD revision. (This has recently been changed to A81.0 and F02.1 in the 10th ICD revision.) If possible

the clinical and pathological details are reviewed and if it is appropriate, relatives of the case will be interviewed. Evidence suggests that very few cases of CJD are missed when these multiple methods of cases ascertainment are used.[125,126]

7.2: PATIENT REFERRAL AND VISIT

Patient's studied in this project included several from the Oxford prospective study and the intervening retrospective analysis but the majority are from the ongoing UK surveillance project.

The majority of referrals to the NCJDSU are in-patients, made by the physician in charge of their care. In order not to miss atypical cases, physicians are encouraged to refer any suspected case as well as "typical" cases of CJD. If appropriate, and if the patient and their relatives agree, a research registrar and research nurse will travel to the hospital or patient's home. The patient (if cognitively able) and their relatives must be aware that a diagnosis of CJD is being considered.

During a visit the relatives will be asked to complete an epidemiological questionnaire with the aid of the research nurse. A research nurse has been in post since 1998. Prior to this, the research registrar completed the questionnaire. On occasion, if the nurse cannot attend, the research registrar will still complete the questionnaire. Ideally, for reasons of consistency in the ongoing case-control study, one member of the family answers the questions.

The questionnaire details the patient's past medical history including previous surgery, hospital attendances, drug history and vaccinations. If possible a list of previous residences, schools and jobs with dates, dietary history, family history and details of any exposure to animals is obtained.

The registrar examines the patient neurologically and a second questionnaire is filled out. This details the history of the patient's illness (with further information obtained

from the patient, if possible, and their relatives), examination on admission and at the time of visit, past medical and drug history and results of investigation. Where possible, the EEG and scans are reviewed. The patient is then classified according to current NCJDSU criteria. (See Appendices C and D)

It is not always possible to visit the patient. They may die before the team can make a visit or the family may not feel able to see members of the unit. In other cases a diagnosis is not made until post mortem or the unit may only become aware of the case after the biannual review of death certificates. If this occurs, where possible, the relatives are visited to obtain details of the clinical history and epidemiological details. The case notes will also be requested and reviewed. Sometimes it is not possible to contact a family or they do not feel able to see us, in which case if possible, the case notes are reviewed. Cases of familial and iatrogenic CJD are not generally visited.

In view of this, the available information on each of the reviewed cases in the study is variable. In most there will be a full epidemiological and clinical questionnaire as well as case notes, results including CSF analysis, EEG, MRI and genetic analysis. On a few, some or most of this will be missing. In addition the results have been collected at various intervals over the last 20 years, during which time the questionnaires and relevant information analysed have varied.

Since 1990, 740 cases of definite and probable CJD of all types have been referred to the NCJDSU. In total 1483 referrals of suspect CJD have been made up to 4/2/02. Only 151 cases have been included in this study as these are the cases on which full genetic and protein isotypic data is available. They have not been selected in any way other than because brain tissue was held and permission was available to use the tissue for research purposes. (Hence allowing protein isotyping.) It is possible that the cases used in this study were subject to selection bias and this is discussed later.

In each case, all the available notes have been searched to look for clinical information. An attempt has been made to obtain MRI, EEG, CSF and genetic analysis on each patient. In addition, a pathological report from the unit has been reviewed in most cases. In the remaining cases, an autopsy report from the referring hospital was available for review.

7.3: CLINICAL SYMPTOMS/ SIGNS

The information collected in the NCJDSU notes is based on the history obtained from relatives and perusal of the case notes at the time of the research registrar's visit. A decision about the presence or absence of a particular symptom or sign was made at the time of the registrar's visit and this could usually be confirmed by re-examining the case notes. In the remaining cases that were not seen in life, the clinical details were obtained from the available cases notes that varied from full in-patient notes to a discharge summary.

A decision was made about:

- Date of onset
- Duration of illness
- Presence of various symptoms and signs
- Timing of each symptom/ sign if present
- Presenting symptom

A: DATE OF ONSET

The date of onset was estimated from the clinical history, usually obtained from the relatives and hospital case notes at the time of the registrar visit. Occasionally the patient was able to give a history. In some cases, General Practitioner (GP) notes were available at a later date and the date of onset may have been altered at that time if further information became available.

If it was suggested that the symptoms began at the beginning or end of a month then the first or last day were used respectively. If it was not clear at which point in the month the illness began the 15th of the month was used. The timing was often difficult to establish and a "best guess" estimate from the first attendance at the patient's GP was often used.

B: DURATION OF ILLNESS

Clearly the date of death was more readily available, and from these two dates the duration of illness was calculated in months.

C: PRESENCE OF SYMPTOMS AND SIGNS

The presence and timing of clinical symptoms and signs was more difficult to obtain. For example, a patient may have been described as unsteady at onset, but there may be no reference to cerebellar signs in the case notes. By the time of examination the patient may have been bed bound and unable to co-operate. In this case it would be unclear whether the unsteady gait was apraxic, ataxic, extrapyramidal or other in nature. Clearly the description of the gait is also a matter of interpretation by the individual examining.

If a clinical sign has not been noted or has been stated as absent it has been designated "don't know". It cannot be assumed from review of case notes that a sign was truly absent if it is simply not mentioned as it may not have been recorded in error. Similarly, if for example cortical blindness was not present when the research registrar visited the patient, it is possible that cortical blindness developed at a time after the visit. For this reason only the definite presence of symptoms or signs has been used. The absence of a particular symptom or sign has not been analysed.

Certain terms have been interpreted as suggestive of the presence of a particular symptom or sign; e.g. "walking as if drunk" has been used to indicate the presence of cerebellar signs, but "unsteady" has not, because there are a number of different

causes of an unsteady gait. Clearly, in cases where there is some doubt a decision has to be made in a fairly arbitrary way about the nature of a particular symptom and sign. This was sometimes difficult but if there was sufficient doubt then the symptom was classified "don't know". The categories assessed and some of the terminologies used that were considered sufficiently indicative of a symptom or sign are detailed in Appendix A.

D: TIMING OF SYMPTOMS AND SIGNS

An attempt was made to time the onset of each symptom or sign from review of the history and case notes and their interpretation. If the patient was noted to be ataxic on admission and the clinical history suggested they were unsteady for two weeks prior to admission it has been assumed that onset of cerebellar symptoms was two weeks prior to admission. This is obviously not a terribly accurate assessment. Symptoms or signs have been designated as occurring at onset if they were the first symptom or sign to be reported. Otherwise, they were designated as occurring early, mid or late based on their timing in the first, second or third portion of the overall course of the illness. The best possible approximation of their timing was used.

E: PRESENTING SYMPTOM

A decision was made about the symptom at onset of the disease in each patient. This is because it is perhaps the most reliable symptom that is obtainable from the history. In addition it might give some information about the beginnings of the disease process within an individual. In most cases there was more than one symptom present. If the onset was monosymptomatic and purely cognitive, cerebellar or visual this was analysed separately.

The psychiatric symptoms in variant CJD are a prominent feature of the illness[222] and have been analysed in more detail than in sporadic, familial and iatrogenic CJD.

7.4: INVESTIGATIONS

The investigation of cases referred to the unit is at the discretion of the physicians in charge of the patient's care, although advice may be asked of the NCJDSU based on the unit's experience. For several reasons not all patients will have had an EEG, CSF analysis and MRI performed. The relevant results are documented at the time of a visit by the NCJDSU. In those cases notified after death, the case notes are requested and investigation results are documented at that time.

Where possible the EEG and MRI are viewed at the time of a visit and copies are requested. It has not been possible to obtain a copy of the MRI in all cases in which it has been performed for various reasons e.g. archive copies may have been destroyed, or we were unable to trace the films. In addition MRI was not widely available 10 years ago and was not performed in many of the earlier cases. Similarly, 14-3-3 protein analysis has only been used in the last few years and so has not been tested in all of the patients.

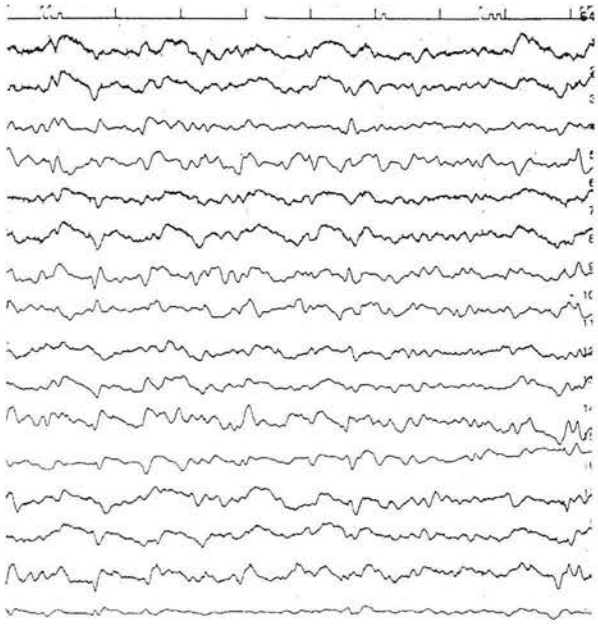
The following investigations were analysed in detail:

- EEG
- CSF analysis
- MRI
- Genetic Analysis

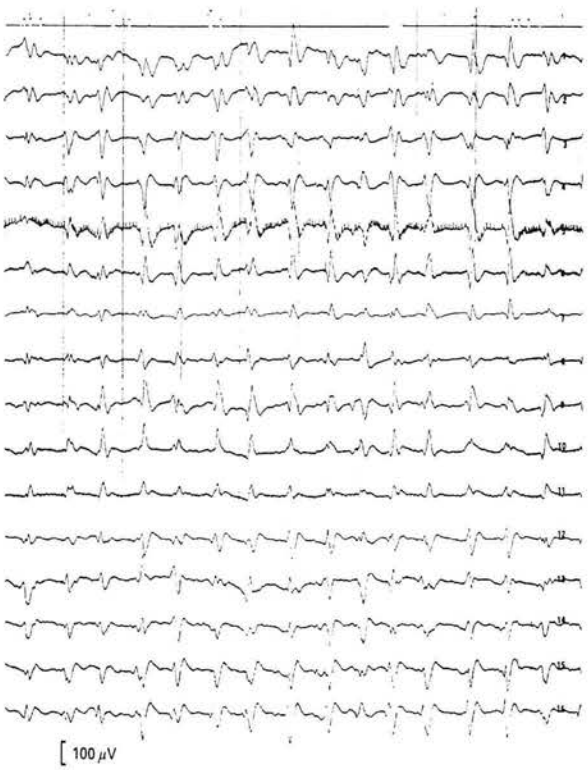
A: EEG

I reviewed all available EEGs myself and reclassified them for the purposes of this study. (See Appendix B) If there was no EEG available for review, the classification made by the research registrar at the time of the visit was used or failing that an interpretation of the EEG report was made. For statistical purposes a "highly suggestive" or "typical" EEG was classified as diagnostic. This is because in a

Figure 7.1: EXAMPLE OF A SUGGESTIVE AND TYPICAL EEG



Suggestive EEG



Typical EEG

clinical setting a "highly suggestive" EEG would carry much the same weight as a "typical" EEG in supporting a diagnosis of CJD. (See Figure 7.1)

It was not possible to do a blinded analysis of the EEGs.

B: CSF analysis

Dr Alison Green performed analysis of the CSF for brain specific proteins.[460] The methods are set out in Appendix E. (Some early cases were analysed in the USA by Dr Paul Brown before the test was fully validated.)

C: MRI

All MRIs were requested from the original hospitals if they were not already available. The planes and sequences of the scans were dependent on local radiological practice. Dr D Collie, consultant neuroradiologist at the Western General Hospital in Edinburgh, reviewed the scans. He was blinded to a diagnosis of sporadic or variant CJD or to an alternative diagnosis.

Each scan was assessed for the presence of high signal in the putamen, caudate nucleus and pulvinar and graded +, ++ or +++. For statistical purposes, a positive scan in sporadic or iatrogenic CJD showed high signal in the putamen and caudate nucleus graded ++ or +++, and a positive new variant scan had the same grade of changes in the pulvinar. A possible scan was noted in some sporadic CJD cases in whom + high signal was noted in the putamen and/ or the caudate. White matter changes and atrophy were also noted in the sporadic CJD scans.

D: GENETIC ANALYSIS

Blood is taken for genetic analysis at the time of a visit by the research registrar. This is only done if the patient or their relatives give informed consent. Matthew Bishop then performs analysis on the blood sample at the NCJDSU. Previously, Lisa

Strain and Kathy Estibeiro have performed genetic analysis. Prof J Collinge from the Prion Unit in St Marys Hospital, London has also provided results.

The deoxyribonucleic acid (DNA) was purified from peripheral blood leucocytes and amplified by polymerase chain reaction (PCR) using primers A2 and A3 (which are specific for the prion protein open reading frame (ORF)) and standard cycling conditions. Polyacrylamide gel electrophoresis was used for fragment separation and the product analysed for mutations.

The Met/Val codon 129 polymorphism was distinguished by digesting the PCR product with the restriction enzyme digest *Nsp*I.

7.5: PATHOLOGY

Preparation of brain tissue for pathological analysis is not always done at the NCJDSU. Tissue is received in a variety of ways. Professors Ironside or Bell may perform the entire autopsy or a fixed brain may be sent for preparation. Sometimes pre-prepared slides are sent for an opinion. Prof Ironside or Prof Bell report the prepared histology from whichever source.

3 separate processes are involved:

- Histopathology
- Immunocytochemistry
- PrP^{res} Isotyping

A: HISTOPATHOLOGY

For the purposes of this study the areas most affected by spongiform change and the degree of neuronal loss and gliosis were estimated. In addition, the presence of other abnormalities, such as kuru plaques, were noted. These details were taken from the

pathology report. Some cases were reviewed with Professor Ironside. The changes were not documented by any quantitative methods for the purposes of this study.

B: IMMUNOCYTOCHEMISTRY

Serial sections were also stained with immunocytochemistry using two anti-PrP antibodies and a consensus protocol. The pattern of PrP deposition was taken from the report and again other changes such as plaque deposits were noted. The results were not analysed by a quantitative method.

The pattern seen was described as:

Vacuolar: granular positivity around and between confluent vacuoles

Diffuse/ synaptic/ reticular: Generalised staining of the neuropil with small dot-like granular deposits of chromagen

Neuronal: pericellular punctuate or granular immunopositivity around unstained neuronal perikarya. Sparse intracellular punctate immunoreactivity

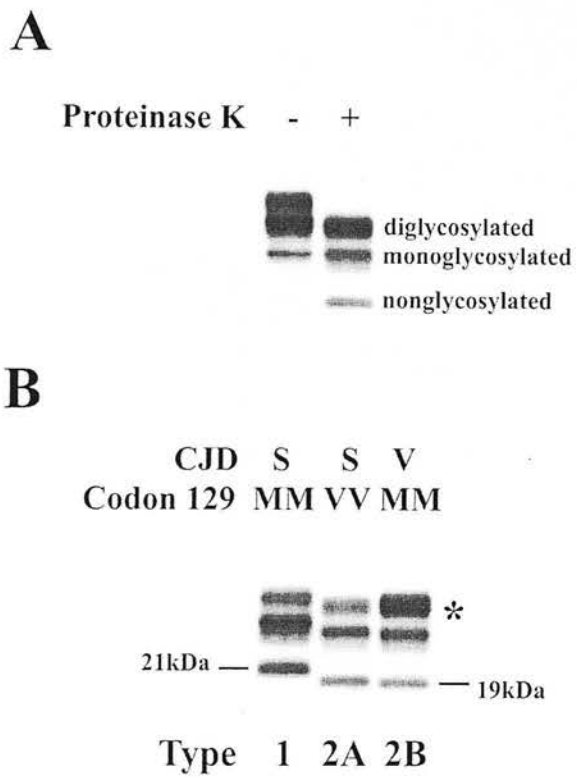
Immunoreactive granular deposits and plaque-like deposits: dense positivity in a granular or plaque-like formation not visible in H&E sections, occasionally surrounding a solid centre

C: PrP^{res} ISOTYPE

PrP^{res} isotyping was performed by Dr Mark Head and Tristran Bunn, with the help of Suzanne Lowry.

The results were classified according to electrophoretic mobility: Type 1 (21kDa) and Type 2 (19kDa). The proportion of the three glycoforms were assessed (di-, mono- and non-glycosylated PrP), further classifying each isotype as A or B. (See figure 7.2) Predominantly di-glycosylated PrP^{res} is present in type 2B, seen in variant CJD. Types 1 and 2A are seen in sporadic, familial and iatrogenic CJD. The glycosylation pattern is an estimate and not a qualitative result at this time although work is being done at the unit to quantify the ratio.

Figure 7.2: Examples of PrP Isotype 1, 2A and 2B



A: Western blot analysis of prion protein (PrP) in variant CJD brain tissue with (+) or without (-) prior digestion with proteinase K. Proteinase K results in complete degradation of PrP^{C} and in N-terminal truncation of PrP^{Sc}. The remaining protease-resistant PrP (PrP^{res}) occurs in di-, mono-, and non-glycosylated forms.

B: Western blot analysis of protease-resistant prion protein (PrP^{res}) in sporadic (S) and variant (V) CJD brain. Nonglycosylated PrP^{res} occurs as either a 21kDa band (termed type 1) or a more extensively truncated 19kDa band (termed type 2). Variant CJD exhibits a characteristic predominance of the diglycosylated band (*) and this protein isotype is termed type 2B to distinguish it from the type 2A isotype seen in sporadic CJD where the diglycosylated form does not predominate. The cases shown were homozygous for methionine (MM) or valine (VV) at codon 129 of the *PRNP* gene.

7.6: CLASSIFICATION AND DIAGNOSIS

When a member of the NCJDSU sees a patient a decision is made which type of CJD the case is most likely to fall into. Generally this relates to whether the case is sporadic or variant.

Secondly the case is classified on the likelihood that this is CJD, i.e. whether they would be defined as definite, probable or possible CJD or CJD is unlikely. The classification of sporadic CJD has changed slightly over the years of surveillance although it has remained broadly similar (see Appendix C). The most recently agreed criteria were used to classify each case during this assessment. (Rotterdam 1998). A classification of probable sporadic CJD carries approximately 95% specificity.

Classification criteria for vCJD have recently been published and validated.[153] (See Appendix D) To date the specificity of these criteria is 100% and the sensitivity has recently been reassessed using the up to date MRI data at the NCJDSU, giving a sensitivity of 92%. (Personal communication, NCJDSU)

CLASSIFICATION

A): Type of CJD	Sporadic
	Variant
	Familial
	Iatrogenic
B): Category	1.0 Definite
	2.0 Probable
	3.0 Possible
	4.1 CJD unlikely
	4.2 Not CJD- Patient improved or other clinical diagnosis
	4.3 Not CJD- Other pathological diagnosis

7.7: STATISTICAL ANALYSIS

Statistical analysis was performed by Dawn Everington. The clinical features and results of investigations were analysed using both parametric and non-parametric tests depending on distribution. Because of the different group sizes it was not always possible to perform a statistical analysis. In addition a large number of statistical tests were performed because of the large number of symptoms and signs considered. A correction should be used to take account of multiple testing in this context. However, this would mean using a p-value far less than the usual level of 5% and this has not been done. The relevant statistical test used is detailed with each set of results because different tests were used throughout.

CHAPTER 8: RESULTS

A total of 151 cases of CJD were included in the study. Full genetic, neuropathological and protein isotypic data were available on 99 sporadic CJD cases. There were a further 3 sporadic cases who were classified as intermediate and 6 sporadic cases who had been isotyped in another centre and classified differently. Genetic and isotypic data were also available on 43 variant cases. (See Table 8.1):

Table 8.1: BREAKDOWN OF 151 CASES

	TOTAL CASES	**of which**	
Sporadic	**108**	99	NCDSU classification
		3	Intermediate
		6	Alternative classification
Variant	**43**		

All but 4 of the 151 cases were from the current NCJDSU project. The timing of these cases span over the 10 year period of CJD surveillance in the UK. Two sporadic cases were from the retrospective study and 2 sporadic cases were from the Oxford study (1of intermediate mobility), data having been collected prior to 1990 retrospectively. This means that patients and relatives were interviewed by a succession of research fellows from the NCJDSU. I have seen approximately one third of the sporadic and variant CJD cases in this series.

I studied all cases in which full genotype and isotype data were available. They were therefore preselected and were chosen simply because the results were available for analysis. However, they would have been selected by the NCJDSU for various reasons: There would have been permission from the family for blood to be analysed for genetic mutations; In addition pathological material would have to be available

and permission would have been given for further analysis to be performed. It is possible that more atypical cases would have been selected by this process as clinicians and relatives may be more likely to proceed to post mortem if the diagnosis in life is uncertain. Furthermore if the pathological changes were atypical the case might be more likely to be referred to the NCJDSU for a second opinion, meaning material is available for future study. This potential bias is discussed later.

The statistics of the NCJDSU sporadic CJD cases collected since 1990 are as follows:

Median age at onset	65 years	(range 15-94)
Median age at death	67 years	(range 20-95)
Median duration of illness	4 months	(range 1-74)

Methionine homozygotes	67%
Heterozygotes	16%
Valine homozygotes	17%

This is similar to the statistics of the cases included in this study.

The NCJDSU data were also compared with data on cases that were isotyped according to a different classification, termed group B. The numbers of cases in each of these groups were too small to perform a statistical analysis and these cases are discussed in appendix F.

8.1: SPORADIC CJD: MAIN RESULTS

A: CASE DATA

83 (84%) of the 99 cases of sporadic CJD were examined in life by a member of the NCJDSU. The relatives were interviewed directly in 91 cases (92%) and the hospital notes were available for perusal in 68 cases (69%). All but 3 cases were from the current NCJDSU project. Of the remaining 3 cases, 1 VV-1 case was from the Oxford study and an MM-1 and MV-2A case were from the retrospective study.

The 99 cases of sporadic CJD were divided according to their *PRNP* codon 129 genotype:

	No cases	
MM	64	65%
MV	17	17%
VV	18	18%

The genotype at codon 129 was available in all of the cases, but full sequencing of *PRNP* had not been performed in 12 cases because consent was not available. The relatives had been interviewed in 10 of these cases, and there was no family history of dementia. In the others a family history was available from case notes and again there was no record of a dementing illness in the family history. In addition, the pathology in each case was not suggestive of hereditary CJD.

In one case there was a polymorphism at codon 117. This is a silent mutation and has not been shown to be disease associated, nor to influence disease phenotype. [451]

The distribution of the PrP^{27-30} isotype was as follows:

Type 1	62	63%
Type 2	37	37%

It was therefore possible to analyse six different groups, according to genotype and isotype:

Table 8.2: SPORADIC CJD: CLASSIFICATION OF CASES

		GENOTYPE: MM	MV	VV
ISOTYPE	1	**54 (55%)**	**5 (5%)**	**3 (3%)**
ISOTYPE	2A	**10 (10%)**	**12 (12%)**	**15 (15%)**

In general all statistical analyses were complicated by the fact that the MM-1 group was very large (54 cases) compared to all the other smaller groups. In the sections where it has been possible to make a meaningful analysis the statistical significance will be mentioned.

B: GENDER DISTRIBUTION

The ratio of males to females varied from 0.5:1 to 2:1, with a varying ratio in the 6 groups. (See Table 8.3) The overall ratio was 0.8:1 reflecting the general ratio seen in sporadic CJD.

Table 8.3: SPORADIC CJD: GENDER DISTRIBUTION

GROUP	MALES	FEMALES	RATIO M:F
MM-1	**24**	**30**	**0.8:1**
MM-2A	**4**	**6**	**0.7:1**
MV-1	**2**	**3**	**0.7:1**
MV-2A	**4**	**8**	**0.5:1**
VV-1	**2**	**1**	**2:1**
VV-2A	**8**	7	**1.1:1**
TOTAL	**44**	**55**	**0.8:1**

There was a slight excess of males in the valine homozygotes (M:F 1.1-2:1), whereas in all other groups there was a slight excess of females (M:F 0.5-0.8:1). This result did not achieve statistical significance.

C: AGE AT ONSET AND DEATH

Age at onset followed a normal distribution. The overall median age at onset and death was in the early 60s in the MM-1, MV- 2A and VV-2A groups, broadly similar to that reported in sporadic CJD. (See Table 8.4)

The Kolmogorov-Smirnoff test shows a significant difference between the groups ($p=0.044$). The VV-1 group had a significantly younger median age of onset at 41. However, there were only 3 cases in this group with a large range in age; the cases were aged 26, 41 and 78 at disease onset. The MM-2A group was also younger at onset, the median being 54 years. (This result was statistically significant when a pairwise comparison was made with the MM-1 group, $p=0.001$ and the MV-2A group, $p=0.009$.)

Table 8.4: SPORADIC CJD: AGE AT ONSET AND DEATH

	AGE AT ONSET (years)			AGE AT DEATH (years)		
	median	mean	range	median	mean	Range
MM-1	**64**	**65**	**43-86**	**65**	**66**	**44-86**
MM-2A	**54**	**54**	**39-72**	**55**	**56**	**41-72**
MV-1	**75**	**62**	**15-79**	**76**	**63**	**20-80**
MV-2A	**64**	**65**	**51-78**	**65**	**66**	**54-78**
VV-1	**41**	**48**	**26-78**	**42**	**49**	**28-78**
VV-2A	**66**	**63**	**46-79**	**64**	**63**	**47-80**

In contrast, the MV-1 cases had a median age of onset at 75 years, older than the other groups. Again this was a small group of only 5 cases with a range of 15-79 years at onset. However, the data is skewed by one very young case. The others were

aged 61, 75, 78 and 79 at onset, suggesting this group does predispose to an older age at onset.

The age at death followed a similar pattern to the age at onset and again, the VV-1 and MM-2A groups also had a younger median age at death (42 and 55 years respectively), and the MV-1 group had an older median age at death (76 years).

D: DISEASE DURATION

The MM-1, MV-1 and VV-2A groups were all of short duration, median 3 months (MM-1) to 5 months (MV-1, VV-2A). In the MM-1 group this was significantly lower than all other groups except for the MV-1 cases. (Mann-Whitney test, $p<0.001$-0.009). The VV-2A group were also of significantly shorter duration than the other groups except for the MM-1 and MV-1 cases (Mann-Whitney test, $p<0.001$-0.009) The duration of illness in the remaining groups was considerably longer, the median from 11 months in the MV-2A and VV-1 groups to 14 months in the MM-2A group. Again this was associated with a wide range in each group, with considerable overlap between the groups. (See Table 8.5)

Table 8.5: SPORADIC CJD: DISEASE DURATION

	Median (months)	Average (months)	Range (months)
MM-1	**3**	**4**	**1-17**
MM-2A	**14**	**17**	**8-37**
MV-1	**5**	**14**	**2-54**
MV-2A	**11**	**16**	**3-51**
VV-1	**11**	**16**	**8-29**
VV-2A	**5**	**5**	**2-11**

However, the distribution of disease duration did not follow a Normal distribution. Of the overall cases, 84% of cases were of duration 12 months or less, 10% of cases were of duration between 1 and 2 years, 3% were of duration between 2 and 3 years,

and the remaining 3% were of durations 37, 51 and 54 months. When the data were divided like this into each subgroup there were marked differences. (See Table 8.6)

The majority of MM-1 (87%) and VV-2A (80%) cases had durations of less than 6 months and nearly all of the cases in these groups had durations of illness less than 1 year (94% and 100% respectively). The MV-1 group may have a similar distribution: 4 of 5 cases (80%) of duration less than 1 year. However, 1 of these, of duration of only 2 months, died suddenly from a pulmonary embolus and the group also includes the case of longest duration. The number of cases is too small to draw firm conclusions.

Table 8.6: SPORADIC CJD: DISEASE DURATION BY SUBGROUPS

	n	<6 mths	%	<1 yr	%
MM-1	53	46	**87**	50	**94**
MM-2A	10	0	**0**	5	**50**
MV-1	5	3	**60**	4	**80**
MV-2A	12	1	**8**	6	**50**
VV-1	3	0	**0**	2	**67**
VV-2A	15	12	**80**	15	**100**

The MM-2A and MV-2A groups seem to have similar distributions to each other. No cases were of less than 6 months duration, 50% were of between 6 months and a year and the remaining longer than this. The VV-1 group may also follow this pattern but there are too few cases to be sure.

When the data are transformed by taking logs a Normal distribution is achieved (with the exception that the cases of duration 51 and 54 months are still outliers). A highly significant difference between the groups is seen ($p<0.001$). The MM-1 group has the shortest duration and differs significantly from the MM-2A, MV-2A and VV-1 groups. (Kolmogorov-Smirnoff test, $p<0.001$)

There seemed to be a significant correlation between duration and age of onset; the older ages tending to have a shorter duration of illness. However, scatter plots of the data showed that the significance of this result is largely because relatively few cases were of long duration. There were 6 cases with durations over 2 years, only one of whom was aged over 60 years. Of the cases with durations less than 2 years, 70% were aged over 60 years.

If the MM-1 group, by far the largest, is analysed there was no association between duration and age at onset. It was not possible to say whether this was a feature of the subgroup itself, or that this relationship does not apply to the cases with a shorter duration of illness. When an analysis of covariance was used to adjust for possible differences in age distribution between the groups, it was shown that the overall shorter duration of the MM-1 group was not due to the older ages within this group.

E: SYMPTOMS AND SIGNS

Each group was analysed for the overall presence of various symptoms and signs. The timing in relation to the onset of the illness was also assessed. As with the analysis of all the variables in this study, it was difficult to quantify the statistical significance of these results because of the relative sizes of each subgroup. However, the presence of rapidly progressive dementia, cerebellar or visual symptoms at onset was looked at to see if statistical significance was achieved.

These particular symptoms are of interest because of the well described Brownell-Oppenheimer (cerebellar symptoms at onset) and Heidenhain variants (visual symptoms at onset leading to cortical blindness) of sporadic CJD. In addition, the onset of the disease is probably the symptom that can be analysed most accurately in terms of timing and nature. It might give an indication of where the pathological process begins in the disease, which might in turn be influenced by protein isotype.

i): Rapidly Progressive Dementia

Dementia was present in 98 of 99 cases (99%). 1 of the MM-1 group was not clearly demented from examination of the notes. A rapidly progressive dementia was defined as a dementia with a progressive course in which a significant deterioration occurred over a year or less, leading to significant functional impairment. All the cases in whom dementia was present fulfilled these criteria, despite an illness of greater than 1 year's duration in several cases. The overall incidence of a rapidly progressive dementia was therefore also 99%. (See Table 8.7)

Table 8.7: SPORADIC CJD: PRESENCE OF RAPIDLY PROGRESSIVE DEMENTIA

	RPD present	%	Sole Presentation	%
MM-1	**53**	**98**	**23**	**43**
MM-2A	**10**	**100**	**8**	**80**
MV-1	**5**	**100**	**1**	**20**
MV-2A	**12**	**100**	**3**	**25**
VV-1	**3**	**100**	**3**	**100**
VV-2A	**15**	**100**	**3**	**20**

Dementia as the sole presenting symptom was also analysed. There was an excess of MM-2A cases with a purely dementing onset; 8 cases were observed but 4 were expected. (Fishers Exact test p=0.007.) All 3 of the VV-1 cases also presented with a pure dementia. The figures are so small in each group that this would not be considered a reliable analysis.

Table 8.8: SPORADIC CJD: TIMING OF DEMENTIA

	Early N=	%	Mid N=	%	Late N=	%	Not noted N=	%
MM-1	38	**70**	14	**26**	1	**2**	1	**2**
MM-2A	9	**90**	1	**10**	0	**0**	0	**0**
MV-1	5	**100**	0	**0**	0	**0**	0	**0**
MV-2A	6	**50**	6	**50**	0	**0**	0	**0**
VV-1	3	**100**	0	**0**	0	**0**	0	**0**
VV-2A	9	**60**	6	**40**	0	**0**	0	**0**

Dementia appeared early on in the illness in 70 cases (71%). There was a suggestion that it appeared less early on in the MV-2A and VV-2A groups. (See Table 8.8)

ii): Cerebellar symptoms and signs

Cerebellar problems were definitely present in 62 of all 99 cases (63%). They definitely occurred in 34 (63%) of the MM-1 group. (See Table 8.9) There were an additional 11 cases (20%) who probably had cerebellar signs but it was not possible to be sure. 2 of these cases may have been dyspraxic. The others were noted to be unsteady or un-coordinated but the underlying nature was not clear. In 22 cases (41%) the symptoms occurred within the first few weeks of the illness. In 11 cases (20%) the signs were first noted midway. 1 case (2%) did not develop signs until late on.

In the MM-2A group cerebellar symptoms were noted in 3 cases (30%) midway into the illness. A further 5 cases (50%) were noted to be unsteady or clumsy but there was no definite mention of cerebellar signs. 1 of these cases was thought to be apraxic but this was not clear and so the case was classified "don't know".

Table 8.9: SPORADIC CJD: CEREBELLAR SYMPTOMS AND SIGNS

	Cerebellar onset N=	%	Cerebellar present N=	%
MM-1	**7**	**13**	**34**	**63**
MM-2A	**0**	**0**	**3**	**30**
MV-1	**0**	**0**	**3**	**60**
MV-2A	**4**	**33**	**11**	**92**
VV-1	**0**	**0**	**0**	**0**
VV-2A	**3**	**20**	**11**	**79**

In the MV-1 group cerebellar symptoms were noted early on in 1 patient (20%) and after some weeks in 2 further cases (40%). The remaining 2 cases became unsteady early on but were classified "don't know" as there was insufficient information.

Table 8.10: SPORADIC CJD: TIMING OF CEREBELLAR SYMPTOMS

	Early N=	%	Mid N=	%	Late N=	%	Not noted N=	%	Don't know N=	%
MM-1	22	**41**	11	**20**	1	**2**	9	**17**	11	**20**
MM-2A	0	**0**	3	**30**	0	**0**	2	**20**	5	**50**
MV-1	1	**20**	2	**40**	0	**0**	0	**0**	2	**40**
MV-2A	10	**83**	1	**8**	0	**0**	0	**0**	1	**8**
VV-1	0	**0**	0	**0**	0	**0**	2	**67**	1	**33**
VV-2A	9	**60**	2	**13**	0	**0**	1	**7**	3	**20**

10 of the MV-2A group (83%) had cerebellar symptoms at onset or early on. 1 other case developed loss of balance at onset but was classified "don't know" as the cause was unclear. 1 further case (8 %) developed cerebellar symptoms and signs midway.

None of the VV-1 group had definite cerebellar changes. 1 case was unsteady after some months but the cause was unclear.

9 (60%) of the VV-2A group had definite early cerebellar problems. A further 3 were classified don't know and of these 2 were unsteady from the onset. 2 cases (13%) developed cerebellar problems after some months. (See Table 8.10)

A purely cerebellar onset was noted in 7 (13%) of MM-1, 4 (33%) of MV-2A and 3 (20%) of VV-2A. This might suggest the MV-2A group cases are more likely to develop cerebellar problems at onset but the result did not achieve statistical significance. However, analysing the presence of cerebellar signs or symptoms at any point during the illness, 92% of MV-2A cases did have cerebellar signs and symptoms at some point. This result achieved statistical significance. (Fishers exact test, p=0.009)

iii): Visual Symptoms and Signs

46 cases (46%) had visual symptoms at some point. An onset of illness with purely visual symptoms was present in 10 MM-1 cases (19%) and 1 VV-1 case (7%). 61% of the MM-1 group, 40% of each of the MM-2A, MV-1 and VV-2A groups and 1 MV-2A case (8%) developed visual symptoms at some point during the illness. MM-1 cases were shown to be statistically more likely to have visual symptoms (Fishers exact test, p=0.006) (See Table 8.11)

Visual symptoms were variable, ranging from burred vision, smearing across vision to diplopia. 20 (37%) of MM-1 cases complained of an upset in vision early on in the illness but 1 case of MM-2A and MV-1 (10% and 20% respectively) and 2 VV-2A cases (13%) also complained of symptoms early on. 11 cases (20%) of MM-1 complained midway and 3, 1, 1 and 4 cases of MM-2A (30%), MV-1 (20%), MV-2A (8%) and VV-2A (27%) respectively complained of symptoms as the illness progressed. 4 (2%) of MM-1 cases developed visual symptoms late on. (See Table 8.12)

Table 8.11: SPORADIC CJD: VISUAL SYMPTOMS

	Visual Present N=	%	Visual Only N=	%
MM-1	33	**61**	10	**19**
MM-2A	4	**40**	0	**0**
MV-1	2	**40**	0	**0**
MV-2A	1	**8**	0	**0**
VV-1	0	**0**	0	**0**
VV-2A	6	**40**	1	**7**

Cortical blindness was noted in 28 cases (52%). An additional 9 cases (17%) were classified don't know. 21 of the 28 cases (75%) had visual symptoms prior to developing cortical blindness and 7 (25%) did not. There was little difference in the number of cases developing cortical blindness between the groups. (See Table 8.13)

Table 8.12: SPORADIC CJD: TIMING OF VISUAL SYMPTOMS

	Early N=	%	Mid N=	%	Late N=	%	Not noted N=	%	Don't know N=	%
MM-1	20	**37**	11	**20**	2	**4**	0	**0**	21	**39**
MM-2A	1	**10**	3	**30**	0	**0**	6	**60**	0	**0**
MV-1	1	**20**	1	**20**	0	**0**	3	**60**	0	**0**
MV-2A	0	**0**	1	**8**	0	**0**	11	**92**	0	**0**
VV-1	0	**0**	0	**0**	0	**0**	3	**100**	0	**0**
VV-2A	2	**13**	4	**27**	0	**0**	9	**60**	0	**0**

The Heidenhain variant of sporadic CJD is characterised by early visual symptoms leading to cortical blindness, in association with a rapidly progressive dementia and other features of sporadic CJD. 11 MM-1 cases (20%) would fit with this description.

Table 8.13: SPORADIC CJD: CORTICAL BLINDNESS

	C. Blindness N=	%	Number who had visual symptoms N=	%	Dont know N=	%
MM-1	18	**33**	15	**83**	7	**13**
MM-2A	3	**30**	2	**67**	0	**0**
MV-1	2	**40**	1	**80**	0	**0**
MV-2A	1	**8**	0	**0**	1	**8**
VV-1	0	**0**	0	**0**	0	**0**
VV-2A	4	**27**	3	**75**	1	**7**

2 VV-2A cases (13%) complained of diplopia early in the illness (1 of these had a purely visual onset), long before dementia developed. Both were noted to have trouble with dizziness and coordination and 1 complained of visual blurring, blaming the problem on new spectacles. Both went on to develop cortical blindness, although it is not clear at what point in the illness this occurred. These cases might have been the Heidenhain variant but it is not certain.

Other cases complained of visual symptoms as the illness progressed and some of these went on to develop cortical blindness but this would not fall within the definition of the Heidenhain variant.

iv): Other Symptoms

All cases were examined for the presence of other symptoms. There were a large number of variables to consider and once again the odd sizes of each subgroup made statistical analysis of the results difficult. (See Table 8.14) As explained in Methods, it was difficult to know if symptoms and signs were absent or, for various reasons, not recorded in the notes. This means that there is likely to be an under-reporting of symptoms and signs. The definitions of each category are given in Appendix A.

The following symptoms and signs were looked at:

- Onset of illness
- Sensory symptoms
- Hearing
- Hallucinations
- Delusions

Table 8.14: SPORADIC CJD: OTHER SYMPTOMS

	P n=	%	Di n=	%	S n=	%	He n=	%	Ha n=	%	De n=	%
MM-1	14	**26**	12	**26**	7	**13**	1	**2**	15	**28**	2	**4**
MM-2A	2	**20**	0	**0**	3	**30**	0	**0**	1	**10**	2	**20**
MV-1	3	**60**	1	**20**	1	**20**	0	**0**	0	**0**	0	**0**
MV-2A	2	**17**	0	**0**	1	**8**	1	**8**	7	**58**	2	**17**
VV-1	1	**33**	0	**0**	0	**0**	0	**0**	0	**0**	1	**33**
VV-2A	5	**33**	7	**47**	2	**14**	1	**7**	6	**40**	1	**7**

P=prodrome; Di=dizzy; S=sensory; He=hearing; Ha=hallucinations; De=delusions

Onset of Illness

3 MM-1 cases (6%) had a sudden onset of illness, described as "stroke-like". There was a suggestion of a prodrome (see Appendix A) in 27 cases (27%). No particular subgroup was affected.

Dizziness was another common complaint early in the illness. 20 cases (20%) complained of dizziness at some point. Dizziness was present at onset in all the cases who complained of this symptoms except for 1 MM-1 and 1 VV-2A case. They are distributed as follows:

MM-1	12 cases	26%
MV-1	1 case	20%
VV-2A	7 cases	47%

Sensory Symptoms

14 cases (14%) complained of a sensory upset at some point. In 10 cases (10%) this was in the early stages of the disease. They are distributed as follows:

MM-1	7 cases	13%
MM-2A	3 cases	30%
MV-1	1 case	20%
MV-2A	1 case	8%
VV-2A	2 cases	14%

Of the MM-1 cases, 5 cases (9%) complained of a sensory upset at onset or early on in the illness. 2 cases (4%) developed the symptoms midway. 3 cases complained of a numb limb, 1 case complained of burning feet. 2 cases complained of tingling in a limb and 2 complained of cold feet. (1 case had both tingling in the right upper limb and cold feet).

Of the MM-2A cases, 2 cases (20%) were at onset. 1 case had a painful arm and the other complained of a sensation of "blood-boiling". 1 further case complained of non-specific back-pain and headaches some months into the illness. (She had a history of kyphosis and had a Harrington rod inserted as a teenager). 1 MV-1 case (20%) had paraesthesia in the fingers of the right hand at onset. 1 MV-2A case (8%) had paraesthesia in the legs early on in illness. There were no sensory symptoms in the VV-1 group. 2 VV-2A cases (14%) had sensory symptoms 1 complained of paraesthesia from onset (7%) and the other complained of pain in the arms and legs. This occurred early in the illness and was initially intermittent but became persistent.

Sensory features are a prominent symptom in vCJD, often occurring early in the illness. Because of this the cases were looked at to see if there was a bias towards asking about sensory symptoms after vCJD was described in 1996: 6 of the 14 cases (43%) were seen prior to 31/12/1995 (by which time it is assumed the NCJDSU registrar would have been aware of the symptoms of vCJD). The remaining 8 cases (57%) all died and were seen after 1/1/1996.

In addition, vCJD tends to occur in a younger age group. The cases seen before the end of 1995 had a median age at onset of 60 years (range 43-79), however, the cases seen after the end of 1995 had a median age of onset of 55 years (range 42-76). This does not support the theory that sensory symptoms might be looked for preferentially in the younger cases.

Hearing

A disturbance of hearing is unusual in CJD. Only 3 cases (3%) overall had symptoms involving hearing. 1 MM-1 case (2%) complained their hearing was "not right" in the early stages of their illness. 1 MV-2A case (8%) developed cortical deafness and 1 VV-2A case (7%) complained of a buzzing in the ears at the onset of their illness.

Hallucinations

29 cases (29%) complained of hallucinations. They are distributed as follows:

MM-1	15 cases	28%
MM-2A	1 case	10%
MV-2A	7 cases	58%
VV-2A	6 cases	40%

The hallucinations usually developed after some months of illness, except for 1 of the MV-2A cases, who had audio-visual hallucinations, developing early on in the illness. If the nature of the hallucinations were defined (21 of the 29 cases), they were nearly always visual in nature. 1 of the VV-2A cases had audio-visual hallucinations in addition to the MV-2A case already mentioned.

Delusions

8 of the cases (8%) developed delusions, distributed as follows:

MM-1	2 cases	4%
MM-2A	2 cases	20%
MV- 2A	2 cases	17%
VV-1	1 case	33%
VV-2A	1 case	7%

The delusions were persecutory or paranoid in nature in all the cases, usually developing after some weeks to months except for 1 MM-2A case who was paranoid from early on in their illness.

v): Other Signs

Many of the sporadic CJD cases were seen late on in their illness, by which time a number of clinical signs, both extrapyramidal and pyramidal were present. In addition the examination of a severely demented patient, who is unable to cooperate can be difficult.

The following signs were looked for in the case notes. (See Table 8.15)

- Pyramidal signs
- Lower motor neurone signs
- Extrapyramidal signs
- Primitive reflexes
- Myoclonus
- Other movement disorders
- Eye movements

Table 8.15: SPORADIC CJD: OTHER SIGNS

	P n=	%	E n=	%	PR n=	%	M n=	%	MD n=	%	EM n=	%
MM-1	32	**59**	18	**33**	38	**70**	51	**95**	23	**43**	13	**24**
MM-2A	5	**50**	3	**30**	8	**80**	9	**90**	2	**20**	1	**10**
MV-1	3	**60**	0	**0**	5	**100**	5	**100**	1	**20**	2	**40**
MV-2A	2	**17**	5	**42**	8	**67**	9	**75**	1	**8**	0	**0**
VV-1	1	**33**	1	**33**	3	**100**	3	**100**	2	**67**	0	**0**
VV-2A	9	**60**	6	**40**	10	**67**	13	**86**	4	**27**	6	**40**

P=pyramidal; E=extrapyramidal; PR=primitive reflexes; M=myoclonus;
MD=movement disorder; EM=eye movement disorder

Pyramidal signs

Pyramidal signs were noted in 52 cases (53%). There seemed to be a slightly lower percentage of MV-2As with pyramidal signs. (Only 2 cases (17%)). In general about half of each group had evidence of pyramidal tract abnormalities.

Lower motor neurone signs

Lower motor neurone signs were seen in 5 cases (5%) of whom 2 were MM-1 cases (6% of this group.) 1 case was diagnosed with motor neurone disease due to the presence of widespread fasciculations, reduced tone and reflexes. 1 case had a history of Guillain-Barré Syndrome and had absent reflexes.

3 of the VV-2A cases (20%) also had lower motor neurone signs: 1 case had lower motor neurone type muscle wasting, 1 had fasciculations and another had both muscle wasting and fasciculations. In general these changes were reported on by the local clinicians and the definition of lower motor neurone signs were not always strictly defined.

Extrapyramidal signs

Extrapyramidal abnormalities, i.e. bradykinesia, or tremor and rigidity of an extrapyramidal nature (including gegenhalten) were noted in 33 cases (33%). The distribution was approaching a third in each group apart from the MV-1 cases in whom extrapyramidal signs were not noted in any of the cases.

Primitive reflexes

Some or all of the various primitive reflexes (i.e. grasp, rooting, palmomental and pout reflexes) were noted in 72 cases (73%). They were noted in 67-100% of each group.

Myoclonus

86 of the 99 cases (87%) developed myoclonus. The majority of cases in each group developed this sign in the mid to late stages of the disease. 6 (11%) of the MM-1 cases and 1 (10%) of the MM-2A cases developed myoclonus in the early stages of the disease or at onset.

Other Movement Disorders

Some form of chorea or dystonia was noted in 33 cases (33%). This was usually described as a dystonic posturing of the upper limb or choreoathetoid movements of the trunk and limbs. A movement disorder was seen in 20-67% of each subgroup, except for the MV-2A group, in which only 1 case (8%) developed a dystonia. In 2 VV-2A cases and 1 MM-1 case facial grimacing was described. In 1 MV-1 case (20%) eyelid apraxia was noted.

Alien limb has been described as a presenting feature of sporadic CJD.[154,155] It was not always possible to be sure whether alien limb was present or not but it was definitely noted in 1 VV-2A case, and 2 MM-1 cases. It was not the presenting feature of any of these cases.

Eye Movements

22 cases (22%) were noted to have abnormalities of eye movement. In 65 cases (66%) however there was no mention of eye movements at all. Reduced upgaze or a general paucity of eye movements was noted in the majority of these cases (i.e. 6 VV-2A cases, 1 MM-2A case and 3 MM-1 cases). However, 2 MV-1 cases and 9 MM-1 cases were noted to have nystagmus. One other MM-1 case had jerky pursuit.

vi): Swallowing and Artificial Feeding

Swallowing abnormalities were noted in 38 cases (38%) in the terminal stages of the disease. Others could have developed problems after the research registrar saw them and this information could have failed to reach the NCJDSU. 1 MM-1 and 1 VV-2A

case developed swallowing problems early on but in the other cases, the problems did not seem to appear until late in the illness. There was no particular group with predominance or under representation of swallowing problems.

There was information on artificial feeding, either nasogastric (NG) or parenteral gastrostomy (PEG) in a limited number of cases. These cases were analysed in relation to their age and duration of illness. (See Table 8.16)

Table 8.16: SPORADIC CJD: NASOGASTRIC FEEDING

AGE AT ONSET (years)	DURATION OF ILLNESS (months)	GENOTYPE
60	15	MM-1
64	3	MM-1
72	17	MM-1
76	5	MM-1*
67	2	MM-1
63	2	MM-1
64	9	MM-1**
71	8	MM-1
62	3	MM-1
15	54	MV-1
54	6	VV-2A

* NG tube sited day before death

** Case notes discuss planned NG tube but no information whether definitely sited

Of the 11 cases in which there is definite information about parenteral feeding, 6 (55%) had an illness of duration greater than 6 months. The majority were MM-1 cases. Only 2 cases were aged less than 55 years. The 15-year-old case was seen 5 months into their illness and a PEG was inserted not long after. They survived in a state of akinetic mutism for 49 months after this. Obviously, swallowing problems are a reflection of bulbar problems and not always a sign of disease progression to near terminal stages.

vii): Akinetic Mutism

Many of the cases were seen before they reached a state of akinetic mutism. It was often not possible to establish whether or not patients went on to develop this state after the registrar had visited. Other cases were noted to be either mute or immobile but the term "akinetic mutism" was not strictly used. Akinetic mutism occurred in all of the subgroups with relatively similar frequencies. (See Table 8.17)

Table 8.17: SPORADIC CJD: AKINETIC MUTISM AND NG FEEDING

	Swallowing N=	%	NG/ PEG N=	%	Akinetic Mutism N=	%
MM-1	22	**41**	9	**17**	31	**57**
MM-2A	1	**10**	0	**0**	3	**30**
MV-1	1	**20**	1	**8**	2	**40**
MV-2A	3	**25**	0	**0**	5	**42**
VV-1	2	**67**	0	**0**	2	**67**
VV-2A	8	**53**	1	7	9	**60**

F: INVESTIGATIONS

The following investigation were reviewed:

- EEG
- CSF
- MRI

i): EEG

I reviewed the EEG in 61 of the 99 cases and reclassified the record according to the NCJDSU criteria set out in appendix B. 10 cases had 2 EEGs performed (4 MM-1, 1 MM-2A, 1 MV-1, 1 VV-1 and 3 VV-2A cases) and 3 cases had 3 EEGs (1 MM-1, 1 MM-2A and 1 VV-2A cases). In all of these cases, the final EEG or the final report (if the EEGs were not available for review) was used in the analysis. No one had a second or third EEG performed if they had an initial diagnostic or highly suggestive EEG. In all but 1 case in whom more than one EEG was performed, there was some

progression of the EEG (although not necessarily a change in classification). In 1 MV-1 case the first EEG done at 3 months was suggestive and the second, done 19 days later, was non-specific.

The EEG was classified as typical, highly suggestive, suggestive or non-specific. Of the EEGs in the 41 MM-1 cases seen by me, the classification would be different according to the criteria used in this study in 13 cases. 10 would be classified as highly suggestive (originally classified as typical) and 3 would be classified as suggestive (originally typical).

In the other groups I reviewed the EEG in 20 further cases. (6 MM-2A, 3 MV-1, 3 MV-2A, 2 VV-1 and 6 VV-2A cases.) In these groups I agreed with the classification given at the time of review.

In 31 cases the EEG was not seen by me but reported locally or by the person who visited the patient: 10 MM-1, 3 MM-2A, 1 MV-1, 7 MV-2A, 1 VV-1 and 9 VV-2A cases. 4 of the MM-1 cases were reported as typical. All other cases were reported as non-specific or suggestive. The classification of these EEGs is unlikely to have affected the overall classification of the patient. However, in 3 cases the EEGs seen at the time of the NCJDSU visit were classified as typical, but were reclassified suggestive by me. It is therefore possible that 1 or 2 cases might be over classified. It is unlikely that any of the cases were under classified, as I did not increase the classification in any case.

Six cases apparently did not have an EEG performed, or at least there was no record of an EEG result in the NCJDSU case notes.

Taking the final EEG in each case. The EEG was reported as highly suggestive or typical in 33 cases (33%). 32 cases were MM-1 (59%) and 1 case was an MV-1 (20%). The EEG was reported as suggestive or non-specific in 60 cases (60%). This

included 20 (37%) of MM-1 cases, 3 (60%) of MV-1 cases, and in all other cases in whom an EEG was performed. A diagnostic EEG was therefore significantly associated with the MM-1 group. (chi square test, $p<0.001$)

Table 8.18: SPORADIC CJD: EEG RESULTS

	T n=	%	HS n=	%	S n=	%	NS n=	%	ND n=	%	N n=	%
MM-1	13	**24**	19	**35**	14	**26**	5	**9**	3	**6**	10	**19**
MM-2A	0	**0**	0	**0**	5	**50**	5	**50**	0	**0**	3	**30**
MV-1	0	**0**	1	**20**	1	**20**	2	**40**	1	**20**	1	**20**
MV-2A	0	**0**	0	**0**	4	**33**	6	**50**	2	**17**	7	**58**
VV-1	0	**0**	0	**0**	1	**33**	2	**67**	0	**0**	1	**33**
VV-2A	0	**0**	0	**0**	1	**7**	14	**83**	0	**0**	9	**60**

T=typical; HS=highly suggestive; S=suggestive; NS=non-specific; ND=not done: N=not seen

The MV-1 case with a highly suggestive EEG was done 3 months into an illness of 5 months duration. The MM-1 cases were done a median of 2 months (range 1-10 months) into an illness of median duration 3 months (range 1-17 months).

ii): CSF

The CSF was often reported as normal but the exact values were not necessarily given. In addition the laboratory upper limit of normal was not always specified. An arbitrary protein level of 0.7 mg/L was regarded as significantly raised in the absence of greater than 5 red cells. A white cell count of greater than 5 was also considered as abnormal.

Of the MM-1 cases, the CSF results were missing or not analysed in 15 cases. In 4 cases the protein was raised (0.72, 0.8, 0.78, 1.22 mg/L) with no note of a raised red cell count. The white cell count was not raised in any cases without a significantly raised red cell count. In the other groups, 10 cases had no available result or the CSF

was not analysed. In all the other cases the CSF results were within the above parameters.

14-3-3 and S-100b

14-3-3 and S-100b was tested in 31 cases. (See Table 8.19) In 2 of these S-100b was not tested: 1 MV-1 and 1 VV-2A case. In total the 14-3-3 protein was positive in 21 cases (68%). There was a trace positive in 2 cases and the fluid was heavily bloodstained in 2 cases. The result was negative in 6 cases (19%).

The positive results were performed a median of 3 months (1-11) in an illness of median duration 5 months (2-11). The equivocal results were performed a median of 3.5 months (3-4) into an illness of median duration 6.5 months (5-9), and the negative results were performed a median of 12.5 months (7-25) into an illness of median duration 19 months (8-54).

Table 8.19: SPORADIC CJD: 14-3-3 AND S-100b RESULTS

		14-3-3						**S-100b**			
	N	+ve	%	-ve	%	+/-	%	inc	%	norm	%
MM-1	15	12	**80**	0	**0**	3	**20**	14	**93**	0	**0**
MM-2A	4	1	**25**	3	**75**	0	**0**	3	**75**	1	**25**
MV-1	3	1	**33**	1	**33**	1	**33**	1	**33**	1	**33**
MV-2A	1	0	**0**	1	**100**	0	**0**	1	**100**	0	**0**
VV-1	1	0	**0**	1	**100**	0	**0**	1	**100**	0	**0**
VV-2A	7	7	**100**	0	**0**	0	**0**	6	**86**	0	**0**

+ve= positive; -ve= negative; +/- = equivocal; inc=increased; norm=normal

12 of the MM-1 cases had a positive 14-3-3 (80%). Of the 3 equivocal cases 2 were bloodstained, 1 so heavily that S-100b could not be analysed either, and 1 showed a trace of 14-3-3. All were associated with a raised S-100b.The positive results were performed a median of 2 months (1-5) into an illness of median duration 3 months (2-10). The equivocal results were performed at a median of 3 months (3-4) into an illness of duration 5 months (5-9).

All 7 of the VV-2A group that were assessed (100%) had a positive 14-3-3. 6 were for S-100b and it was raised in each case. The test was done at a median of 5 months (1-11) into an illness of median duration 6 months (4-11).

In the remaining groups very few cases were tested. One (25%) of the MM-2A group had a positive 14-3-3 (performed at 6 months into an illness of 8 months duration) and 3 had a raised S-100b. 3 of the MM-2A cases (75%) were negative, tested for at a median of 17 months (8-14) into an illness of median duration 17 months (16-21).

One case (33%) of the MV-1 group was positive, associated with a raised S-100b (performed at 3 months into an illness of 5 months duration), and another showed a trace of 14-3-3 protein, the S-100b was normal (performed at 4 months into an illness of 8 months). One case had a negative result, performed at 25 months into an illness of 54 months.

Both the MV-2A and VV-1 groups had 1 case tested. In both cases the result was negative with a raised S-100b. The MV-2A case was tested at 7 months in an illness of 8 months and the VV-1 case was tested at 16 months in an illness of 28 months duration.

The S-100b was only raised if the 14-3-3 was positive in all groups apart from the MV-2A and VV-1 cases, in which there was a raised S-100b in the absence of a 14-3-3 result.

iii): MRI

The MRI was available for analysis at the NCJDSU in 44 cases. Fourteen of these (32%) were classified positive. Fifteen (34%) were classified possible (i.e. mild high signal in the caudate and/or the putamen), and the remaining 15 scans (34%) were negative. The high signal seen in the caudate nucleus and putamen was at times

asymmetrical. One case had a positive scan reported locally but the changes in the putamen and caudate were not reported in any of the other cases. (See Table 8.20)

Overall, the timing of the scans was:

Positive	3 months (1-22)	illness duration 5.5 months (3-29)
Possible	3 months (1-19)	illness duration 5 months (2-54)
Negative	3 months (1-18)	illness duration 5 months (1-37)

Table 8.20: SPORADIC CJD: MRI RESULTS

	N=	positive	%	possible	%	negative	%
MM-1	22	6	**27**	9	**41**	7	**32**
MM-2A	6	1	**17**	1	**17**	4	**66**
MV-1	2	0	**0**	2	**100**	0	**0**
MV-2A	4	3	**75**	0	**0**	1	**25**
VV-1	2	1	**50**	1	**50**	0	**0**
VV-2A	8	3	**38**	2	**25**	3	**37**

A scan was available for review in 22 MM-1 cases:

Scan result	N=	%	Median date (months)	Median duration (months)
Positive	6	27	2 (1-4)	3.5 (3-4)
Possible	9	41	2 (1-6)	5 (2-10)
Negative	7	32	2 (1-7)	3 (1-9)

Other abnormalities included white and grey matter high signal in 9 cases. Atrophy was noted in 14 cases. The pulvinar had some slight high signal in 2 cases. One scan was normal. Only 1 of these scans was reported as positive by another centre. (Reported in 1998. The Finkenstaedt review of the MRI in sporadic CJD was published in 1996.[8])

In 25 cases no scan was performed or there was no report available and we were unable to obtain a copy of the scan. The remaining 7 cases had a scan but were not reviewed by us. Of these 3 had non-specific white matter changes or atrophy, 1 had a mucus retention cyst, 1 had a porencephalic cyst and 2 were reported normal.

Six MM-2A cases had a scan available for review by the NCJDSU:

Scan result	N=	%	Median date (months)	Median duration (months)
Positive	1	17	12	21
Possible	1	17	4	16
Negative	4	66	6 (2-18)	14 (8-37)

Other abnormalities noted included mild atrophy in 3 cases. Two cases had high signal within the cortex. One scan was considered normal. Of the remaining MM-2A cases in whom the scan was not seen by us, 2 scans were normal and 1 showed some atrophy. The final case had no scan performed.

Two MV-1 cases had a scan available for review. Both had slight change in the putamen and would be considered possible. They were performed after 3 and 19 months of illness. The illness duration was 8 and 54 months respectively. Other changes included atrophy in both and 1 had some white matter changes. One further scan, not see by us, was reported as normal and 2 other cases did not have a scan performed.

In 4 of the MV-2A cases we were able to review a scan. Three of these (75%) had a positive scan. The final scan was the wrong weighting and so could not be assessed. (The scan was performed at 2 months in an illness of duration of 3 months.) In the other cases the median time to scanning was 5 months (range 3-11 months). The median illness duration was 9 months (range 7-21 months).

Other changes included atrophy in all 3 cases, high signal in the cortex in 1 case and in the grey matter in another. Two cases also had some high signal in the pulvinar. No scan was performed in 6 cases. In the remaining 2 cases, in whom we could review the radiology report, 1 had ischaemic changes and 1 was atrophic.

Of the 2 VV-1 cases who had a scan available 1 was strongly positive at 22 months, (duration of illness 29 months) and 1 would be considered possible after 1 month, (duration of illness 11 months). Both had atrophic changes. The final case had no scan performed.

Eight VV-2A cases had a scan performed:

Scan report	N=	%	Median date (months)	Median duration (months)
Positive	3	37	3 (3-5)	6 (5-11)
Possible	2	25	3 (1-5)	4 (2-6)
Negative	3	37	4 (1-5)	7 (5-7)

Additionally, atrophy was seen in 2 cases and 1 case had slight signal change in the pulvinar. Two cases had normal scans. Of the remaining cases a scan report was available in 3: 2 had atrophic change and 1 had minor ischaemic change.

The scan was performed more than once in 7 cases. In 4 of these both scans were negative. (2 MM-1, 1 MM-2A and 1 MV-1 case) In 2 other cases (1 MM-1 and 1 VV-2A) the scan was positive on both occasions. Finally, in 1 VV-1 case the first scan at 10 months was negative, but the second scan, performed 1 year later, 22 months into the illness was positive.

G: PATHOLOGY

i): Histopathology (See Table 8.21)

MM-1: In general there was microvacuolar and areas of confluent spongiform change throughout the cortex in all cases. The changes were noted to be patchy in 2

cases. In 33 cases the occipital lobe was noted to be one of the areas most affected. In 10 cases the temporal lobe was particularly affected, in 6 cases the parietal lobe and in 9 the frontal lobe. Two cases commented on marked involvement throughout the cortex. (Some cases had marked involvement in more than 1 lobe.)

Five cases had areas of status spongiosis. The median duration of illness in these cases was 15 months (1-17 months), in comparison to the overall median duration of illness of 3 months (1-17 months) of the MM-1 group as a whole. An MRI was available for review in only 1 of these cases, in whom widespread severe atrophy was noted.

In general, involvement of the hippocampus was patchy if present at all. Involvement of the basal ganglia was patchy in 4 cases but otherwise tended to mirror the spongiform change of the cortex. In 2 cases the basal ganglia changes were more marked than in the cortex. 1 of these cases had marked dystonic posturing during the illness. The other was a classical Heidenhain variant. Gegenhalten was noted but no other extrapyramidal features. In addition this case was the only MM-1 with marked thalamic spongiform change. There were no particular thalamic features to the illness. Thalamic involvement in most cases was generally less marked and was patchy in 23 cases.

These findings were similar in the cerebellum where 30 cases were noted to have patchy involvement, although 1 case was noted to have the most marked spongiform change in the cerebellum. Brainstem involvement was only commented on in 12 cases and was invariably mild and patchy.

There were no amyloid plaques in any case. Gliosis and neuronal loss was prominent in the cortex and often the cerebellum and basal ganglia. In 4 cases the thalamus had prominent gliosis and neuronal loss, in 2 cases this was the most striking area.

Other changes noted included age-related changes or evidence of arteriosclerosis in 20 cases. Three cases had β amyloid plaques and the changes of Alzheimer's disease. One case had a calcified 4th ventricle meningioma.

MM-2A: In 1 case only a frontal lobe biopsy was available. This merely showed spongiform change. In the remaining cases histology was available from all areas of the brain. Spongiform change was present throughout the cortex. In 5 cases this was reported as confluent. In 2 other cases it was patchy. The frontal and/or temporal lobes were the most heavily involved areas in 6 patients. In 3 patients the occipital lobes were heavily involved and in 1 patient the parietal lobe showed marked change.

Spongiform change was present in the basal ganglia in all 9 cases, in general the changes were less marked than in the cortex and particularly so in the thalamus. However, in 1 patient the basal ganglia showed marked change. Spongiform change was present in the cerebellum but to a lesser extent and in 8 cases it was patchy. Three cases were noted to have slight involvement of the brainstem.

In 5 patients neuronal loss and gliosis was present throughout the cortex, concentrated usually in the area of most marked spongiform change. One case had marked neuronal loss and gliosis in the thalamus and stem. In this case spongiform change was patchy except in the temporal cortex. There were no amyloid plaques seen.

One case was noted to have an incidental small vascular malformation.

MV-1: All 5 cases had tissue available for examination. One case of 54 months duration showed the changes of status spongiosis. The remaining 4 cases had spongiform change throughout the cortex. (3 mainly in the occipital lobes, 3 in the frontal lobes, 2 in the temporal lobes and 1 in the parietal lobe; most cases with more

than 1 area overlapping.) In 1 case this was microvacuolar, in another it was confluent and in another it was both. Spongiform change was present in the basal ganglia, thalamus and cerebellum but here the changes were in general patchier. Gliosis was present in areas of the cortex in 4 cases and in the cerebellum in 3 cases. Neuronal loss was roughly similar but was also present in areas of the deep grey matter in 3 cases. Kuru-like plaques were noted in the cerebellum in 1 case prior to staining.

Two cases were noted to have the changes of arteriosclerosis in the brain.

MV-2A: The spongiform change was mild to moderate throughout the cortex in all 12 cases. The change was confluent in 2 cases and there were changes in the hippocampus in 8 cases. Spongiform change was more marked in the basal ganglia than in the cortex in 9 cases and in general the changes were patchier in the thalamus. There was no excess of extrapyramidal signs in the MV-2A cases. One case had marked spongiform change in the hypothalamus. The changes in the cerebellum were patchy in 4 cases and in the remaining cases there was generally less marked spongiform change in the cerebellum. The brainstem was involved in 10 cases but only slightly.

Gliosis and neuronal loss was most marked in the cortex in 7 cases. In 2 other cases both the cortex and the cerebellum were involved. In 1 case the cerebellum was severely involved. In 2 cases the caudate and putamen changes were the most marked. 5 cases had kuru type plaques in the cerebellum and cortex, and in a further case kuru type plaques were also seen in the hippocampus. In 3 cases the plaques were predominantly in the cerebellum. Three cases had no plaques.

Three cases had age related senile plaques in the hippocampus and another had β-amyloid plaques and Hirano bodies in the hippocampus. One case had the changes of

moderately severe arteriosclerosis and another had pallidal siderosis and lacunar infarcts.

VV-1: There were only 2 cases with histology available in this group. One case, whose illness duration was 11 months, had changes of status spongiosis throughout the cortex and basal ganglia. In the other case spongiform changes were present throughout the cortex but were most marked in the basal ganglia. The changes were patchy in the thalamus and cerebellum in both cases. There was neuronal loss and gliosis in the cortex in both cases and also in the basal ganglia in the case with status spongiosis.

VV-2A: Spongiform change was present throughout the cortex in all cases. The changes were patchy in 3 cases. In all but 1 case the changes were more marked in the basal ganglia. The final case had only patchy spongiform change in the basal ganglia. In general the changes in the thalamus and cerebellum were less marked. 9 of the cases were noted to have slight patchy spongiform change in the brainstem. Neuronal loss and gliosis was variable and present in most areas. No cases had any amyloid plaques.

Table 8.21: SPORADIC CJD: PATTERN OF SPONGIFORM CHANGE

	CORTEX	BASAL GANGLIA	THALAMUS	CEREBELLUM	AMYLOID PLAQUES
MM-1	Occipital	Some patchy	Patchy	Patchy	No
MM-2A	Frontal/temporal	Present	Patchy	Patchy	No
MV-1	Frontal/occipital	Present	Patchy	Patchy	Present
MV-2A	Present	Most marked	Patchy	Patchy	Present+
VV-1	Present	Most marked	Patchy	Patchy	No
VV-2A	Present	Most marked	Patchy	Patchy	No

ii): Immunohistochemistry (See Table 8.22)

MM-1: Immunohistochemistry was not done in 1 case. In the other cases there was a reticular and perivacuolar deposition of PrP. In occasional cases there were granular or perineuronal deposits. In 29 cases the occipital lobe was again noted to have the most marked involvement. A further 3 cases were noted to have more marked involvement of the frontal lobe, 6 in the temporal lobe, 1 in the parietal lobe and 1 throughout the cortex. (Again, some cases had marked involvement of more than 1 lobe.) These changes were mirrored in the basal ganglia but tended to be more reticular in the thalamus and often less marked. A few cases had a perivacuolar deposition in the thalamus.

Deposits in the cerebellum also tended to be reticular but more granular in the granular layer. Eight cases had some perivacuolar deposits and 1 case had some perineuronal deposition. In 27 cases slight patchy, predominantly reticular deposition was seen in the brainstem.

MM-2A: Immunohistochemistry was not performed in the frontal biopsy case. In the remaining 9 cases deposition in the cortex was perivacuolar and synaptic, usually concentrated in the area of greatest spongiform change: 2 with changes most marked in the occipital lobe, 1 in the frontal lobe, 1 in the frontal and temporal lobes and 2 throughout the cortex. One case also had perineuronal deposition. In the basal ganglia and thalamus, deposition was synaptic and perivacuolar, again 1 case also had some perineuronal deposits. In the cerebellum, 2 cases had perivacuolar and plaque-like deposits. One case had some focal deposition of plaques. Two other cases had some synaptic and focal deposits. The brainstem had patchy involvement noted in 1 case.

Three of the MM-2A cases were remarked on for their unusual pathology. One had change of the thalamic variant, 1 had unusual PrP deposition in the occipital and temporal cortex and in 1 case the pathology was similar to GSS.
MV-1: Immunohistochemistry was done in all cases. Again the staining pattern usually mirrored the areas of greatest spongiform change. 4 cases had marked involvement of the occipital lobe, 2 the frontal lobes and 1 the temporal lobe, again more than 1 area was involved in each case. In the cortex, synaptic deposits were present in 4 cases with additional perivacuolar changes in 2 of the cases. Two cases had plaque-like deposits. The changes elsewhere tended to be more synaptic in the deep grey nuclei and cerebellum, with the same 2 cases showing plaque-like deposits throughout. One case also had plaque-like deposits in the brainstem.

MV-2A: Immunohistochemistry was performed in all cases. PrP was deposited in the cortex in a variable reticular, perivacuolar and perineuronal pattern. 8 cases had plaque-like deposits or plaques. The pattern was similar in the basal ganglia, thalamus and cerebellum although the pattern tended to be more reticular and 10 cases had plaques or plaque-like deposits in this area. These were particularly marked in the cerebellum. Most cases had reticular deposits in the brainstem although these were less marked and plaques were deposited in 5 cases. One case was noted to have an unusual GSS type of staining.

VV-1: Immunohistochemistry for PrP was present in a variable reticular, perivacuolar and perineuronal pattern in the cortex, basal ganglia and cerebellum. Deposition was also present in the thalamus in the case with status spongiosis.

VV-2A: Immunohistochemistry showed a predominantly linear and perineuronal deposition of PrP in the cortex. Eleven of the 15 cases had plaque-like deposits. Five of the cases had greatest involvement in the temporal lobes, 3 in the frontal lobes and 2 in the occipital lobes (with overlap). The hippocampus was the area of greatest involvement in 3 cases.

The changes in the basal ganglia were generally more reticular with a perivacuolar pattern also present. Plaque-like deposits were present in 7 cases. In the thalamus the changes tended to be reticular with plaque-like deposits in 8 cases. Plaque-like deposits were present in the granular layer of the cerebellum in all 15 cases and reticular deposition was present in the molecular layer. Seven cases were noted to have some reticular deposition in the brainstem.

Table 8.22: SPORADIC CJD: PATTERN OF IMMUNOHISTOCHEMISTRY

	Cortex	Basal Ganglia	Cerebellum	Plaques
MM-1	Reticular	Reticular	Patchy	No
MM-2A	Synaptic/ Perivacuolar throughout			No
MV-1	Synaptic	Synaptic	Synaptic	Plaque-like
MV-2A	Reticular	Reticular	Reticular	Plaques+
VV-1	Reticular	Reticular	Reticular	No
VV-2A	Linear	Reticular	Plaque-like	Plaque-like

H: CLASSIFICATION

Each case was reclassified by me and given the highest possible classification in life (without neuropathological information) according to the most recent criteria. (See Appendix C).

i): Probable cases

An EEG result of "typical" or "highly suggestive" was used to indicate a probable case. In all, 46 cases (49%) were classified probable. Of these, 37 (69%) belonged to the MM-1 group. Seven VV-2As (47%) were also classified as probable. Only 1 MM-2A and 1 MV-2A would have been probable cases in life. (See Table 8.23)

Table 8.23:SPORADIC CJD: DIAGNOSTIC CRITERIA

	PROBABLE	%	POSSIBLE	%	CJD unlikely	%
MM-1	37	**69**	14	**26**	3	**6**
MM-2A	1	**10**	7	**70**	2	**20**
MV-1	1	**20**	3	**60**	1	**20**
MV-2A	0	**0**	9	**75**	3	**25**
VV-1	0	**0**	1	**33**	2	**67**
VV-2A	7	**47**	7	**47**	1	**6**

ii): Possible cases

Possible cases fulfilled the clinical criteria for sporadic CJD but did not have supportive investigations, i.e. the EEG was not typical or the 14-3-3 protein result was negative. Their results were as follows:

In the MM-1 group 10 cases were "possible".

No EEG/ 14-3-3	1 case
EEG non-specific/ no 14-3-3	2 cases
EEG suggestive/ no 14-3-3	10 cases
EEG suggestive / 14-3-3 trace	1 case.

In the cases with a suggestive EEG the record was performed at a median of 4 months (range 2-4 months). The non-specific EEG records were performed at 1 month. One of these cases had a positive MRI result.

Seven MM-2A cases were classified as "possible":

4 had a non-specific EEG performed at 6, 7, 9 and 14 months.

3 had a suggestive EEG performed at 4,12 and 12 months.

The 14-3-3 was not performed in 4 cases and in the remaining 3 cases it was negative. None had a positive MRI.

Three of the MV-1 group were classified "possible":

1 case did not have an EEG or 14-3-3 performed.

1 case had a suggestive EEG after 1 month and no 14-3-3 performed.

1 case had a non-specific EEG at 4 months and a trace of positive 14-3-3 protein.

The MRI was negative in all these cases.

Nine of the MV-2A cases were classified as "possible":

1 case did not have an EEG or 14-3-3 performed.

4 had a non-specific EEG at 3, 5, 11 and 19 months.

4 had a suggestive EEG at 2, 9, 12 and 13 months.

14-3-3 was not analysed in all 9 cases.

2 of these cases had a positive MRI scan.

Only 1 VV-1 was a "possible" case:

The EEG was suggestive at 6 months and 14-3-3 was not analysed. No MRI imaging was performed.

Seven of the VV-2A group were classified as "possible" cases:

In all the cases the EEG was non-specific, performed a median of 3 months (range 1-12 months).

14-3-3 was not analysed in any of these cases.

None of the cases had a positive MRI scan, although 1 case had some very slight high signal in the caudate nucleus.

iii): CJD Unlikely

Fourteen cases were given a classification of "CJD unlikely". This included only 3 (6%) of the MM-1 cases and 1 (6%) of the VV-2A cases. Their details follow.

MM-1 cases:

Patient 1: 69-year-old male whose illness lasted 2 months. A member of the NCJDSU did not see the patient in life and the relatives were not interviewed. There was no note made of a dementing process in the case records. There were cerebellar features but also lower motor neurone changes and a working diagnosis of motor neurone disease was made. The EEG was non-specific.

Patient 2: A 53-year-old woman who died after 3 months of illness. She was examined by a member of the NCJDSU, and the relatives were interviewed but the hospital notes were not available. She had an early dementia and visual and cerebellar features were present but no myoclonus, pyramidal or extrapyramidal signs were noted and she was not seen at a time when there was akinetic mutism. The EEG was non-specific and CSF was not analysed for the presence of 14-3-3 protein but the MRI was positive.

Patient 3: A 63-year-old woman who died after 2 months of illness. There was only limited information of her case from the interview of her relatives. The patient was not seen during life and the hospital notes were not available. She had an early dementing illness and was unsteady but there is no definite note of cerebellar, pyramidal or extrapyramidal features. She developed visual symptoms late on but it is not clear if she became cortically blind or developed akinetic mutism. She developed myoclonus late in the illness. The EEG was highly suggestive but 14-3-3 protein was not performed and the MRI results are unavailable.

VV-2A case

A 65- year-old woman with an illness of 6 months duration. She was seen in life by the NCJDSU and the relatives were interviewed. The hospital notes were also available. She presented with dizziness and cerebellar signs. Dementia appeared after some weeks. There was no note of pyramidal or extrapyramidal problems, myoclonus, cortical blindness or akinetic mutism however. The EEG was non-specific, 14-3-3 was not done and the MRI would be considered possible.

8 other cases were classified as "CJD unlikely": 2 of these cases (20%) were in the MM-2A group. Both had an illness of duration greater than 2 years. A member of the NCJDSU saw neither case, but both presented with dementia. One case developed definite cerebellar problems and had some choreoathetoid movements but these may have predated the illness, as the patient had been on neuroleptic medication for many years. The other case was unsteady but no specific cerebellar features were noted. Myoclonus developed late on in the illness and so it is conceivable they may have been classified as "possible". Neither case had a typical EEG and 14-3-3 protein was not analysed. Only 1 MRI was available for review but neither was reported as showing high signal in the putamen or caudate nucleus.

One (20%) MV-1 case was a classified as unlikely. This was simply because the duration of illness was greater than 2 years, other wise she would have been classified as "possible". The EEG was non-specific, the 14-3-3 was negative and the MRI was also "possible".

Three (25%) of the MV-2A cases were classified as "CJD unlikely". Two of these cases would have been classified as possible but for the long duration of illness. The EEG was only done in 1 case and showed non- specific changes. 14-3-3 was not analysed and an MRI was not performed in either. The third patient was a 61-year-old woman who died after 8 months of illness. Only the hospital notes were available. She presented with cerebellar features and dementia but there was no note

of pyramidal, extrapyramidal signs or cortical blindness or akinetic mutism. There was no note of myoclonus although startle was commented on. The EEG was non-specific, 14-3-3 was not performed and the MRI was reported as showing only atrophy.

Two (67%) of the VV-1 cases were given the classification "CJD unlikely". One case would have been classified as possible but for the long duration. The EEG was non-specific and the 14-3-3 was negative but the MRI was strongly positive. The second case was a 41 years old man who died after 11 months. The presentation was with dementia and he was noted to be unsteady but there were no definite cerebellar, pyramidal or extrapyramidal signs. Myoclonus was noted late on but he was not noted to develop cortical blindness and akinetic mutism was not stated although he became mute and immobile. The EEG was non-specific and 14-3-3 was not performed. The MRI was considered possible.

8.2: SPORADIC CJD; INTERMEDIATE CASES

A: CASE DATA

Three sporadic cases were of intermediate mobility, i.e. between 19 and 21 kDa. Two of these cases were Met homozygous and the other was Val homozygous. In 2 cases a member of the unit had examined the patient and the hospital notes were available in 1 case. One case was from the Oxford study and the other 2 were from the current study.

B: GENDER DISTRIBUTION

There were 2 males and 1 female (ratio 2:1).

C: AGE AT ONSET

The median age of onset was 76 years (range 68-82).

D: DISEASE DURATION

The median duration of illness was 5 months (range 3-9). The case whose duration of illness was 9 months was fed by PEG.

E: SYMPTOMS AND SIGNS

The onset involved cerebellar symptoms in all 3 cases. One case also had visual symptoms at onset and a hemisensory burning sensation, which progressed to pins and needles. Dementia and myoclonus developed in all 3 cases.

F: INVESTIGATIONS

All 3 cases had a typical or highly suggestive EEG, although in the case with a typical EEG I did not review the record. None had 14-3-3 protein analysis and 1 had an MRI reported as showing cerebellar atrophy.

G: PATHOLOGY

There were no distinguishing features to the pathology in any of the 3 cases. There was microvacuolar spongiform change throughout the cortex. In some areas it was confluent. One case (of 9 months duration) had areas of status spongiosis. Spongy change was less marked in the deep grey matter and cerebellum. There were areas of neuronal loss and gliosis but no amyloid plaque formation. PrP immunochemistry was not performed in 1 case. In the other 2 there was a reticular deposition throughout the cortex and deep grey matter. In 1 case there were perivacuolar areas in the cortex. Staining was more granular in the cerebellum.

Table 8.24: SPORADIC CJD: INTERMEDIATE CASES

Geno -type	Onset years	Duration months	Clinical onset	EEG	MRI	Pathology
VV	76	5	Cerebellar	Typical (not seen)	Not done	
MM	68	9	Sensory, cerebellar	Highly suggestive	Negative (not seen)	Status spongiosis
MM	82	3	Dementia	Highly suggestive	Not done	

H: GROUP B CLASSIFICATION

Both the Met homozygous cases were classified "type 2" by Group B. The Val homozygous case was not classified by Group B. (See Appendix F)

8.3: SPORADIC CJD: GENOTYPE ONLY

As previously discussed, the genotype alone seems to influence significantly the phenotype of cases of CJD. The Group A cases were therefore analysed, considering genotype only.

A: CASE DATA

The cases were distributed as follows:

MM	64	65%
MV	17	17%
VV	18	18%

As discussed previously, the Met homozygotes and heterozygotes had a greater proportion of females but the Val homozygotes were more likely to be male. This was statistically significant ($p<0.5$) (See Table 8.25)

C & D: AGE AT ONSET & DISEASE DURATION

Age at onset was not significantly different between the genotypes; there was considerable overlap between all 3 groups. (Kruskall-Wallis test, $p=0.5$) (See Table 8.25) However the MV group had a significantly longer duration of illness than the MM and VV groups. (Kruskall-Wallis test, $p=0.002$)

Table 8.25: SPORADIC CJD: GENOTYPE ONLY: PATIENT STATISTICS

	Age Onset years	Duration months	M:F ratio
MM	**63** (39-86)	**3** (1-37)	**0.8:1**
MV	**65** (15-79)	**9** (2-54)	**0.6:1**
VV	**60** (26-79)	**6** (2-29)	**1.3:1**

E: SYMPTOMS AND SIGNS

Most of the cases in each group presented with a rapidly progressive dementia (24-48%). A cerebellar presentation was also seen in each group but it seemed to be commoner in the heterozygotes (24%) and the Val homozygotes (18%). Presentation with visual upset was seen in 17% of the Met homozygotes. One Val homozygote (6%) also presented with visual symptoms. This result did not achieve statistical significance. (Fishers exact test, p=0.1) (See Table 8.26)

Table 8.26: SPORADIC CJD: GENOTYPE ONLY: CLINICAL FEATURES AT ONSET

	RPD only N=	%	Cerebellar only N=	%	Visual only N=	%
MM	31	**48**	7	**11**	10	**17**
MV	4	**24**	4	**24**	0	**0**
VV	6	**33**	3	**18**	1	**6**

The disease progression is set out in tables 8.27, 8.28 and 8.29. Dizziness was more common in the Val homozygotes and visual upset was more common in the Met homozygotes. In addition, swallowing problems seemed to be more common in the Val homozygotes.

Table 8.27: SPORADIC CJD: GENOTYPE ONLY: CLINICAL SYMPTOMS

	P	%	Di	%	RPD	%	S	%	He	%	V	%	Ha	%	De	%
MM	16	**25**	12	**19**	63	**98**	10	**16**	1	**2**	37	**58**	16	**25**	4	**6**
MV	5	**29**	1	**6**	17	**100**	2	**12**	1	**6**	3	**18**	7	**41**	2	**12**
VV	6	**33**	7	**39**	18	**100**	2	**11**	1	**6**	6	**33**	6	**33**	2	**11**

P=Prodrome, Di=Dizzy, RPD=Rapidly Progressive Dementia, S=Sensory, He=Hearing, V=Visual upset, Ha=Hallucinations, De=Delusions

Table 8.28: SPORADIC CJD: GENOTYPE ONLY: CLINICAL SIGNS

	C	%	P	%	E	%	PR	%	M	%	MD	%	EM	%
MM	37	**58**	37	**58**	21	**33**	46	**72**	60	**94**	25	**40**	14	**22**
MV	14	**82**	5	**29**	5	**29**	13	**72**	14	**82**	2	**12**	2	**12**
VV	11	**61**	10	**56**	7	**39**	13	**72**	16	**89**	6	**33**	6	**33**

C=Cerebellar, P=Pyramidal, E=Extrapyramidal, PR=Primitive Reflexes, M=Myoclonus, MD=other Movement Disorders, AL=Alien Limb, EM=Eye Movements

Table 8.29: SPORADIC CJD: GENOTYPE ONLY: TERMINAL STAGES

	Swallowing	%	Cortical Blindness	%	Akinetic Mutism	%
MM	23	**36**	21	**33**	34	**53**
MV	4	**24**	3	**18**	7	**41**
VV	10	**56**	4	**22**	11	**61**

F: INVESTIGATIONS

Of the records that I reviewed, the EEG was typical or highly suggestive in 52% of Met homozygotes, 7% of heterozygotes and no Val homozygotes. This result was statistically significant. (Chi-square test, $p<0.001$) (See Table 8.30)

14-3-3 protein was positive in 68% of Met homozygotes and 88% of Val homozygotes. Only 33% of heterozygotes had a positive 14-3-3 analysis.

Table 8.30: SPORADIC CJD: GENOTYPE ONLY: INVESTIGATIONS

	EEG typical N=	%	14-3-3 +ve N=	%	MRI +ve N=	%
MM	32 /61	**52**	13/19	**68**	7/28	**25**
MV	1/14	**7**	1/4	**33**	3/6	**50**
VV	0/18	**0**	7/8	**88**	4/10	**40**

Half of the heterozygotes had a positive MRI and 25% and 40% of the Met and Val homozygotes respectively also had a positive MRI.

G: PATHOLOGY

One Met homozygote did not have PrP staining and one Val homozygote had no pathological tissue for analysis. (See Table 8.31)

i): Histopathology

In general spongiform change was seen predominantly in the cortex in Met homozygotes, and more prominently in the basal ganglia in heterozygotes and Val homozygotes. Kuru-type plaques were seen prior to PrP staining in only the heterozygotes. These were seen in 10/17 cases (59%).

ii): Immunohistochemistry

After PrP staining, 2 Met homozygous cases had plaque-like aggregates in the cerebellum and 1 had them in the basal ganglia. Of the Val homozygotes, 15 (83%) had plaque-like deposits in all or some of the cortex, basal ganglia and thalamus. Plaques and plaque-like deposits were seen in the cerebellum. Eleven (65%) of the heterozygotes had plaques and plaque-like deposits throughout the brain.

Table 8.31: SPORADIC CJD: GENOTYPE ONLY: PATHOLOGY

Genotype	Spongiform change	Kuru-plaques	%	PrP Plaque-like & plaques	%
MM	**cortex**	0	**0**	3/63	**5**
MV	**basal ganglia**	10/17	**59**	11/17	**65**
VV	**basal ganglia**	0	**0**	15/18	**83**

8.4: SPORADIC CJD: ISOTYPE ONLY

In order to assess whether changes seen could relate purely to isotype, the Group A data were analysed for trends relating to protein isotype 1 and 2A.

A: CASE DATA

Of all the cases, 62 were classified type 1 (63%), and 37 (37%) were type 2A.

B: GENDER DISTRIBUTION

The male to female ratio was 0.8:1 in each group. (See Table 8.32)

C & D: AGE AT ONSET AND DISEASE DURATION

The median age of onset was 64 years (range 15-86) and 61 years (range 39-79) in types 1 and 2A respectively. This was not statistically significant. (Mann-Whitney test, p=0.070) The duration of illness was 3 months (range 1-54) and 8 months (2-51) respectively. This was statistically significant (Mann-Whitney test, $p<0.01$)

Table 8.32: SPORADIC CJD: ISOTYPE ONLY: PATEINT STATISTICS

	Age Onset years	Duration months	M:F ratio
Type 1	**64** (15-86)	**3** (1-54)	**0.8:1**
Type 2A	**61** (39-79)	**8** (2-51)	**0.8:1**

E: SYMPTOMS AND SIGNS

44% of the type 1 group presented with dementia only and 38% of the type 2A had this presentation. A purely cerebellar onset was seen in 11% and 19% of types 1 and 2A respectively and 16% and 3% respectively of each group presented with a visual onset. This last result is of borderline significance (Fishers exact test, p=0.049) (See Table 8.33)

Table 8.33: SPORADIC CJD: ISOTYPE ONLY: CLINICAL FEATURES AT ONSET

	RPD onset N=	%	Cerebellar onset N=	%	Visual onset N=	%
Type 1	27/62	**44**	7	**11**	10	**16**
Type 2A	14/37	**38**	7	**19**	1	**3**

The clinical symptoms and signs are set out in tables 8.34, 8.35 and 8.36. Most of the symptoms and signs occurred with similar frequency in both groups. However, visual upset occurred in type 1 more than type 2A (56% compared to 30%). In general all the symptoms were noted more frequently in the type I group, except for hallucinations, which were more frequent in the type 2A group. However, in all cases the signs were noted more frequently in the type 1 group.

TABLE 8.34: SPORADIC CJD: ISOTYPE ONLY: CLINICAL SYMPTOMS

	P	%	Di	%	RPD	%	S	%	He	%	V	%	Ha	%	De	%
Type1	18	**29**	13	**21**	61	**99**	8	**13**	1	**2**	35	**56**	16	**24**	3	**5**
Type 2A	9	**24**	7	**19**	33	**100**	6	**16**	2	**35**	11	**30**	14	**38**	5	**14**

P=Prodrome, Di=Dizzy, RPD=Rapidly Progressive Dementia, S=Sensory, He=Hearing, V=Visual upset, Ha=Hallucinations, De=Delusions

TABLE 8.35: SPORADIC CJD: ISOTYPE ONLY: CLINICAL SIGNS

	C	%	P	%	E	%	PR	%	M	%	MD	%	EM	%
Type 1	37	**60**	36	**60**	19	**51**	46	**74**	59	**95**	26	**42**	15	**24**
Type 2A	25	**68**	16	**43**	13	**38**	26	**70**	31	**84**	7	**19**	7	**19**

C=Cerebellar, P=Pyramidal, E=Extrapyramidal, PR=Primitive Reflexes, M=Myoclonus, MD=other Movement Disorders, EM=Eye Movements

Table 8.36: SPORADIC CJD: ISOTYPE ONLY: TERMINAL STAGES

	Swallowing	%	Cortical Blindness	%	Akinetic Mutism	%
Type 1	25	**40**	20	**32**	35	**56**
Type 2A	12	**32**	8	**22**	17	**46**

F: INVESTIGATIONS

The EEG was typical or highly suggestive in 57% of the type 1 group but none of the type 2A. This was statistically significant (Chi-square test, $p<0.01$) 14-3-3 analysis was positive in 68% of the type 1 cases, and 67% of the type 2A cases, and the MRI was positive in 27% and 39% of the types 1 and 2A respectively. (See Table 8.37)

Table 8.37: SPORADIC CJD: ISOTYPE ONLY: INVESTIGATIONS

	EEG typical N=	%	14-3-3 +ve N=	%	MRI +ve N=	%
Type 1	33/58	**57**	13/19	**68**	7/26	**27**
Type 2A	0/35	**0**	8/12	**67**	7/18	**39**

G: PATHOLOGY

1 of the type 1 cases had no pathological tissue for analysis and 1 had no PrP staining. The results of the pathological changes are set out in table 8.38

i): Histopathology

The majority of type 1 cases had spongiform change predominantly in the cortex. In the type 2A cases about half were concentrated in the cortex and half in the basal ganglia. Kuru plaques were seen in 1 type 1 case (2%) and 9 type 2A cases (37%).

ii): Immunohistochemistry

PrP staining revealed plaque-like deposits and plaques in 3 of the type 1 cases (5%). These were sparse in the basal ganglia, thalamus and cerebellum. of the type 2A cases, 25 (68%) had these deposits.

Table 8.38: SPORADIC CJD: ISOTYPE ONLY: PATHOLOGY

Isotype	Spongiform change	Kuru-plaques	%	PrP Plaque-like & plaques	%
Type 1	**cortex**	1/61	**2**	3/60	**5**
Type 2A	**Basal ganglia/ cortex**	9/37	**24**	25/37	**68**

8.5: SPORADIC CJD; GROUP B CLASSIFICATION

48 sporadic CJD cases were classified by Prof J Collinge at the Prion Diseases Unit, St Marys Hospital, London. He has published his results in several papers and has proposed several more isotypes than Parchis' group.[20] The classification differs slightly from the NCJDSU isotypes (see Chapter 6). The London classification is termed Group B and the NCJDSU classification Group A.

The results of this analysis are set out in appendix F. There are a larger number of groups and only a few cases in each group so statistical analysis was not possible. The data at this stage add little to this analysis but are discussed in chapter 9 as they might become more meaningful in the future when more cases can be analysed.

SUMMARY OF SPORADIC CJD FINDINGS

- The MM-1 cases form the largest group and are typical of classically described sporadic CJD.
- In these cases the age at onset is typically in the 50-70 age group and the duration of illness is a few months. The disease follows a rapidly progressive course, characterised by dementia. Myoclonus, pyramidal and cerebellar problems typically develop. A state of akinetic mutism is often seen prior to death. The EEG is usually positive.
- The VV-2A cases tend to present with cerebellar signs and follow a fairly typical course. The EEG is usually negative but 14-3-3 seems to be positive in most cases.
- The MV-1 cases are also similar to classical sporadic CJD. 14-3-3 protein or the EEG may be useful in this group.
- The MV-2A cases also seem to present with more cerebellar signs but 14-3-3 protein and the EEG do not appear to be helpful in investigation.
- The MM-2A group tends to include cases of younger onset and of longer illness duration. These cases usually followed an atypical course and investigations were largely unsupportive of sporadic CJD.
- The VV-1 group was very small, making it difficult to comment on trends. These cases weRe generally atypical.
- Pathological changes were largely attributable to codon 129 genotype only.
- There was considerable overlap between groups and it was not possible to establish the influence of isotype or genotype alone on the data as a whole.

8.6: VARIANT CJD

A: CASE DATA

Full genetic analysis and protein isotyping was available in 43 cases of variant CJD. Five patients were not seen by a doctor from the NCJDSU. The relatives were interviewed in all cases. The full hospital notes were not available in 2 cases. All the cases come from the current surveillance project.

B: GENDER DISTRIBUTION

The number of males and females was 24 and 19 respectively ratio (1.3:1).

C: AGE AT ONSET

The median age at onset was 26 years (range 14-53).

The median age at death was 27 years (range 15-54).

D: DURATION OF ILLNESS

The median duration of illness was 14 months (range 7-39).

E: SYMPTOMS AND SIGNS

The clinical course in all the cases was similar to the features of vCJD when it was first described.[100]

i): Prodrome

In 2 cases (5%) the family noted some form of prodromal illness: one patient had an ear infection and lost his hearing and another had a flu-like illness prior to the onset of the first symptoms of vCJD. One patient had Bells palsy prior to the onset of their illness and another was diagnosed and treated for thyrotoxicosis at the onset of their first symptoms of vCJD.

ii): Psychiatric Symptoms

Psychiatric symptoms were part of the presenting complaint in all but two patients (95%). One of these cases presented with a hemi-sensory disturbance but very quickly developed depressive symptoms. In the other there was insufficient data to be sure that psychiatric symptoms were present from early on but the patient was anxious and irritable later on in the illness. The presenting symptoms were often non-specific, for example moodiness, in some put down to normal teenage behaviour. Patients went on to develop more marked psychiatric symptoms. (See Table 8.42)

Depression was present in 30 cases (70%), often diagnosed by the GP and anti-depressant medication was prescribed in some cases. The depression was sometimes thought to be reactive, in 2 cases, related to the death of a relative. In another case depression developed and the break up of a relationship ensued. In this case the relationship break up may also have been related to the onset of other features of vCJD.

Anxiety symptoms were present in 30 cases (70%). This varied from panic attacks and tearfulness to preoccupation with things that would not normally have been of concern. For example, in one case the patient was excessively worried about forth coming exams and despite passing the exams held a fixed belief he had failed. This was very out of character. Five patients suffered from anxiety and fear mainly at night.

Sleep patterns were commonly altered. 21 patients (49%) were excessively tired and would sleep during the day as well as sleeping all night. 11 cases (26%) suffered from insomnia. One of these patients went on to become excessively sleepy later in the course of their illness. Two cases were unable to sleep because they were awaking screaming and crying in the night. Another patient couldn't sleep later in her illness because she suffered from nocturia and frequency.

Anorexia and weight loss was present in 23 patients (53%). Two of the 23 cases complained of dysgeusia and 1 other had this symptom but we have no record of anorexia or a change in weight. Two of the patients with anorexia initially ate excessively but subsequently lost weight. There is a suggestion of possible weight gain in one patient.

Anger or aggressive was noted in 25 patients (58%) usually towards the beginning of their illness. Behaviour ranged from being rather bad tempered to outbursts of aggression. In all but 2 cases this was extremely uncharacteristic.

Hallucinations were present in 15 cases (35%) and were possibly present in a further 2 cases. In 9 cases the hallucinations were visual and in 1 case they were auditory. In the remaining cases they were mixed visual and auditory hallucinations.

Delusions were present in 16 cases (37%). They were predominantly paranoid but one person thought she was pregnant and another thought he was on a plane. One case had hallucinations and delusions from the onset of their illness but in most cases the symptoms appeared some months into the illness.

Table 8.42: VARIANT CJD: PSYCHIATRIC SYMPTOMS

Symptom	N=	%
Depression	30	**70**
Anxiety	30	**70**
Hypersomnia	21	**49**
Insomnia	11	**26**
Anorexia	23	**53**
Aggression	25	**58**
Hallucinations	15	**35**
Delusions	16	**37**

Three patients had a past medical history of psychiatric illness, ranging from long-term emotional and psychological problems to a previous history of psychotic illness 4 years prior to the onset of vCJD. Another had a family history of learning difficulties and epilepsy and had had problems with learning difficulties himself. One other patient had a history of cerebral palsy and behavioural problems. In some of these patients the onset of vCJD was often thought to be related to their past medical history and made initial diagnosis more complicated.

ii): Dementia

Dementia was present in all cases of vCJD but was only present at onset in 6 cases (14%). Most cases presented with psychiatric symptoms with or without sensory symptoms. It was sometimes difficult to establish at what point dementia appeared and the nature of the symptoms. As the age of the variant cases tends to be younger, relatives were sometimes reluctant to accept symptoms of confusion or memory loss as dementia. For example one patient did very badly in exams he was expected to do well in. However, the family was insistent that he was functioning normally and did not feel this was indicative of cognitive problems.

iii): Sensory Symptoms

Persistent sensory symptoms were present in 28 cases (65%) and in 6 (14%) the symptoms were present from the onset. In a further 7 cases sensory symptoms were possibly present but it was unclear whether or not they were persistent so they have been classified as "don't know". The sensory symptoms varied from limb pain, often poorly described and localised to pins and needles or dysaesthesia. Symptoms were sometimes lateralised.

The sensory symptoms of the first 50 cases of vCJD were reviewed by myself and have been published elsewhere.[223] These showed that sensory symptoms were present in 63% of cases and in 31% these were present from the onset of the illness. The symptom most commonly described was limb pain, which was often non-

specific and poorly localized. Other symptoms included cold feelings, dysaesthesia, paraesthesia and numbness. These symptoms are believed to be of thalamic origin.

iv): Visual upset

Twelve cases (28%) complained of visual upset, 2 of these from the onset of the illness. Two of the patients complained of blurred vision but the others complained of double vision. Many of those with diplopia could be seen to shut one eye some of the time.

v): Auditory upset

Only 2 patients (5%) complained of hearing problems. One patient became very sensitive to noise midway through their illness and the other patient complained of hearing loss following an ear infection at the onset of the illness.

Table 8.43: VARIANT CJD: OTHER SYMPTOMS

Symptom	N=	%
Dementia	43	**100**
Sensory	28	**43**
Visual	12	**28**
Hearing	2	**5**

vi): Cerebellar problems

Cerebellar signs, predominantly ataxia, were present in all but 2 of the cases (95%). In these 2 cases, neither was seen until she was bed bound. Both their families noted they were unsteady on their feet. However, cerebellar signs were not specifically mentioned in the case notes and so it cannot be assumed they were definitely present. Three (7%) of the cases had symptoms of unsteadiness, later found to be ataxic, from the onset of their illness. Of the others, 9 (21%) were unsteady from early on and 27

(63%) of the others developed ataxia in the middle months of illness. In the final 3 (7%) ataxia was not noted until late on.

vii): Pyramidal signs

Pyramidal signs were noted in 37 (86%) of the patients.

viii): Primitive reflexes

Primitive reflexes were noted in 25 cases (58%).

ix): Extrapyramidal signs

Extrapyramidal signs were only noted in 3 cases (7%).

x): Movement Disorders

Myoclonus was noted in 33 patients (77%) but other movement disorders were also prominent. Choreaform and dystonic movements were noted in 29 of the cases (68%). In 5 cases (12%) chorea or dystonia was present in the absence of myoclonus.

xi): Eye movements

Twenty cases (47%) had abnormal eye movements: in 2 of these cases, pursuit was noted to be jerky but the others had a reduction in up gaze, there was a suggestion of this in one other patient.

xii): Cortical Blindness

Cortical blindness was noted in only one case (2%).

xiii): Akinetic Mutism

Twelve cases (28%) went on to develop akinetic mutism and another cases was noted to be mute. However, no note was made of immobility.

Table 8.44: VARIANT CJD: OTHER SIGNS

Sign	N=	%
Cerebellar	41	**95**
Pyramidal	37	**86**
Myoclonus	33	**77**
Movement disorder	29	**68**
Extrapyramidal	3	**7**
Eye movement	20	**47**
Cortical blindness	1	**2**
Akinetic mutism	12	**28**
Swallowing	19	**44**

xiv): Swallowing problems

Abnormal swallowing was noted in 19 cases (44%) and at least 9 patients were fed by a nasogastric tube or by PEG feeding (21%). In 1 further patient a PEG was sited but could not be tolerated and was removed. The median duration of illness of cases who were NG or PEG fed was 18 months (range 11-39 months). In comparison, the cases in whom we have no information (it is likely that some of these cases were NG fed but we do not know about it) had a median duration of 13 months (range 7-29 months).

F: INVESTIGATIONS

i): EEG

All cases had at least one EEG performed during their illness. The EEG was not diagnostic in any cases of vCJD. In 2 of the 43 cases it was reported as normal. In 39 cases it was described as non-specific. Two cases had an EEG that could be regarded as suggestive of CJD by our criteria, i.e. there were triphasic complexes but these were not sustained or truly periodic. The EEG was performed a median of 9 months (range 2-24 months) into the illness of median duration 14 months (7-39). The final EEG record was used if more than one EEG was performed.

ii): CSF Analysis

The basic CSF glucose, protein and cell count was considered normal or non-specifically abnormal in all cases. The value of CSF protein was missing in 3 cases and reported normal in 3 cases but there is no record of the exact value. In the remaining cases protein ranged from 0.09 to 1.61 mg/L (with 119 red cells). In 8 cases the protein was greater than 0.6 mg/L. In 6 cases the white cell count is reported as "normal". The result was missing in 5 other cases and in the remainder there were less than 5 white cells.

14-3-3 was analysed in 33 cases. In 15 cases the result was negative (45%) and it was positive in 13 cases (30%). One result was positive but heavily bloodstained. The remaining 4 cases were analysed twice. In 2 cases the first analysis showed a trace and the second result was positive. In one case the result showed a trace the first time and the second was negative. The final case was negative in both analyses. This gives an overall positive value in 15 cases (45%).

The level of S-100b was analysed in 29 cases. A level greater than 0.38μg/L is considered abnormal. The result was normal in 3 cases, all of whom had a negative 14-3-3 result. The result was raised in the remaining 24 (83%), ranging from 0.41 to 2.71μg/L. In 13 of these cases the 14-3-3 was also positive. Two cases were tested twice. In 1 case the 14-3-3 was negative on both analyses but the S-100b was 0.25μg/L and 0.84μg/L. In the second case the 14-3-3 result was trace then positive and the S-100b was 0.41 then 0.69μg/L.

Looking at only the results analysed once, the positive 14-3-3 results were associated with a mean S-100b of 1.46μg/L. The negative 14-3-3 results associated with an abnormal 14-3-3 were in general lower with an average result of 0.88μg/L.

iii): MRI

All 43 cases had an MRI of the head performed during their illness. In 33 cases (77%) significant high signal was present in the pulvinar area of the thalamus and the scan would be regarded as positive. Three of the cases (7%) had a negative scan. A further case had high signal in the pulvinar but also in the putamen and caudate. Two other cases were reported as equivocal (5%). The scans were performed a median of 9 months, range 2-24 months into the illness.

More than 1 scan was performed in 10 cases. In 5 the first scan was positive. In 1 case both were negative and in another both were equivocal. In 2 the second scan was positive. In these cases, the first scan (negative) was performed at 2 and 6 months and the second scan (positive) was performed at 7 and 8 months. Finally one case had a positive first scan. The second scan was also probably positive but was technically difficult to interpret.

Of the 39 scans reviewed by the NCJDSU, 85% were definitely positive. The MRI was not seen by the NCJDSU in 4 cases. Of these, 2 were reported normal, 1 was reported positive and there is no report available for the final scan. The 2 that were reported normal were performed and reported on prior to the publication of the MRI findings in vCJD. It is possible that in retrospect these scans would be considered positive.

Table 8.44: VARIANT CJD: INVESTIGATIONS

Investigation	N=	%
Typical EEG	0/43	**0**
Positive 14-3-3	15/33	**45**
MRI positive	33/39	**85**

G: PATHOLOGY

To date the pathological findings in all the cases of vCJD have been remarkably consistent. Tissue blocks or the whole brain parts thereof were available for analysis in all cases. Significant cortical atrophy was seen in 4 cases, all of whom had a long duration of illness (25, 29, 33 and 39 months). Mild to moderate cerebellar atrophy was seen in 11 cases. (The cerebellum was not available in all cases). An incidental cavernous angioma was found in 1 case.

i): Histopathology

Mild to moderate spongiform change was seen throughout the cortex in all but 1 case, in whom there was status spongiosis. The changes were patchy but in areas of severe change there was neuronal loss and gliosis. The occipital cortex was the area of the cortex most affected in 20 cases, along with the frontal cortex in 2 cases, the temporal cortex in 1 case and the parietal cortex in 1 case. In 1 other case the fronto-parietal cortex was the worst affected. In general the hippocampus was rarely involved.

The basal ganglia were the area of most severe spongiform change, in particular the caudate nucleus and putamen. This was often accompanied by marked neuronal loss and gliosis. In the thalamus there was severe neuronal loss and gliosis, often with only mild spongiform change, although the spongiform change was marked in 9 cases. In 3 cases there was also marked neuronal loss and gliosis in the hypothalamus.

In 12 cases there was mild spongiform change in the brainstem, in 1 case associated with marked neuronal loss and gliosis. Five other cases had marked neuronal loss and gliosis in the brainstem. The cerebellum was involved to varying degrees, again with associated neuronal loss and gliosis.

ii): Immunocytochemistry

The most striking feature in all the vCJD cases was the widespread deposition of florid plaques. These were present throughout the cortex and deep grey matter and also in the cerebellum. Plaques were particularly concentrated in the basal ganglia. More plaques were demonstrated on PrP immunohistochemical staining.

In addition there was widespread staining, usually pericellular and perivascular in the cortex and cerebellum. The staining was more linear in the basal ganglia, sometimes with a synaptic and perineuronal picture.

H: NCJDSU CLASSIFICATION

Thirty-two cases (74%) fulfilled criteria for probable vCJD during life.
Eight cases were considered possible; in these cases we were unable to view the scan in 2 cases, 2 patients had an equivocal scan and 3 had a negative scan.
Three cases were classified "CJD unlikely". In 2 there was insufficient information to be sure of cerebellar and sensory signs and symptoms and in 1 there were insufficient data to be sure of a psychiatric onset and there were no persistent sensory symptoms. Two of these cases had a positive scan.

I: GROUP B CLASSIFICATION

All cases of vCJD have been classified type 4 by Group B. I have therefore not compared the results.

SUMMARY OF VARIANT CJD CASES

- The variant cases have a relatively distinct clinical and pathological phenotype.
- This is fairly consistent in all cases.
- Cases tend to be of younger onset and of longer illness duration.
- Psychiatric symptoms are prominent at the onset.
- Sensory symptoms, usually painful and persistent are also common.
- The terminal stages of the illness are similar to sporadic CJD.
- The EEG is usually non-specific.
- 14-3-3 protein supports a diagnosis of CJD but does not distinguish from the sporadic form of the disease.
- The pulvinar sign on MRI is highly specific and sensitive in variant CJD.
- The pathological changes are distinct from sporadic CJD.

CHAPTER 9: DISCUSSION

9.1: INTRODUCTION

The clinical and pathological features of sporadic CJD have been well described for many years. Most cases follow a typical clinical course and have characteristic pathology, however unusual cases are well described. In 1996 a new variant of CJD was reported, subsequently linked to BSE.[100] Recently, analysis of the genotype and protein isotype in cases of TME has suggested a molecular basis for phenotypic changes seen in cases of this TSE. This study was extended to human spongiform encephalopathies and it has been suggested that the codon 129 genotype and protein isotype are responsible for the phenotypic variation seen in cases of CJD.[399] It has also been suggested that the protein isotype is a surrogate marker of agent strain, and that the different phenotypes seen in sporadic CJD are due to different strains of the agent. To date, only one protein isotype has been identified in vCJD, associated with a reasonably consistent clinical course and pathology. This supports the theory that this may be due to a single, different strain of prion agent. However, an alternative UK study has categorised the protein isotypes differently and has suggested further molecular variation and further evaluation of the methodology and typing of protein isotyping is required.[20]

Two large European studies have noted trends in six genotype and protein isotype groups related to variations in age at onset, duration of illness, clinical features, investigation results and pathology in sporadic CJD, familial and iatrogenic CJD.[399,455] The data from these studies were pooled from North American and European monitoring data. Few of these countries have a dedicated surveillance system and it is unlikely that many of the cases were seen in life. No information was given about the source of data. In addition it is not stated how investigation results were obtained and if they were reviewed again for the purposes of the study.

This study extends the analysis of the genetic and protein isotypic influence on cases of sporadic CJD seen by the UK surveillance system. Cases date back to 1990 when surveillance began at the NCJDSU, and also includes several retrospective cases from an Oxford based study prior to this date, and retrospective cases from the intervening years. I have reviewed all the records available at the NCJDSU in each case. In as many cases as possible this includes records of a detailed interview with relatives or the patient and detailed examination of the patient performed by a dedicated NCJDSU registrar. In about a third of cases this is myself. In addition I have reviewed and reclassified as many EEG records as possible and I have obtained the original MRIs and reviewed them with a neuroradiological colleague. It is believed that the data in this study is as complete as possible for such a rare disease.

In the following sections the discussion aims to:

9.2 Highlight the problems of surveillance of CJD and the problems encountered in this study

9.3 Look for trends in the 6 groups to support a relationship between the clinico-pathological phenotype and protein isotype and genotype. The results of this study are compared to an amalgamation of data from previous studies.

9.4 Look at analysis of genotype or isotype only

9.5 Compare with analysis by Group B

9.6 Discuss the variant CJD cases and the differences compared to atypical sporadic CJD cases.

9.7 Discuss the basis for protein isotype as a surrogate marker of strain variation

9.8 Discuss future possible research

9.2: PROBLEMS

A: DATA COLLECTION

This study uses data collected on over 10 years of UK surveillance of cases of CJD. Some cases predate this: 3 cases originate from the Oxford study and 5 cases are retrospective cases from the intervening years. A member of the NCJDSU saw the majority of cases in life.

In a population of 56 million people approximately 50 to 60 cases of sporadic CJD would be expected each year. This means that the number of cases for analysis of such a rare disease is relatively small. Additionally a proportion would not have a post mortem, or permission would be given for only a limited autopsy and not further research. This means that brain tissue would not be available for protein isotyping in all cases referred to the NCJDSU, further reducing the number of cases available for review. It is conceivable that some atypical cases have died and not had an autopsy; potentially skewing the data towards "typical" sporadic CJD although look-back studies have suggested this is unlikely to be the case.[125,126]

Clinical information was available in most cases from interview with relatives, case notes and the CJD registrar visit. However, for various reasons, not all of the cases were willing to be seen or would have relatives who were prepared to be interviewed. Case notes were not always easily obtainable. Of the 99 sporadic cases in this study, 16% of patients were not seen or examined by a member of the NCJDSU, 8% of relatives were not interviewed and in 32% there were no case notes available for review. Case notes were normally photocopied at the time of the NCJDSU registrar visit; information on subsequent clinical course was rarely available, as the notes were not requested again after death.

B: ASSESSMENT OF SYMPTOMS AND SIGNS

One of the difficulties inherent in a surveillance system for CJD is making an exact assessment of the presence or absence of any symptom or sign and the timing of it.

Many of the observations on any particular variable were made at the time of a visit by the CJD registrar. Following the visit, the NCJDSU team did not see the patient again and there was often little communication, if any, with the hospital staff looking after each individual. It is therefore possible that symptoms or signs that were absent at the time of the visit developed later in the disease.

Often the history related to events before being seen by a doctor, and so the onset and presence of symptoms and signs was difficult to define. Many of the patients were cognitively impaired by the time they were seen and so were not usually reliable historians. For this reason the history was normally obtained from the case notes and a relative, who may not have lived with the patient, or have been around for some of the illness. In the cases not seen in life by the NCJDSU there may only be a history available from a relative. In some cases the interview was conducted months, if not years, after the death of the relative and so the accuracy of the history was further potentially decreased.

For example, a relative may have stated that the patient was unsteady for about 2 months prior to admission to hospital. The accuracy of timing the onset of this was often rather vague, influencing the duration of the illness. Also, unsteadiness may be due to cerebellar, extrapyramidal, apraxic or other problems, making it difficult to be sure of the signs at onset. In such a case there could be uncertainty about whether a case was truly a Brownell-Oppenheimer variant (i.e. presenting with progressive cerebellar ataxia).

The onset of psychiatric and cognitive problems in vCJD cases was particularly difficult to define. For example, in one case a student was living away from home at the onset of their illness and the history from a member of the family was deduced from telephone conversations with the patient. In another case, the family denied any cognitive problems in their son but it later became evident that he had failed exams he was expected to pass at a time when the family believed there were no cognitive

problems at all. For cases that were not seen in life there was often even less information.

For these reasons, the timing of the illness onset and development of symptoms and signs was at times inaccurate and a "best guess". Additionally, the frequency of the various symptoms and signs was probably underestimated in the study, often being classified "don't know" if there was doubt about their presence. In view of the fact that as many as possible cases were seen in life in this study, and the registrar had as long as they wanted to interview the patient or their family, unlike in a busy clinical setting, it is difficult to see how more accurate information could be obtained.

It is possible that some of the symptoms and signs looked at were more susceptible to bias than others, potentially biasing the results. Signs such as akinetic mutism, which appear late in the illness, may not have developed by the time of the NCJDSU visit and information about their development after the visit may be absent. Additionally, once a diagnosis of CJD is reached there may be a tendency to examine a patient less, in order not to cause unnecessary stress, meaning that signs appearing late in the illness are likely to be under-estimated.

Allowing for the above difficulties in categorising data, age of onset and duration of illness are easily measurable. Previous studies have consistently suggested a relationship with the genotype and protein isotype. Clearly there are other possible reasons for this. The duration of illness could be influenced by other factors, such as whether supportive feeding was used, if infections were treated or if other underlying diseases or conditions were present.

As sporadic CJD progresses the diagnosis often becomes more obvious; there are not many conditions affecting the nervous system associated with such relentless progression, involving so many areas of the brain, in the presence of a normal scan and basic CSF analysis. After some weeks or months the EEG has often evolved, or

14-3-3 protein may have been looked for, suggesting a diagnosis of CJD. The presentation of sporadic CJD is therefore of greater importance, both in establishing a diagnosis early, and in trying to differentiate the condition from other more treatable diseases. For this reason the onset of the disease was assessed in each case.

In addition, for the reasons stated above, the first symptoms in the illness might be the most accurately reported and recorded in comparison to the development and timing of later features of the illness.

Finally, it is possible the first symptoms might be of particular importance as they may be indicative of the site of the onset of pathological changes within the brain. This in turn might influence the progression of the disease other changes such as the duration of illness. If the disease process starts in the brainstem, it is possible that death will occur earlier as this site might be subject to more degeneration.

If possible a decision was made if the onset of disease was "purely dementing", "purely cerebellar", as in the Brownell-Oppenheimer variant, or a "purely visual" onset leading to cortical blindness, suggesting the Heidenhain variant. The presence of other symptoms and signs at onset and during the course of the illness were recorded and these criteria have also been examined in the other studies of protein isotype, (possibly with less emphasis on the onset of illness). However, for the reasons listed above, many of the signs seemed to be present in most cases and more importantly, the absence of a particular sign cannot be taken to mean it was definitely absent. There may have been an omission in the recording of its presence or absence, or it may have appeared subsequent to the registrar visit.

C: AMALGAMATION OF DATA FROM OTHER STUDIES

The data from the Parchi studies were pooled from European and North American surveillance. Each country has its own methods of surveillance and not all countries visit the cases and examine the patients themselves, relying on information from case

notes. For this reason, it is possible the data from these studies may also have problems in the timing of symptoms and interpreting comments made in clinical notes. It is possible that the data in the UK study is more accurate as in the majority of cases the patient was interviewed and examined for the purposes of the ongoing study. In addition the EEG and MRI were reviewed personally in all the cases in which records and scan were available. Parchi et al. do not state whether they reviewed all records themselves but if not, this is another area for potential error.

It was not possible to combine the data on age at onset and duration of illness, as it was not clear whether the values in the paper were a mean or median. In addition, the data from the smaller European studies were pooled from various countries, including the UK, and so some cases may have been included twice in the amalgamation of results in chapter 6.

D: STATISTICAL ANALYSIS

To further compound the analysis of data, there is one large group and 5 smaller groups. This makes any statistical analysis non-parametric and unreliable with large confidence intervals. This is particularly marked when comparing the MM-1 to VV-1 group; i.e. 54 cases and 3 cases respectively. Clearly, in a group of 3 cases, the presence or absence of any particular sign in all or any of the patients may well be due to chance.

However, this may be the best we can do as the UK system, which has been set up to see all cases of sporadic (and latterly variant) CJD in the UK, had only 3 VV-1 cases in a 10 year time period. Surveillance would have to continue for possibly 20-30 years in order to obtain a statistically significant number of cases in each of the smaller groups. (Bearing in mind this is a new technique and it is hoped that more cases will be isotyped with time). Parchi et al encountered the same problems, with only 3 VV-1 cases in their 300 cases.[399]

E: ATYPICAL CASES

It is likely that this study over represents atypical cases as there may be a bias towards protein isotyping of cases with unusual clinico-pathological phenotypes. These cases are likely to have been selected for further analysis because of unusual features of the disease, or indeed concerns over the type of CJD affecting the patient. In the young atypical cases there is often an underlying concern that the case is variant rather then sporadic CJD, again selecting them preferentially for protein isotyping.

The cases that have been selected for PrP isotyping in this study will have been included because neuropathological material is available at the unit and permission has been given for autopsy by relatives. This may be a reflection of the degree of certainty of the diagnosis by the clinicians in charge of the patients care; i.e. atypical cases are less likely to be given a diagnosis of "probable CJD" during life and relatives and clinicians will be more inclined to proceed to autopsy to establish a cause of death.

If a random selection of sporadic CJD cases were to undergo genotyping and protein isotyping, it is likely that a greater proportion than was seen in this study would be MM-1 cases and that the other groups have been overestimated. However, the case statistics were compared to the statistics of the NCJDSU data as a whole and were broadly similar in terms of age at onset and illness duration.

9.3: CLINICO-PATHOLOGICAL PHENOTYPE OF SPORADIC CJD

A: CASE DATA

99 cases of sporadic CJD were studied. 65% of the cases were Met homozygous, 17% were heterozygous and 18% were Val homozygous. The percentage of heterozygotes and Val homozygotes is slightly higher than that seen in some studies and is probably a reflection of the selection of atypical cases for isotyping. There are 12 cases included in whom there was no genetic sequencing to exclude hereditary CJD. There was no family history of dementia in any of the cases and the clinico-pathological phenotype was not suggestive of hereditary CJD, but an inherited form of the disease has not been definitively excluded.

Nearly two thirds of the cases were isotype 1 and the remainder was type 2A. This is similar to the distribution seen in other studies. This implies that the case distribution in Parchi's and this study is similar and so the differences seen may be related to methodology. When the cases were further distributed according to genotype and isotype the distribution was again similar to that seen in previously published studies; the majority of cases fell into the MM-1 group (55%). As has been noted previously, very few of the type 1 cases are heterozygotes (5%) or Val homozygotes (3%), however, the type 2A group distributes fairly evenly between all 3 genotypes: 10% were Met homozygotes, 12 % were heterozygotes and 15% were Val homozygotes.

B: GENDER DISTRIBUTION

The overall ratio of males to females was 0.8:1, similar to that seen in most epidemiological analyses of sporadic CJD. There was a slight excess of males in the Val homozygotes (1.25:1). The significance of this is uncertain, and did not achieve statistical significance. As there were only 3 VV-1 cases and 15 VV-2A cases it is likely to be due to chance.

C: AGE AT ONSET

The age of onset has been related to genotype in various studies. Recent analysis of protein isotype with genotype has also suggested a relationship. There was a wide range of ages in each of the 6 groups, and so the data overlapped considerably. The MM-1, MV-2A and VV-2A groups had a median age of onset of 64, 64, and 66 years respectively. This would fall within expected limits. However, both the VV-1 and MM-2A groups had a younger median age of onset (41 years and 54 years respectively) and both these results achieved statistical significance. In contrast, the MV-1 cases had an older median age of onset of illness, 75 years. This figure is skewed somewhat by one very young case, whose age of onset was 15 years, the remaining cases developed the first signs of illness at 61, 75, 78 and 79 years.

Although it was not clear if the data were mean or median values in Parchi's study,[399] they also showed a younger age of onset in the MM-2 group (58.3 years) and the VV-1 group (39.3 years), in whom it was statistically significant. However, the age of onset of MV-1 cases was 62.1 years, slightly younger than expected (in contrast to this study where the cases were older).

When the Zerr[455] and Parchi[399] studies are combined with the isolated case reports and the age at onset in this study, the majority of cases fall into the late 50s, early 60s age group. Only the VV-1 cases stand out as being significantly younger than would be expected. (See Table 9.1)

Because of the wide age range within each group, it seemed reasonable to look at how many young cases there were. A younger age of onset is often associated with an atypical phenotype of sporadic CJD; only 3 of the MM-1 cases were aged less than 50 years. There were also 3 of the MM-2A cases less than 50 years, 1 each of the MV-1, MV-2A and VV-2A groups and 2 of the VV-1 group. These cases will be discussed later in more detail.

Table 9.1: AGE AT ONSET OF CASES

	Parchi et al	N	Zerr et al	N	Isolated cases	N	This study	N
MM-1	**65.5**	203	**68**	70	**67**	10	**64**	54
MM-2A	**58.3**	12	**63**	3			**54**	10
MV-1	**62.1**	8	**63**	8	**66**	1	**75**	5
MV-2A	**59.4**	27	**62**	10	**68**	1	**64**	12
VV-1	**39.3**	3	**27**	2	**49**	1	**41**	3
VV-2A	**61.3**	47	**62**	15	**65**	3	**66**	15

NB: Parchi data not clear if median or mean. All other data is a median value.

D: DURATION OF ILLNESS

One of the characteristic features of sporadic CJD is the relentless progression of disease and the short duration of illness. As with the age of onset, one of the most consistent findings in relation to genotype and, latterly, protein isotype, is that the more unusual genotype/ isotype cases have a longer duration of illness. (See Table 9.2)

In this study a shorter median duration of illness was seen in the MM-1 cases (3 months) and the MV-1 cases and VV-2A cases (both 5 months). In contrast the MV-2A and VV-1 cases were of 11 months duration and the MM-2A cases were of median 14 months duration. Once again there was a large overlap when the range of values was analysed in each group, making the statistical significance of these findings difficult to quantify.

This trend was also seen in the Parchi[399] and Zerr[455] studies, the MM-1, MV-1 and VV-2A cases seem to have a more characteristic short duration of illness. One isolated report of an MV-1 case had a rather longer than expected illness duration of 12 months. It is interesting to note that the median duration of illness was generally considerably longer in other studies compared to that seen in this study. The reason for this is unclear.

Table 9.2: DURATION OF ILLNESS

	Parchi et al	N	Zerr et al	N	Isolated cases	N	This study	N
MM-1	**3.9**	203	**5.2**	70	**7**	10	**3**	54
MM-2A	**15.7**	12	**14**	3			**14**	10
MV-1	**4.9**	8	**3.4**	8	**12**	1	**5**	5
MV-2A	**17.1**	27	**17.4**	10	**29**	1	**11**	12
VV-1	**15.3**	3	**25.5**	2	**15**	1	**11**	3
VV-2A	**6.5**	47	**7.5**	15	**9**	3	**5**	15

NB: Parchi data not clear if median or mean

All other data is a median value.

When the data were subdivided into the number of cases of duration of illness of less than 6 months this trend was reinforced. 60% of MV-1 cases, 80% of VV-2A cases and 87% of MM-1 cases had an illness of duration less than 6 months and almost all the cases in each group were of less than 1 year duration (80%, 100% and 94% respectively). In contrast 50% of each of the MM-2A and MV-2A groups and 67% of the VV-1 groups were of duration greater than 1 year. Only 1 MV-2A case and no MM-2A or VV-1 cases were of less than 6 months duration.

This may reflect a true genotype/ isotype influence but the data are likely to be biased by a number of factors: It is possible that the cases of longer duration had naso-gastric (NG) or parenteral gastrostomy (PEG) feeding. Unfortunately information about NG or PEG feeding was unavailable in most cases. Recent questionnaires comment on the presence of an NG tube or PEG however, some cases are ambulant with an intact gag reflex when they are visited by a member of the NCJDSU. They may survive for many months after a visit and artificial feeding may have been introduced later in the illness. This information may not necessarily reach the NCJDSU. Of course, this does not exclude the possibility that PEG feeding might be commenced because the patient survives for longer

From personal observation, it seemed that some cases who had a longer duration of illness were fed artificially, but there was only information about 11 cases in this study. Only 55% of these were of illness duration greater than 6 months. Of these, 9 were in the MM-1 group who had the shortest overall median duration of illness (3 months). The median duration in the cases who were artificially fed was 5 months (range 2-17 months). However, only 3 of the MM-1 cases were of greater than 1 years illness duration and 2 of these cases were NG fed. Similarly, the only MV-1 case whose duration was greater than 1 year was also NG fed. In contrast, 3 of the VV-2A cases were of illness duration greater than 6 months (7, 11 and 11 months). None of these cases were NG fed as far as we know. One VV-2A case was NG fed, of duration 6 months. (The median duration of the group as a whole was 5 months.)

It may also be that younger cases are more likely to be artificially fed for more emotive reasons. Families sometimes find it more difficult to let a younger relative die and may wish to keep them alive in the hope of a recovery or a cure. Sometimes relatives feel that an older person has had an opportunity to live their life, or may not wish to see them continue any longer in a state of akinetic mutism. However, of the 11 cases in whom there is information about NG feeding, only 2 were less than 60 years. One was 15 years old and one was 54 years. The median age of onset of all the 11 cases was 64 years.

It is also possible that younger cases live for longer irrespective of supportive measures such as artificial feeding. They may have a greater capacity to fight infection, the terminal event in many cases of sporadic CJD. Or a decision may be made to treat infection in a younger person whereas in an older person treatment may be withheld for the same reasons given above. There is no information about whether infection was treated or not in the majority of cases.

Another possibility is that younger patients may have a greater "brain reserve" so that they can survive a progressive neurodegenerative process for longer. Initial

analysis of the data had suggested a significant correlation between duration of illness and age of onset but only 6 cases were of duration greater than 2 years, making any interpretation of these data difficult. (Although all but one case was aged less than 60 years.) Overall, 70% of cases that had an illness of duration less than 2 years, were over 60 years. When the MM-1 cases alone were analysed for an association between age at onset and illness duration, none was found. The other groups are too small to look for a trend.

E: SYMPTOMS AND SIGNS

i): Onset

The symptoms at onset were felt to be very important in the analysis of clinical phenotype. Firstly, information about the onset of the illness was probably the most accurate in interviews with relatives. The timing of the development of pyramidal signs was very difficult in a patient who already had marked dementia and was bed-bound, but their first symptom, be it visual upset, cognitive impairment or anything else was often well remembered and clearly marked in case notes.

Secondly, the onset of the illness might give some important information about where the pathological process began. This might be related to the strain of the agent or might be due to other factors, not yet known. The Brownell-Oppenheimer and Heidenhain variants are historically well recognised and should be recognizable from the case history. Establishing that each phenotype is related to a different protein isotype would support the working hypothesis of this study.

Dementia, classically "rapidly progressive", is one of the defining characteristics of sporadic CJD. 6 cases had a duration of illness of greater than 2 years and so were excluded as possible cases because of the strict diagnostic criteria. However, these cases still had a rapidly progressive dementia, deteriorating significantly in function in less than a 1 year period.

One of the MM-1 cases did not clearly have a dementing illness and so was classified "don't know". 41 cases (41%) presented with purely dementing symptoms, the majority of these were MM-1 cases (43% of this group). All of the VV-1 cases and 80% of the MM-2A cases presented with dementia as the only clinical feature. This latter result achieved statistical significance, although the group is small, casting doubt on the significance of this.

A purely cerebellar onset was only seen in the MM-1, MV-2A and VV-2A groups, 14 cases (14%) in all. Again the figures are small but a third of the MV-2A group (4 cases) presented with this onset and a fifth of the VV-2A group (3 cases) had purely cerebellar symptoms at onset. 13% (7 cases) in the MM-1 group presented with only cerebellar symptoms and signs.

Perhaps more significant, a purely visual onset was seen in only 11 cases (11%) and all of these apart from 1 case were MM-1 cases (19% of this group). One VV-2A case also presented with purely visual symptoms. They complained of diplopia from the onset, progressing to complaints of visual blurring. However, another VV-2A case complained of diplopia at onset. The second case did not complain of loss of or deterioration of vision however, although, like the first case they complained of dizziness and were seen to be poorly co-coordinated. Both developed cortical blindness.

This illustrates the difficulties in classification. The first VV-2A case would be considered to be a Heidenhain variant. It is less clear in the second case: Patients sometimes describe a blurring of vision as double vision. Dizziness (not vertigo) and poor coordination can be seen if the vision is poor, but can also be seen in cerebellar problems. Therefore this case could not be definitely classified as a Heidenhain variant. This is unfortunate, as a second Heidenhain variant in the VV-2A group would make an association of this presentation with the MM-1 group less significant.

In the MM-1 cases it was difficult to establish if these cases presented with cortical blindness (i.e. classical Heidenhain variant). They usually presented with visual symptoms first and at least one case had cataract surgery before other dementing features became apparent and a diagnosis of sporadic CJD was made. All but 3 of these cases were noted to develop cortical blindness in the course of their illness. Once again, failure to note the presence of cortical blindness does not necessarily mean it was absent.

One of the hypotheses relating to genotype and protein isotype, and the clinico-pathological phenotype of sporadic CJD, was that the well-described variants of the illness might relate to the different subgroups. However, a purely dementing onset was seen in some cases from each group. Similarly, the Brownell-Oppenheimer variant was seen in the MM-1, MV-2A and VV-2A groups. Finally, the Heidenhain variant was seen in only the MM-1 cases and 1 or possibly 2 VV-2A cases. This would require analysis of future cases to assess if this is a chance result.

Parchi et al.[399] also analysed the clinical signs at onset, although they did not analyse the strict definition of a sole presentation used in this study. They noted that cognitive impairment was invariably present in both VV-1 and MM-2 cortical cases, but was absent in most VV-2 cases. This study also identified dementing features at onset in 90% of the MM-2A cases and 100% of the VV-1 cases. However, 54% of the MM-1 cases, 20% of MV-1 cases and 50% of MV-2A cases all had dementing symptoms from the onset of the illness as well as 47% of the VV-2A cases.

They also noted ataxia was common in the VV-2A and MV-2A cases, but lacking in the VV-1 and MM-2 cortical groups. We also found similar trends, although less marked; 60% of MV-2A cases and 33% of VV-2A cases had cerebellar signs as one of the symptoms at onset, in contrast to 81% and 100% of the Parchi cases. They noted visual signs only in 26% of MM-1 cases and 12% of MV-1 cases, in contrast to the findings of this study.

A stroke-like onset has been described in some cases of sporadic CJD. In this group it was seen in 3 cases, all MM-1. A prodrome was seen in less than one third of cases in each group except for the MV-1 cases in whom 3 cases (60%) had a prodrome. Dizziness was seen in the MM-1 group (26%) and the MV-1 group (20%). However 47% of the VV-2A group experienced dizziness. This may be a reflection of the preponderance of cerebellar symptoms in this group. All the cases who complained of dizziness developed cerebellar problems. A disturbance of hearing was unusual and occurred rarely in the MM-1, MV-2A and VV-2A groups.

Alien limb was also looked for because it has been described as a presenting symptom of sporadic CJD, however this was not seen as a presenting symptom in any of the cases in this series. Indeed, it was only seen in 3 cases in total.

ii): Clinical Symptoms and Signs During Evolution

Dementia

If each symptom is considered in turn, dementia appeared early on in the majority of cases but half of the MV-2A cases and 40% of the VV-2A cases did not develop dementia until some months into the illness. Unlike the Parchi study,[399] dementia was a feature of the illness in all cases apart from one MM-1 case, in whom there was insufficient information to be sure. In addition it usually appeared early on in the illness. Parchi et al.[399] noted dementia was mild and late in the VV-2A group. This was not seen in this study.

Cerebellar problems

Cerebellar symptoms and signs were noted in nearly two thirds of cases. They occurred early on in 60% of the VV-2A cases and 83% of the MV-2A cases and 41% of the MM-1 group. It is possible that the former 2 groups tend to present earlier with cerebellar signs. If the presence of cerebellar signs and symptoms alone is looked at, 92% of the MV-2A group and 79% of the VV-2A group had these

problems. They were also present in 63% of the MM-1 group and 60% of the MV-1 group. However less than a third (30%) of the MM-2A group and none of the VV-1 group had cerebellar problems. The figures could easily be skewed by the large number of cases in which there was no note made of cerebellar problems (14%) or in whom there was insufficient evidence to be sure whether problems with gait were due to disturbance of the cerebellar system (23%).

Parchi et al.[399] also found that the VV-2A and MV-2A cases were the most frequently affected by cerebellar symptoms and signs. In addition, they noted the lower frequency in the VV-1 cases. They found that the MM-2 cortical cases rarely had cerebellar signs but the MM-2 thalamic cases did. As will be discussed later, the thalamic/ cortical distinction was less apparent in this study, however, cerebellar problems were present in only 30% of MM-2A cases.

Visual problems

There was a similar problem with lack of information about visual problems. Probably because the cases were often not seen until they were severely demented and bedbound, by which point it would not be possible to establish if cerebellar problems were present and it would be difficult for the patient to report visual problems. Cortical blindness may have developed following the visit, and may be difficult to assess in the later stages of the illness; and so there may be under-reporting of this sign. The presence of visual symptoms did not necessarily mean that cases went on to develop cortical blindness, although this may simply be an artifact of under-reporting of cortical blindness. In the majority of all cases (54%) there is insufficient information to comment on the visual symptoms and indeed their presence was only noted in 46%.

As stated earlier, a purely visual onset was seen in 19% of the MM-1 cases and in 37% of this group visual symptoms appeared early on. In contrast a smaller number of other cases were noted to have visual problems, 40% of MM-2A, MV-1 and VV-

2A cases and 8% of MV-2A cases. These symptoms usually appeared early or midway into the illness. Visual symptoms were not noted in any of the VV-1 cases. Cortical blindness was only a feature of a small number of cases, from 8% of the MV-2A cases to 40% (i.e. 2 cases) of the MV-1 cases. This is likely to be due to under-reporting.

Parchi et al.[399] did not comment on cortical blindness separately. They included visual loss, visual field defect, visual distortion, abnormal colour vision and cortical blindness in the one category. These symptoms were only noted in the MM-1 and MV-1 cases.

Sensory Symptoms

Sensory symptoms are of interest because of vCJD in which they are a prominent feature. It is possible that sensory symptoms are over represented in cases of sporadic CJD since the description of vCJD, because a member of the NCJDSU may ask more specifically about them. However many of the cases in this series occurred before vCJD was described in 1996, and so this is unlikely. In all, 14 cases complained of sensory symptoms. In 10 of these cases this was in the early stages of the disease. There was no particular preponderance of sensory symptoms in any of the groups, occurring in 13% of MM-1, 30% of MM-2A, 20% of MV-1, 8% of MV-2A and 14% of VV-2A cases. No VV-1 cases had a sensory complaint noted.

The nature of the symptoms was similar to that seen in vCJD i.e. burning and tingling or coldness, perhaps suggestive of a thalamic origin. However it was not often clear from the records whether these symptoms were persistent or not.

Hallucinations

29% of all the sporadic cases had hallucinations. These were nearly always visual in nature. In the majority of cases they developed mid to late into the illness.

Hallucinations were not seen in the MV-1 and VV-1 cases. In contrast they were seen in 40% of the VV-2A and 58% of the MV-2A cases.

Delusions

Delusions were seen in a small number of cases, 8% of cases in total. Again no particular group was affected. The delusions tended to be persecutory and again did not appear until some months into the illness.

Clinical signs

It is more difficult to be definitive about clinical signs. As stated previously it is possible that patients developed certain signs after a member of the NCJDSU made a visit. In some cases many of the signs were classified "don't know" because there was insufficient information to be sure they were present, underestimating the incidence of each sign. For this reason it is impossible to make any statistical analysis of the results and it is possible to comment only on trends.

Pyramidal signs were seen in over half of all cases. It is possible that there were fewer cases with pyramidal signs in the MV-2A group as only 17% had pyramidal signs. About a third of all cases had extrapyramidal signs. None of these were MV-1 cases, and this may reflect a difference in this group. Lower motor signs are uncommon in sporadic CJD although historically they were believed to be present in an amyotrophic variant of the condition. In this series they were seen in only 5 cases (5%).

In general as any form of CJD progresses the disease tends to be associated with the development of hypertonia, (sometimes in the form of gegenhalten), hyper-reflexia and primitive reflexes, seen in this series in 73% of cases. These signs would not be particularly useful as discriminators between different forms of the disease or indeed between different subtypes of sporadic CJD. Nearly two thirds of cases went on to

develop akinetic mutism. Again this is probably under-estimated because cases may well have developed this sign after a member of the unit saw them.

The type of movement disorder may also be helpful. Myoclonus is a feature of all forms of CJD and develops at some stage in the majority of cases. In this series it was seen in 87% of all the cases, usually in the late stages of the disease. Myoclonus was only seen early in the disease in a few cases, 1 of these was an MM-2A case and the remaining 6 (11%) were MM-1 cases.

31% of this series experienced choreaform or dystonic movements. 43% of the MM-1 cases were affected but only 1 or 2 cases in the other groups apart from the VV-1 group in which 2 cases (67%) and the VV-2A group, in which 4 cases (27%) were affected. There was often little information about the nature of the movement disorder in the case notes.

Parchi et al.[399] noted that myoclonus was a prominent feature of the MM-1 and MV-1 cases but less so in the other groups. This was not noted in this study, in which over 75% of each group was noted to develop myoclonus. They also noted myoclonus appeared earlier in these 2 groups whereas in this study only a small proportion of the MM-1 and MM-2A cases developed myoclonus early on and in the other cases it tended to be noted later on in the illness. Like this series, pyramidal and extrapyramidal features were present in a percentage of each group and there were no remarkable trends.

E: INVESTIGATIONS

i): EEG

A decision was made to include a highly suggestive or typical EEG in the criteria for "probable" sporadic CJD. This was for several reasons: From the perspective of clinical management, a highly suggestive EEG is often sufficient to support a diagnosis of sporadic CJD in the appropriate clinical setting. However, for the

purposes of epidemiological surveillance clearly strict criteria would have to be employed to ensure a diagnosis of "probable" CJD is as secure as possible.

Secondly, the EEG classification differs from study to study in the literature, and is sometimes less strict than the criteria used for a "typical" EEG in this study. It therefore seems not unreasonable to include a "highly suggestive" EEG, as it would often be classified in the literature as sufficient to support a diagnosis of sporadic CJD. However, in all possible cases the EEG was reclassified personally and according to stated criteria, avoiding inter-individual variation. Retrospective analysis of the EEG in other studies has shown that the classification of the records was at times inaccurate. Since the advent of 14-3-3 protein it is conceivable that the EEG is studied more strictly as CSF analysis for brain specific proteins provides an alternative means of supporting a diagnosis.

In this study a positive EEG was seen in 59% of MM-1 cases. 13 cases did not have an EEG result available, or I did not see it. If those cases are excluded, the EEG was positive in 78% of MM-1 cases. Only 1 other case had a highly suggestive EEG, an MV-1 case. No other group had a positive EEG.

Parchi et al.[399] found a positive EEG in 80% of MM-1 cases and 71.4% of MV-1 cases. Zerr et al.[455] had similar findings, with a positive EEG in 80% of MM-1 cases and 75% of MV-1 cases. They also identified 1 MM-2A case with a positive EEG. Neither study comments on the exact criteria used in the analysis of the EEG and both classify a positive test as showing positive sharp wave complexes on the EEG.

One of the cases in the MV-1 group did not have an EEG result available. However, of the 3 other cases, 2 had a suggestive or non-specific EEG and one record was not seen by me but was reported as non-specific. It is unlikely that there has been under-reporting of the EEG result in this study.

One other consideration is the timing and repeating of EEGs. It is known that with serial recording of EEGs in sporadic CJD the record often becomes more typical with time. It is conceivable that in an atypical case of CJD the diagnosis would not be considered. If the first EEG was not suggestive then the test might not be repeated. Very few cases in this study had more than one EEG. To be sure that the "atypical groups" were not associated with a typical EEG, serial EEG recordings throughout the illness would have to be performed.

ii): 14-3-3 PROTEIN

14-3-3 protein has been used increasingly in recent years to support a diagnosis of sporadic CJD. Because the test has only been validated relatively recently, many of the cases in this study have not been analysed as they date back to before 14-3-3 analysis was available.

Less than a third of the cases were tested: The MM-1 cases were positive in 80% (15 cases). The remaining 3 MM-1 cases that were tested had an equivocal result. Experience with 14-3-3 analysis of cases not included in this study, suggests that retesting cases later in the disease sometimes gives a positive result. The VV-2A cases were positive in 100% (7 cases tested). In the remaining groups there were only isolated results but only 1 MM-2A (25%) and 1 MV-1 case (33%) had a positive result.

In contrast, Zerr et al. [455] found that all groups had a positive 14-3-3 analysis in 96%-100% of cases, except the MV-2A group in whom 3/10 cases (30%) were positive. They tested 107 cases, however like this study, the majority of cases tested were in the MM-1 and VV-2 groups. Parchi et al.[399] did not comment on the use of 14-3-3 analysis.

Analysis of S-100b showed that this protein was raised in all but 2 cases. The level of the protein was often raised despite a negative 14-3-3. Current opinion is that this

test is less specific however and a high S-100b level in the absence of a positive 14-3-3 result could not be taken to be supportive of a diagnosis of sporadic CJD. None of the other studies comment on the use of S-100b. Zerr et al.[455] use NSE however we have also found this a less specific test at the NCJDSU, and no longer use it.

iii): MRI

Finally, high signal in the basal ganglia on MRI imaging has been reported in 79% of sporadic CJD cases.[8] An MRI was available for review at the NCJDSU in 44 cases (44%). The scan was positive in 14 cases (32%). Like 14-3-3 analysis, there are insufficient MRIs available in each group to make a useful statistical analysis. The scan was positive in 3 MV-2A cases (75%) and 1 VV-1 case (50%). Less than half of the cases in each of the other groups had a positive scan.

Most of the scans were requested retrospectively. In many cases, several years after the imaging was performed, and several years before Finkenstaedt et al.[8] published their findings on the use of MRI in sporadic CJD. Only 1 case was reported to have shown high signal in the basal ganglia by the hospital in which the patient was based. It is likely that there will be more positive reports now that MRI changes in sporadic CJD have been published and more modern scanning techniques are available, using different weightings. It is well recognised that different MRI machines can have vary in brightness in areas of the brain, and more modern machines may be more uniform.

Zerr et al.[455] reported the results of 43 scans (40% of the cases in their study). 70% of the scans were positive. Again many of the groups only had scans in 1 or 2 cases in each subgroup available for analysis. They were encouraged by the results because 2 MV-1 cases (100%), 8 MV-2A cases (89%) and 7 VV-2A cases (70%) were positive. German surveillance has been in place since June 1993 and the timing of the MRI images is not clear; i.e. if most of the scans post-date Finkenstaedts' paper on the use of MRI in sporadic CJD.[8]

G: PATHOLOGY

The pathological results for this study were extrapolated from pathological reports on each case. In the majority these were the reports issued by the NCJDSU, and each case had been examined by the NCJDSU, with 2 exceptions. However this was not a quantatative pathological study, unlike the Parchi study[399] and the study by Macdonald et al.[447] (who looked at genotype alone). The results are based only on comments about the distribution of spongiform change and other findings in a routine pathological report.

Spongiform change was prominent in most cases. The occipital lobe was involved extensively in the MM-1 cases, although many of these cases did not have prominent occipital lobe symptoms such as cortical blindness. The frontal and occipital lobes seemed to be involved more in the MM-2A cases and the frontal, occipital and temporal lobes in the MV-1 cases. In the MV-2A cases and both VV groups the basal ganglia were the areas most affected by spongiform change. Only the heterozygotes had kuru-type plaques present prior to staining.

There were exceptions however; In 2 MM-1 cases the changes in the basal ganglia were more marked than in the cortex. 1 of these cases had no extrapyramidal features but was noted to have dystonic movements of the mouth and limbs. The second case did not have evidence of any movement disorder although there was some gegenhalten on examination. The presentation in this case was of the Heidenhain variant. This case also had prominent changes in the thalamus, not seen in any of the other MM-1 cases. Sensory symptoms were not reported.

There did not appear to be a relationship between the areas of more pronounced spongiform change and the presence of symptoms and signs: In the MV-2A group spongiform change was more marked in the basal ganglia than in the cortex in 9 cases (75%). Extrapyramidal signs were not more prominent in this group. In the

VV-2A cases spongiform change was again more marked in the basal ganglia and changes in the cerebellum were not prominent. However this group had prominent cerebellar features in many of the cases and again, extrapyramidal signs were not particularly prominent.

Status spongiosis was seen in 7 cases, although other cases had areas of severe confluent spongiform change. This included 5 MM-1 cases, 1 MV-1 case and 1 VV-1 case. The duration of illness of these cases ranged from 1-54 months. The MM-1 cases were of 1, 4, 15, 17 and 17 month's duration. The VV-1 case was of 11 months duration and the MV-1 case was of 54 months duration. The median duration in the MM-1 cases with status spongiosis was 15 months, in comparison to an overall median duration in this group of 3 months. This suggests that status spongiosis is more likely to be seen in cases of long duration but can occur also in cases of short duration. An MRI was available for review in only 1 of these cases, in whom widespread severe atrophy was noted.

Immunohistochemistry changes were attributable to the genotype and there was no real difference in the patterns seen between the 6 subgroups. The deposition pattern in each area of the brain seemed to reflect the degree of underlying spongiform change. The changes in the cortex and basal ganglia tended to be reticular and perivacuolar. In the cerebellum, the changes were reticular in the molecular layer and granular in the granular layer. The VV-2A cases had a more linear deposition pattern in the cortex.

The MV cases seemed to be associated with the deposition of plaque-like material. In the MV-2A cases these seemed to be kuru-type plaques, whereas in the MV-1 group the deposits were more plaque-like. Plaque-like deposits were also seen in the VV-2A cases. Tissue was available in only two of the VV-1 cases, neither of whom had plaque-like deposits.

Rather surprisingly, a single MM-1 case had plaque-like deposits in the basal ganglia. They had a typical illness but did develop extrapyramidal rigidity and choreiform movements as the illness progressed. Three of the MM-2A cases also had some plaque-like deposits. These were deposited in the cerebellum in all cases. Two of these cases were not seen in life and CJD was considered unlikely, and there is not a great deal of clinical information available. All were unsteady during the illness and two were noted to have definite cerebellar signs.

There were some cases with an unusual pathology. Three of the MM-2A cases were atypical: 1 case had pathological changes of the thalamic variant (see later). 1 case had unusual pathology in the occipital and temporal cortex. The clinical features of this case were a little unusual. The patient presented with confusion and demented over an illness of 17 months. Investigations were not supportive of a diagnosis of sporadic CJD. There were no thalamic features to the illness. She complained of visual upset after some months but was not noted to be cortically blind.

Two cases had plaque-like PrP deposition suggestive of GSS, an MM-2A and an MV-2A case. Very little information is available on the MM-2A case but what is known suggests that the illness was predominantly of a progressive dementia. The patient had been treated with neuroleptic medication for many years. Dystonic movements were a prominent feature but it is not known if these movements were a side effect of medication. The MV-2A case had a prolonged illness, also with prominent dementia and few other signs noted. Investigations were not supportive of sporadic CJD.

In addition one MM-1 case had the changes of the panencephalopathic variant reported initially although the changes were later reported to be of status spongiosis. (The duration of illness was 17 months.) Another was originally reported as Alzheimer's disease and sporadic CJD was not diagnosed until PrP immunohistochemistry was developed. In this case the clinical picture was

suggestive of sporadic CJD and the EEG was reviewed by myself and classified "typical". The duration of illness was 5 months.

Parchi et al.[399] comment on the "thalamic variant" of sporadic CJD and state that these cases are a subgroup of the MM-2A cases. Half of their MM-2A cases fall into this category. One case was identified in the MM-2A group whose pathology was suggestive of the thalamic variant although the clinical picture was not entirely typical of this variant. In 4 of the MM-1 cases there was prominent gliosis and neuronal loss in the thalamus, in 2 of these this was the most prominent feature. However, spongiform change was seen in the cortex and so the pathology would not have been entirely consistent with SFI. The clinical picture was not suggestive of SFI in any of these cases.

Overall, the changes identified by Parchi et al.[399] are probably broadly similar in this study. However, they employed a quantitative approach. In contrast, the pathological findings in this study are observational only and so the two studies are not directly comparable.

H: DIAGNOSTIC CRITERIA.

i): Probable and Possible Cases

The MM-1 cases are typical of sporadic CJD. This is reflected in the fact that 69% of this group would have been classified as probable cases of sporadic CJD during life. 14 cases in this group were classified as possible cases. This means that clinically these cases were suggestive of sporadic CJD but diagnostic tests were not supportive. The EEG was not typical in any of the cases. Previous studies have shown that the EEG evolves during the illness and it is conceivable that the EEG may have become more typical if it had been repeated later in the disease, had it been available. Many of these cases were seen before 14-3-3 protein analysis was available. It is also possible that this result may have been positive. One case had an equivocal 14-3-3 result and subsequent experience suggests these cases become

positive when retested. Finally, one case had a positive MRI result, which may be of significance in the future.

The other group to include a significant proportion of probable cases is the VV-2A group. Again, the clinical features of this group were suggestive of sporadic CJD but the EEG was unhelpful. Before the advent of 14-3-3 protein these cases would have achieved a classification of "possible" in life, however in all 7 cases tested, a positive 14-3-3 result elevated them to "probable cases". Seven further cases were "possible" and so if 14-3-3 had been tested it is conceivable that they would also have been "probable cases". (Zerr et al.[455] also found this result was positive in 100% (15 cases) tested.)

ii): Possible cases

Only 2 further cases were classified "probable" sporadic CJD, one MM-2A case and one MV-1 case. However, 70% of MM-2A cases, 60% of MV-1 cases and 75% of MV-2A cases were "possible". This means that clinically all these cases were suggestive of sporadic CJD, despite the fact they were in the "atypical" groups. Clearly these cases would have been classified "probable" if the results of investigation had been supportive of a diagnosis.

Of the 7 "possible" MM-2A cases all had an EEG performed but it was negative in each case. 3 had 14-3-3 protein assessed and all were negative, 4 had an MRI scan available for review and it was positive in 1 of these cases. This might suggest that even if all 3 investigations had been available in all 7 cases, the results may not have elevated the cases to a "probable" classification.

In the MV-1 cases the EEG and MRI seemed to be generally unhelpful but 14-3-3 was only analysed in one of the 3 possible cases. It showed a trace of positive protein; again with subsequent experience this may have been positive had it been

repeated. Hopefully, as 14-3-3 protein is used more widely, this may prove to be a helpful investigation in the MV-1 cases.

In the MV-2A cases 9 were "possible" sporadic CJD. The EEG was unhelpful in all of these cases but 14-3-3 protein was not analysed in any of them and further information on the use of this investigation in the future may prove that it is of some use. In addition the MRI was positive in the 2 cases that were scanned and further analysis of the use of this investigation is indicated.

Finally the VV-1 group consists of only 3 cases and although the NCJDSU registrar saw 2 of them in life, the clinical information available does not point to a particular pattern of illness. In the single "possible" case 14-3-3 protein and MRI were not performed and the EEG was not supportive.

As suggested previously, it is likely atypical cases will have been particularly selected by the unit for analysis of the PrP isotype. This may reflect the relatively low number of "positive" investigations. These cases are less likely to have been considered "probable" sporadic CJD during life, prompting autopsy and PrP analysis.

Parchi et al.[399] suggested that the EEG was a useful investigation in the MM-1 and MV-1 groups. This study has only shown that it is helpful in the MM-1 group, although 1 MV-1 case (20%) also had a highly suggestive EEG. They do not comment on the use of 14-3-3 protein and MRI in sporadic CJD.

However, Zerr et al.[455] analysed the use of all 3 investigation in the various subgroups. They also found that the EEG was helpful in the MM-1 and MV-1 groups. 14-3-3 protein was useful in all the groups except the MV-2 group. This study cannot support these findings in any of the groups except the MM-1 and VV-2A cases, in whom the majority had a positive result. However, 14-3-3 analysis is

more widely available now and subsequent analysis of future cases may support the findings of Zerr et al.[455] If this is the case, then significantly more cases may achieve a "probable" diagnosis during life.

Finally, Zerr et al.[455] also suggested that a positive MRI scan might be a useful tool in cases that had a negative EEG or 14-3-3 result. To date, the experience of the NCJDSU has not shown the MRI to be such a useful diagnostic tool. The MRI was only positive in 43% of cases, fewer than the 79% reported by the German group.[8] However, as suggested previously, many of the cases in this study were seen many years ago. MRI has become more widely available over the past 15 years and different weighting techniques are now in use which may increase the sensitivity of the test. In addition, the brightness of different structures varies between machines.

It is possible that this is the reason for the lower number of positive scans in this study and that now abnormalities have been described, the sensitivity of the test may increase. The specificity of the test has not been reported, and further prospective evaluation of the use of MRI in sporadic CJD would be necessary before it could be included in the diagnostic criteria.

Were the MRI to be included how much further information would it provide? If, as it has been suggested in studies to date, the EEG is only a useful diagnostic tool in MM-1 and possibly MV-1 cases, then a significant proportion of cases would not be classifiable as anything more than a "possible" case prior to the use of 14-3-3 protein. (40% of this study.)

14-3-3 protein has been shown to be a useful diagnostic tool in sporadic CJD and it seems likely that it will support a diagnosis of sporadic CJD in many of the "atypical" subgroups that the EEG has proved unhelpful in. To date, 14-3-3 has been shown to be a highly sensitive and specific tool in the appropriate clinical setting. However, some of the cases in these other subgroups tend to be "atypical" and a

further investigation might lend more support to a diagnosis of sporadic CJD. This could prevent a patient proceeding to brain biopsy, a highly invasive investigation.

Finally, Zerr et al.[455] found 14-3-3 analysis was only positive in 30% of the 10 MV-2A cases tested. It was also negative in the 1 MV-2A case analysed in this study. However, they found the MRI was positive in 89% of the 9 cases they tested. In this study 75% of the 4 MRIs performed in the MV-2A were positive. This suggests that the MRI may yet prove to be the most helpful investigation in this subset.

iii): CJD unlikely cases

Surprisingly, very few of even the "atypical" groups had many cases that would not have fulfilled "possible" criteria: i.e. the clinical picture fitted with sporadic CJD but the results of investigation were not supportive. The clinical features of these cases are described in Chapter 8: Results.

There are three cases within the MM-1 group who were classified as "CJD unlikely". In two of the cases the clinical syndrome sounds suggestive of sporadic CJD but there is insufficient clinical information to be sure that enough symptoms and signs were definitely present; may of them were classified "don't know". In one case the EEG was highly suggestive and so they would almost certainly have been a "probable" case, had there been more certainty about the clinical syndrome. In the second case the MRI was positive but the other investigations were not supportive. At most this case would have been "possible" unless further investigation was embarked upon.

There is less information available on the final case. Because of lower motor signs a diagnosis of motor neurone disease was made during life. Lower motor signs have been reported in 6% of sporadic CJD cases.[121] There were also cerebellar signs evident from the onset and double vision developed after several weeks of an illness of 2 months duration. Dementia was not remarked upon and sensory symptoms were

prominent, the patient complained of burning feet. There is no information about the terminal stages of the illness. Finally, the EEG was non-specific after 1 month of illness. 14-3-3 analysis and MRI examination were not performed. This case cannot be classified higher than "CJD unlikely".

One VV-2A case was classified "CJD unlikely". Like most of the other cases in this group, she also had a cerebellar presentation, going on to develop dementia. There is insufficient clinical information about whether or not other signs developed to make a "possible" diagnosis and the results of investigations were not supportive of a diagnosis.

Of the 8 other cases, 6 were classified as "unlikely" because the duration of illness was greater than 2 years. 4 of these would have other wise been considered "possible" cases if the duration of illness had been shorter. Another 2 cases were not seen by a member of the unit and there was insufficient information to be sure of the presence of the various signs to confirm a "possible" case (and over-riding the illness duration of greater than 2 years).

Of the two cases of less than 2 years duration, there was insufficient information in one and in the other although they were seen during their illness, there is again insufficient information but from the clinical details they may also have been "possible".

This seems to illustrate that the diagnostic criteria are relatively sensitive and that the main exclusion criterion is that some of the cases are greater than 2 years duration. In the majority of the other cases there is simply insufficient information to be certain of the presence of various symptoms and signs. For those that were classified as "CJD unlikely" it is conceivable that a positive 14-3-3 protein or MRI result might make the diagnosis be considered despite a duration of greater than 2 years.

I: CLINICAL FEATURES OF EACH GROUP

The study of protein isotype in sporadic CJD has evolved from some of the original transmission studies on scrapie, which suggested that different clinical syndromes were transmissible, attributable to different strains of the scrapie agent. Subsequent work in TME and the various TSEs has suggested that there are structural variations within the PrP^{res} molecule and that these properties are transmissible, suggesting different strains of the agent. Western blotting techniques have identified two distinct protein isotypes in sporadic CJD. These isotypes are believed to be surrogate markers of strain. If this is the case are there clinically and pathologically discriminating features to each group?

i): MM-1

The MM-1 cases make up by far the largest group. The clinical and pathological phenotype is similar to well-described classical sporadic CJD. In general, the cases are of short duration and have a predominantly dementing illness. However Brownell-Oppenheimer cases are seen in this group (17%). All but one of the cases presenting with visual symptoms were MM-1 and although there was insufficient information to call all of these cases the Heidenhain variant, there seemed to be a trend towards this phenotype. The EEG and 14-3-3 protein were helpful in the majority of cases.

ii): MV-1

The MV-1 cases also had a short duration of illness. The median age at onset was rather older than that seen in sporadic CJD at 75 years. A prodrome seemed to be more common in this group. Dementia was an early feature of all the cases. Sensory symptoms, hallucinations and delusions were not common and there were no unusual features on examination. The EEG was typical in many of the cases reported by Parchi et al and Zerr et al. This was not supported in this study. 14-3-3 analysis was helpful in many of the cases reported by both these groups. Pathological changes were typical of heterozygotes, with prominent kuru-type plaques.

iii): VV-2A

The VV-2A cases also tended to be of shorter duration. The median age of onset was not significantly different from the MM-1 cases. Cerebellar symptoms were prominent in this group and these were present from the onset in 20% although they were also present from the onset in 13% (7 cases) of the MM-1 cases. (Brownell-Oppenheimer) There was also at least one case with a purely visual onset. Dizziness was seen in 40% of this group, possibly a reflection of the prominent cerebellar problems. In addition hallucinations were prominent, occurring in 40%. The EEG was not typical but 14-3-3 was helpful in all the cases tested. Pathological changes were typical of Val homozygotes, with spongiform change being more typical in the basal ganglia and plaque-like deposits present on immunohistochemistry.

iv): MM-2A

The MM-2A cases had a younger median age of onset and longer median duration of illness. None of the cases were of duration less than 6 months; indeed half of them were of duration greater than 1 year. Dementia appeared in all cases and in 90% this was an early feature of the illness. Cerebellar signs were reported in only 30% but 50% were classified "don't know" so it is not clear if cerebellar features are significantly underrepresented. Visual symptoms and cortical blindness were a feature of the group but none of the cases had a true Heidenhain or Brownell-Oppenheimer presentation. Sensory symptoms however were present in 30%.

Investigation of the cases in this study did not show evidence that the EEG, 14-3-3 analysis or the MRI is particularly helpful. (Although Zerr et al.[455] have suggested otherwise.) Pathological changes were unusual in several of the cases, including one thalamic variant however there were no particular distinguishing features to the group.

Parchi et al.[399] divided the MM-2A group into two subsets, a cortical and thalamic variant. Zerr et al. did not note this distinction and it was not apparent in this study. The cortical variant is characterised by a prominent dementia. Visual and cerebellar signs are typically absent. The thalamic variant has previously been called fatal sporadic insomnia, characterised by progressive insomnia and psychomotor agitation at night. Neuropathology shows prominent thalamic atrophy. The thalamic variant made up 50% of the Parchi MM-2A group.

v): MV-2A

The MV-2A group also had a longer duration of illness and again 50% of cases were of duration greater than 1 year. Dementia presented early on in the majority of cases but 4 cases (33%) had a Brownell-Oppenheimer presentation and cerebellar features were present in 92%, greater than any other group. In this group hallucinations were common, present in over 50%. There were no notable features on examination.

EEG and 14-3-3 analysis were unhelpful but future evaluation of the MRI may show that this investigation is of diagnostic value. Again, the pathological changes were more in keeping with changes related to genotype and there were no findings to distinguish the group particularly from the MV-1 cases.

Parchi et al.[399] report similar findings in their MV-2A cases and an isolated case report of an MV-2A case also noted prominent cerebellar symptoms.

vi): VV-1

Finally in the VV-1 group there were only 3 cases spanning a wide age range. The median age of onset was only 41 years and the group all had a long duration of illness (8-29 months). All the cases presented with a purely dementing illness. There were no cerebellar or visual symptoms or signs noted in any of the cases but 2 of them (67%) had a movement disorder. One case had a positive MRI (50%) but

investigation was generally unhelpful. The pathological changes were again more related to genotype and there were no unusual features within the group.

Parchi et al.[399] also noted similar features with prominent dementing symptoms and the lack of cerebellar signs. However, their cases did not have any form of dyskinesia other than myoclonus. They noted particularly severe pathology in the cortex and striatum. This was present in only 1 of the cases in this study but pathology was only present in 1 other case and so it is not possible to draw conclusions on the pathological changes.

J: GENERAL COMMENTS

These changes may suggest a trend towards distinct clinical phenotypes in each group. There do seem to be some consistencies in the findings of this study and those of Parchi and Zerr. The most consistent finding is the difference in duration of illness; most of the short duration cases are in the MM-1 group, but it is possible that the changes seen in this study are related to genotype alone. In particular, the pathological changes seemed to be only associated with genotype, other than certain atypical cases. (See later)

At this stage, it would not be possible to assess the clinical characteristics and investigative results of a case of probable sporadic CJD and make a confident judgment of the genotype and protein isotype subgroup. In most cases the case would be typical of classically described sporadic CJD and would be considered an MM-1 case. Based on the proportion of cases that are in the MM-1 group, there is a high probability that this would be correct. However, each group contained some cases that were suggestive of typical sporadic CJD, which might easily have been suspected of belonging to the MM-1 group. This was particularly true in the VV-2A and MV-1 groups.

If a cerebellar onset was present or cerebellar signs were prominent, the case might be expected to be in the VV-2A or MV-1 group, but many of the MM-1 cases and indeed cases in all the other groups had prominent cerebellar signs. Again, there would be considerable uncertainty in trying to predict to which group the case belonged.

At this stage, the most consistent finding is that atypical cases, usually of long duration, and often with unsupportive investigation results, would be in a group other than the MM-1 group. Even this statement must be qualified, as there are several "atypical" cases within the MM-1 group, who might easily have been suspected of being in an "atypical" group.

It seems likely that Parchi et al.[399] looked at the 6 different groups and attempted to attribute distinct clinical features to each group. In this study, the case notes were examined once the genotype and protein isotype had been identified, although at that stage each clinical symptoms and sign was not specifically examined to see if they fitted with the study by Parchi et al.[399] However, the data were then examined to try and attribute trends to the genotype and protein isotype subgroups. It would be important to try and allocate several cases to each subgroups, blinded to their genotype and isotype.

Clinically, this is of little importance to the patient or their family. From their perspective they are usually predominantly interested in reaching as certain a diagnosis as possible of "probable" sporadic CJD in life. From the clinicians' point of view, they are keen to exclude other treatable causes of a rapidly progressive dementia. The atypical cases of sporadic CJD might present a cause for concern. These patients may proceed to invasive techniques such as brain biopsy if there is sufficient doubt about the diagnosis.

In fact, rather surprisingly, the majority of atypical cases in this study were considered at least "possible" CJD. In those that did not reach this classification, there was usually insufficient information to be sure of the presence or absence of the clinical signs. In those that were considered "possible" many of them were seen and diagnosed before the advent of 14-3-3 protein and the description of the MRI changes in a series of sporadic cases.

It seems likely, based on the findings of Zerr et al.,[455] that many of those cases might have had a positive 14-3-3 result, had the test been available. These cases would therefore have been classified "probable" sporadic CJD. This classification would be associated with a specificity of about 95%. There are of course reports of false positive results with 14-3-3 analysis, but many of the conditions in which a false positive result was obtained, should be distinguishable clinically from even atypical sporadic CJD.

It is possible that the MRI will yet be validated as a useful investigative tool, although the specificity of this test also needs to be ascertained. Assuming the EEG is usually not typical except in the MM-1 cases. A "positive" MRI in association with positive 14-3-3 analysis may give sufficient confidence of the diagnosis in an atypical case, without proceeding to brain biopsy. In the MV-2A cases the limited data to date suggests that the MRI might be the most useful investigation.

K: INTERMEDIATE CASES.

There were 3 intermediate sporadic cases. No other group has reported cases of intermediate mobility. It is difficult to know the significance of this. It is possible that the intermediate mobility was due to technical difficulties. In particular, copper binding can affect the mobility of the protein and there may have been technical problems in removing the copper from these cases. It has been shown that the protein molecule unfolds more if there is less copper present. In theory if the intermediate cases were run again after treatment with E.D.T.A., which would bind copper, they

would be of type 1 mobility. This theory does not hold if the cases proved to be of type 2 mobility.

Other possibilities are that the underlying mechanism of disease is different in these cases. Alternatively, copper binding in different regions of the brain varies and it may relate to the area of brain sampled. Presumably there are also differences in copper binding between individuals.

Group B classified the 2 Met homozygous intermediate cases as type 2 cases. All the group B MM type 2 cases that were also classified by Group A were MM-1 cases i.e. typical sporadic CJD cases. These 2 cases would have been fairly typical of the MM-1 group, although 1 case had sensory symptoms at onset. Both cases had cerebellar symptoms and went on to develop dementia. The duration of illness was 3 and 9 months. The patient who survived for 9 months had a PEG tube sited from early in the illness. The EEG was highly suggestive in both MM cases, again suggesting that they were MM-1 cases.

The Val homozygote case was part of the Oxford study. The onset at 76 years with cerebellar signs and 5 months duration of illness would suggest this was a VV-2A case. The EEG was reported as typical but was not available for review. There was no pathology report other than the original, when PrP immunohistochemistry techniques were not available. There is no comment on involvement of the basal ganglia.

There are no odd features about any of the cases to suggest that they might belong to a different isotype group of 20kDa mobility (although this study has demonstrated a wide variety of clinical features within the cases in each group anyway). No other group has reported cases of intermediate mobility.

9.4: GENOTYPE AND ISOTYPE ONLY

The sporadic cases were analysed with respect to genotype or isotype alone to look for trends in the clinical picture, investigations and pathology.

A: GENOTYPE ONLY

i): Case Data

It is possible that the changes noted in this study are related to genotype only and protein isotype has no influence on the clinico-pathological picture. The excess of Met homozygotes amongst sporadic CJD cases and the atypical features of the Val homozygous and heterozygous cases are well documented. The NCJDSU distribution of genotype seen in cases referred since 1990 is:

MM	69%
MV	14%
VV	17%

The distribution of genotype in this study was slightly different, probably a reflection of a bias towards studying atypical cases in more detail. However, 65% were Met homozygous. This is still greater than the 42% seen in the population as a whole. There are rather more than expected of heterozygotes (17%) and Val homozygotes (18%).

ii): Gender Distribution

There was a slight excess of male Val homozygotes. In contrast there was a slight excess of females in the Met homozygotes and heterozygotes. The reason for this is not clear and may simply be due to chance.

iii): Age At Onset

As with previous studies, the Val homozygotes tended to be younger (median age 60 years) than the Met homozygotes (median age 63 years) but the heterozygotes

tended to be older (median age 65 years). However, there was a wide range of ages in each genotype, with considerable overlap and the median age of onset varied from 60 years in the Val homozygotes to 65 in the heterozygotes. Exceptionally young cases were present in each group.

iv): Duration Of Illness

Similarly, the Val homozygotes tended towards a longer duration of illness (median 6 months) and the heterozygotes had a significantly longer duration of illness (9 months). There was a wide variation in illness duration in each group. All 3 groups had cases of duration greater than 2 years. They also had cases with a very short duration of illness, typical of sporadic CJD.

v): Clinical Symptoms And Signs

The presenting symptom in each group was analysed. Dementia as a pure presentation was present in all groups, with little difference in frequency. A pure cerebellar presentation was more common in the heterozygotes and Val homozygotes. However 10 Met homozygous cases (17%) presented with a pure visual onset. 1 Val homozygous case also presented with the same onset (6%). Greater numbers would be required before the significance of this result could be ascertained. There was no other significant difference in any of the other clinical symptoms and signs.

vi): Investigations

In this study 52% of the Met homozygous case had a typical or highly suggestive EEG. Only 1 further case had a highly suggestive EEG, a heterozygote. The EEG is strongly associated with the Met homozygous genotype.

However, if 14-3-3 analysis is studied a larger proportion of the other genotypes have a positive result, aiding diagnosis. The numbers are of course smaller and need further prospective study but 68% of the Met homozygotes and 88% of the Val

homozygotes that were tested had a positive 14-3-3 result. Only one of 3 heterozygote cases (33%) had a positive result. This may be chance and with further evaluation the test may prove to be more helpful in the heterozygotes. Alternatively, this may in some way be a reflection of a slight difference in the disease process in the heterozygotes.

The MRI was positive in 50% of heterozygotes that were tested however and if this test was validated it may prove to be the most helpful investigation in this genotype. Again, very few case had an MRI scan available for analysis but only 25% of the Met homozygotes and 38% of the Val homozygotes that were tested had a positive scan.

vii): Pathology

There have been several reports already on the genotypic influence on the pathology of sporadic CJD. Met homozygosity seems to predispose to more spongiform change in the cortex and heterozygosity or Val homozygosity seems to predispose to more change in the basal ganglia. Heterozygotes are the only group to have Kuru-type plaques in this study prior to PrP staining. However after PrP staining plaques and plaque-like deposits are seen in just under a third of the Val homozygotes and 5% of the Met homozygotes as well as 65% of the heterozygotes.

vii): General Comments

The results of this study generally agree with other published data. Met homozygosity predisposes to sporadic CJD and, in general, the Val homozygotes tend to present at a younger age. In addition both Val homozygotes and heterozygotes tend to have a longer duration of illness. The only symptom to show a difference related to genotype was the presence of a visual onset, which was more common in the Met homozygotes. The numbers were too few to achieve significance.

It is also recognized that Met homozygous cases tended to have a positive EEG but this investigation is less helpful in the other genotypes. This study bears this finding out. However, although the results are very small, 14-3-3 protein analysis and MRI may prove to be useful in all the genotypes. Pathological analysis shows that many of the changes seen are related to genotype. In particular, Kuru-plaques are seen only in heterozygotes in this study and case studies in the literature.

B): ISOTYPE ONLY

i): Case Data

The data were also analysed to see if the protein isotype had a significant influence on the phenotype of the disease and pathology, irrespective of genotype. About 2/3 of cases were type 1 and the rest were type 2A. As with so much of the rest of the data, there was considerable overlap between the 2 groups and so it is only possible to comment on the trends apparent.

ii): Gender Distribution

No difference was seen in the gender distribution

ii): Age At Onset

The type 2 cases were slightly younger (61 years compared to 64 years), but again, there was considerable overlap between the groups.

iv): Illness Duration

The type 2A case had a longer duration of illness, 8 months compared to 3 months but the type 1 group had a case of 51 months compared to a case of 54 months duration in the type 2A group, again demonstrating considerable overlap.

v): Symptoms And Signs

When the onset of illness was compared, there was no significant difference when a purely dementing or cerebellar onset was compared. 16% of the type 1 cases

presented with purely visual symptoms, in comparison to 3% of the type 2A cases. This result achieved only borderline significance. There was no significant difference between the two groups in the other variables analysed.

If the clinical signs are compared all signs were in general less common in the group 2A. This is likely to be a reflection of under-reporting in this group because it occurs across the board. Only 16% of the 2A group had movement disorders other than myoclonus in comparison to 40% of the type 1 group. This may be a reflection that these signs are less common in the type 2A group or this may simply be chance due to under-reporting. Again, all signs were present in many of each group so no discriminating clinical features stood out.

vi): Investigations

A typical EEG was strongly associated with the type 1 group. None of the type 2A group had a positive EEG. However, nearly three quarters of the type 1 group and half the type 2A had a positive 14-3-3 analysis and 35% and 39% of the type 1 and 2A groups respectively had a positive MRI scan. This suggests once again that 14-3-3 protein analysis is a useful tool in the absence of a typical EEG, and the MRI may be positive where other investigations are not supportive.

vii): Pathology

When the pathological changes were analysed there was a suggestion that type 2A cases were more likely to have more spongiform change within the basal ganglia. Kuru plaques and PrP plaques and plaque-like deposits were also more likely to be seen in the type 2A cases but could be seen in either.

viii): General Comments

Once again, there are apparent trends. The type 1 cases tend to be more typical of sporadic CJD but there are no discriminating features that are present in only one group. Younger cases or cases of longer duration may be more likely to be in the

type 2A group but this is not exclusively the case. An onset of illness with purely visual symptoms is most strongly associated with a type 1 isotype, but there is one type 2A case with such an onset. It is therefore not a discriminating symptom. The other symptoms and signs are seen in either group and are even less discriminating.

As far as investigation is concerned, the EEG is strongly associated with the type 1 group. However 14-3-3 protein or MRI may be positive in either group. Pathologically, protein isotype appears to have less influence on the pattern of spongiform change and immunohistochemical staining.

9.5: GROUP B CLASSIFICATION

Group B have proposed a different classification system for CJD. They identified the same 2 isotype mobilities but proposed the presence of more glycosylation patterns.[20] This results in a larger number of groups, but a smaller number of cases in each. To date, they have concentrated more on the issue of protein isotype being a marker of strain and have not commented much on the clinical features of each group. Because there are fewer cases to study it was only possible to look for trends in each of the groups and further evaluation with a larger number of cases is required.

A: CASE DATA

Group B classified 48 cases. All but 4 were also classified by the NCJDSU, allowing the data to be compared. They identified 5 different isotypes of sporadic CJD. In conjunction with codon 129 genotype, this meant there were 9 different groups with 1 to 19 cases in each. (Type B1 was only seen in Met homozygotes and the type B6 and B7 cases were 2 isolated cases.) Because of the large number of groups of diverse size, statistical analysis could not be preformed.

B: AGE AT ONSET

The median age at onset was in the mid 60s in all the groups apart from the VV-B2 group (53 years) and the VV-B3 group (58 years). The MM-B3 group had an older age of onset at 71 years.

C: ILLNESS DURATION

Median disease duration varied from 2-13 months, a longer duration of illness being seen in the MV-B3 group (8 months), the MM-B3 group (7 months) and the MV-B7 group (13 months).

D: SYMPTOMS AND SIGNS

When the cases were examined for clinical trends no real differences were apparent. A percentage of each group had a cerebellar or dementing onset and a small percentage of the MM-B1 and MM-B2 cases had an onset with visual upset. It might have been expected that the MM-B6 and MV-B7 cases would be atypical, because they were lone cases in each group: The MM-B6 had a typical Heidenhain onset, choreaform movements developed midway into the illness but there were no other atypical features. The MV-B7 case had a typical course but for a rather long duration of illness. However the case had a cerebellar onset. This case would have been classified as MV-2A by group A and it is interesting to note that it is the only one in that group who had a cerebellar onset.

E: INVESTIGATIONS

68% and 65% of the MM-B1 and MM-B2 cases respectively had a typical or highly suggestive EEG. Unfortunately there are too few 14-3-3 and MRI results to come to a conclusion about the relevance of these investigations.

G: PATHOLOGY

The Pathological changes were of kuru-plaques in the heterozygotes and plaque-like deposits in the Val homozygotes.

H: GENERAL COMMENTS

There are insufficient data to draw conclusions on the Group B data. It is possible that there are more isotypes then Group A suggest but the cases in this study do not have obvious clinical and pathological features which divide them up into discrete sub-groups. More cases would need to be analysed.

The changes seen in these cases could be related to genotype alone: In general the Val homozygotes had a younger age at onset and a longer duration of illness is seen in the heterozygotes. The clinical features of the group are not unusual and very few

of the cases were atypical, the MV-B7 case if of interest because it was an atypical case, but this could be due to the influence of genotype alone. The MM-B6 case is a very typical sporadic CJD case, but for the presence of choreaform movements, which were seen in 33% of sporadic cases in this study.

When the distribution of cases is compared to the Group A cases, all the MM-B1, MM-B2 and MM-B6 cases would have been MM-1 in the NCJDSU classification. All the MM-B3 would have been MM-2A. The VV-B2 would have been either VV-1 or VV-2A in the Group A classification but the VV-B3 would all have been VV-2A. The same is true of the MV-B2 and MV-B3 cases, but for one MV-B7 case.

There are 3 possible reasons for the differences seen:

1. That there are more isotypes based on glycoform ratios that have not been identified by the Parchi and NCJDSU groups to date. There is insufficient evidence at this stage to confirm or discard this theory. If protein isotype is a marker of strain, and if different strains of CJD have different clinico-pathological phenotypes, at this stage the clinical correlates have not been truly identified.
2. That the differences between the 2 groups are due to differences in laboratory technique and interpretation. The analysis of the glycoform ratio by Western blotting in this study has been qualitative. Differences in the preparation techniques used could influence the glycosylation of the PrP^{res} and the relative molecular mass, depending on the chelation of copper and other factors. Again, this needs further evaluation.
3. As discussed previously it is not clear how much the glycosylation of PrP^{res} is influenced by the biochemical milieu within the brain or by agent strain and again the importance of the glycosylation ratio on protein isotyping in sporadic CJD is not clear.

9.6: VARIANT CJD

A: CASE DATA

43 cases of variant CJD were included in this study, although there are now 129 definite or probable confirmed cases. Since the first description of vCJD in 1996, the clinical and pathological picture has changed little. All the cases have been Met homozygous and have had a distinct protein isotype: type 2B, i.e. of 19 kDa mobility and a predominantly diglycosylated pattern. This same pattern has been seen in BSE and the other TSEs that have been linked in aetiology to BSE, such as FSE and exotic ungulate encephalopathy.[104]

B: SYMPTOMS AND SIGNS

The clinical picture has been very consistent, and is broadly similar in all the cases in this study. The median age of onset was 26 years and the median duration of illness was 14 months. The presenting complaint in all cases but one was of psychiatric symptoms. These were non-specific, including depression, anxiety and disturbance in sleep pattern. Primary psychiatric symptoms were less common; hallucinations were present in 35% and delusions were present in 37%.

Sensory symptoms were common early in the illness or at onset. One case presented with sensory symptoms before progressing to depressive symptoms. 14% of cases had sensory symptoms from the onset and symptoms were present in 65% at some point in the illness. These were only included if they were persistent and some cases were classified "don't know" because there was only an isolated mention of sensory upset. The nature of the symptoms were varied and described as non-specific limb pain, dysaesthesia, burning or cold feelings. The symptoms were sometimes lateralised and the aetiology is believed to be of thalamic origin.

Patients went on to develop dementia in all cases but cognitive problems were present from the outset in only 2 cases. All cases became unsteady on their feet and

in all but 2 cases cerebellar signs were noted. In most cases cerebellar signs did not develop until some months into the illness. As the illness progressed pyramidal signs, in particular rigidity with brisk reflexes and extensor plantar responses were common. Primitive reflexes were present in 58% of cases. Myoclonus was noted in 77% and dystonia or chorea was prominent in 68% of cases. 42% of cases had a reduction of upgaze. Cortical blindness was noted in 2% and 28% of cases were noted to develop akinetic mutism. This may be an underestimate, as in general the vCJD cases were seen earlier in their illness and late signs may simply be under-reported.

Although 44% were noted to have abnormal swallowing there is only a note of NG or PEG feeding in 21%, again this may be an underestimate but does not seem to account for the longer duration of illness seen in the vCJD cases as a whole, as the median duration of illness of the cases who were not NG fed was 13 months. It is also noteworthy, but probably coincidental, that fewer of the "later" cases appear to have been PEG fed, possibly because by this time the illness was better known and recognized as incurable.

The median duration of illness of cases who were NG or PEG fed was 18 months. In comparison, the cases in whom we have no information or who were not NG fed had a median duration of 13 months. It seems likely that the cases who were NG fed did live longer. Only 4 of the 34 cases in whom we have no information lived longer than 18 months, the median duration of the assisted feeding cases.

C: INVESTIGATIONS

Again, the results of investigations in vCJD are fairly distinct from sporadic CJD. The EEG was normal in 5% and non-specific in the majority (91%). No cases had a highly suggestive or typical EEG. Routine CSF analysis was normal or non-specifically abnormal in all cases. In a small number of cases the protein was raised,

sometimes in association with a raised red cell count. No cases had an abnormally high white cell count in the CSF.

14-3-3 protein was analysed in 33 cases. A positive result was obtained in 45% (some cases had an initial negative result). S-100b was high in 85% of cases and was usually higher in the cases with a positive 14-3-3 protein. However, this is a less specific test. In general, analysis of brain specific proteins so far, has proved less helpful in vCJD than in sporadic CJD. Whilst a positive 14-3-3 protein might support a diagnosis of variant CJD, there are insufficient data for it to transform a possible case to a probable case as occurs in sporadic disease. In addition, it does not help differentiate sporadic from variant CJD.

However, the MRI has been shown to be a relatively specific and sensitive test in vCJD. 77% of the cases in this study had high signal in the pulvinar area of the thalamus. Four scans were not available for review. Two of these were reported to be normal but they were both reported prior to the publication of the MRI findings in vCJD. It is conceivable that in retrospect these cases may be positive.

If only the cases where the MRI was reviewed by the NCJDSU are considered, 33 of 39 cases were positive i.e. 85%. This fits with the ongoing analysis of cases by the NCJDSU since the MRI paper was published. 92% of cases in whom the MRI is available for review have a positive scan. Although, similar changes have been seen on MRI imaging in other conditions, they do not in general mimic vCJD clinically.

One of the difficulties of diagnosing probable vCJD in life is in cases who fit the clinical picture but in whom the MRI is negative. In two cases a second MRI was positive. Others went on to have tonsil biopsy performed. This is an invasive procedure and is probably unnecessary in the presence of a positive MRI scan.

D: PATHOLOGY

The pathological changes in the variant cases have also been remarkably consistent and to date, distinct from the changes of sporadic CJD. Spongiform change was mild to moderate in most cases and most marked in the occipital lobe of the cortex. in nearly half of cases. (Despite the absence of cortical blindness in all but 1 case.) The basal ganglia were more severely involved in most cases, with marked gliosis and neuronal loss. However, the most striking abnormality in all the cases was the deposition of florid plaques throughout the cortex, deep gray matter and cerebellum. These plaques are similar to those seen in kuru, and have not been noted in any of the sporadic, familial or iatrogenic cases.

E: COMPARING SPORADIC AND VARIANT CJD CASES

An important distinction, particularly from the point of view of patients and their relatives, is whether or not a case of CJD is sporadic or variant. The cases have been very distinct pathologically but it is desirable to make a distinction during life.

To date the clinical phenotype of variant CJD has been relatively distinct from sporadic CJD, supporting the theory that a different single strain of agent is involved. In particular, the age at onset is in general younger and the duration of illness is generally longer. The psychiatric prodrome and prominent persistent sensory features are uncommon in sporadic CJD and the progression of variant CJD, with the development of cerebellar symptoms and signs has been very distinctive. In addition the pulvinar changes on MRI have been a useful diagnostic tool.

It is notable that vCJD has a relatively consistent clinical course in contrast to the sporadic cases, in whom each sub-group has been shown to have some rather atypical cases. However there are some concerns that some "atypical" variant cases may be missed and considered to be sporadic CJD. All vCJD cases to date have been Met homozygous. It is possible that cases of vCJD who are heterozygous or Val homozygous at codon 129 may have a distinct clinico-pathological phenotype. These

cases could be mistaken for an alternative diagnosis in life and if autopsy is not performed could have been missed altogether by the surveillance system. Look-back studies have suggested this is unlikely.[125,126]

Conversely, it is conceivable that some of the young atypical sporadic CJD cases, who are often Val homozygous or heterozygous, are in fact atypical vCJD cases. The results of pathological examination and eventually transmission studies should discriminate between the 2 groups but it is preferable to make the correct diagnosis in life. There are several similarities and differences evident from this study:

i): Age at onset

Although vCJD cases are generally younger, the youngest sporadic case in the study was aged 17 years at onset. In this study the oldest vCJD case was 53 years, younger than the median age at onset of sporadic CJD. However, subsequent to this study a case of variant CJD has been seen in a 74-year-old man.[220] Nevertheless the median age at onset of vCJD is 26 years, in comparison to 65 years in sporadic CJD and only 3 of the sporadic cases in this study were younger than 40 years.

ii): Duration of illness

Again, although vCJD cases are generally of longer duration of illness (median 14 months), 14 sporadic cases were of longer than 14 months duration in this study. 2 cases were of over 50 months duration, longer than any variant case to date. (The longest vCJD case was of 39 months duration.) In addition 18 of the vCJD cases were of 12 months duration or less. Many of the atypical sporadic CJD cases have an illness duration of about a year. The relationship to NG or PEG feeding was analysed but, although there was a suggestion that the cases who had assisted feeding lived for longer, there were insufficient cases to be sure.

iii): Psychiatric symptoms

The long psychiatric prodrome of variant CJD cases is very distinctive. A prodrome was seen in 28 sporadic CJD cases (28%), but in no case did it have the nature of the prominent psychiatric symptoms seen in the vCJD cases, usually consisting of a few weeks of being "under the weather" or having a flu-like illness.

Hallucinations and delusions are also prominent in vCJD, but 29% of all the sporadic cases had hallucinations in contrast to 35% of the vCJD cases. In both groups, these were nearly always visual in nature and usually developed mid to late into the illness.

Delusions were seen in 8% of sporadic cases. They tended to be persecutory and did not appear until some months into the illness. In vCJD however, delusion were seen in 37%. They were similar in nature and timing to the sporadic cases.

iv): Sensory symptoms

Sensory symptoms are a prominent feature of the vCJD cases, occurring in two thirds of cases and present in 14% from the onset. They were seen in only 14% of sporadic CJD cases. The nature of the sensory symptoms was often similar, 1 case complained of burning feet and another of a feeling of their "blood boiling". 2 others complained of cold feet and 3 complained of pains in limbs. Often, these symptoms appeared early on in the illness. The remaining cases complained of numbness, tingling and pins and needles. One of the prominent features of the sensory symptoms in vCJD is the persistence of the sensory upset. In general the sporadic cases decline cognitively much more quickly than the variant cases. It was not clear if the sensory symptoms in the sporadic cases were persistent or not in any of the cases.

It is possible that since the original description of vCJD, sensory symptoms are looked for in sporadic CJD by very directed questioning, in an attempt by the

interviewer to distinguish between sporadic and variant CJD. In this study, of the 14 cases with a sensory upset, 57% were seen after vCJD was described, suggesting that their presence is not due to direct questioning following the description of vCJD.

In young cases of possible CJD it is possible that the presence of sensory symptoms may make vCJD more likely. However, it would not be possible to discriminate from sporadic CJD by the nature of the sensory symptoms, as they are broadly similar in both groups. In addition, as might have been expected, the presence of sensory symptoms does not exclude sporadic CJD. The median age of the cases with sensory symptoms was 56 years, older than the oldest vCJD case in this study.

v): Other symptoms and signs

When considering the clinical signs in sporadic and variant CJD, although cerebellar signs are almost always present in variant CJD, they are so common in sporadic CJD that this could not be used to discriminate between the 2 conditions. Similarly, pyramidal signs and extrapyramidal rigidity are common in both.

Myoclonus seems to be slightly more common in sporadic CJD, occurring in 77% of variant cases, but 87% of sporadic cases. However chorea and dystonia were seen more commonly in the variant cases, seen in 68% of cases compared to 33% of the sporadic cases. This type of movement disorder is often very distinctive in the variant cases, whereas, even if present, is often not a prominent feature in sporadic disease.

Another feature of the vCJD cases was the presence of abnormal eye movements, in particular, reduced up gaze, present in 42%. Comments on the nature of eye movements were missing in about two thirds of sporadic cases. Reduced up-gaze or a paucity of eye movements was noted in 10 cases (10%). Paresis of conjugate upgaze in 5% of cases of sporadic CJD has been described.[121]

The clinical progression of the vCJD cases was similar to sporadic CJD after signs such as cerebellar problems began to appear. Most cases deteriorated rapidly and akinetic mutism was present in many cases although cortical blindness was seen less in vCJD.

vi): Investigations

Whilst, there are obvious differences between the variant and sporadic cases, in particular the prominent psychiatric onset and persistent sensory symptoms, it is clear that there is also some overlap in the clinical features. The use of supportive investigation is helpful in discriminating between the two diagnoses. None of the vCJD cases have had a typical EEG. However, an atypical EEG is usually seen in atypical cases of sporadic CJD, in whom the distinction between variant and sporadic is more in question.

Similarly, the presence of 14-3-3 protein may support the diagnosis of CJD but would not discriminate between the 2 types. A negative 14-3-3 protein would not rule out vCJD, although it would make sporadic CJD less likely.

High signal in the pulvinar area of the thalamus is highly specific for vCJD and has not been seen in cases of sporadic disease. This test is therefore an important discriminator, however if the MRI is negative then more invasive investigation such as tonsil biopsy may be considered. In atypical cases of sporadic CJD, in whom the EEG and 14-3-3 is negative, basal ganglia changes on MRI may prove to be helpful but are yet to be validated.

The final diagnosis rests on neuropathological changes. The distribution of spongiform change is fairly characteristic in vCJD and distinct from the changes of sporadic CJD. The most important finding is the presence of the florid plaque, which has been seen only in vCJD.

9.7: IS PROTEIN ISOTYPE A MARKER OF STRAIN?

Transmission studies of scrapie, extending back to the 1960s suggested that there are different strains of scrapie. There is little information on the clinical features of the disease in sheep and goats but when the disease is transmitted to mice, distinct clinical features are seen; scratching and drowsy.

Most of our understanding of TSE strain variation has come from experimental mouse models. Strains of scrapie with distinct clinical characteristics have been demonstrated. The characteristics are maintained through hosts of different PrP genotypes, or even through hosts of different species. The extent to which these models extend to human correlates is unclear.

i): Molecular basis

The molecular basis for this is poorly understood. However, Western blot analysis of TME identified 2 distinct mobilities of PrP^{res} that corresponded with distinct clinical phenotypes, drowsy and hyper, in transmission to a mouse host. These 2 protein isotypes of 19 and 21 kDA were identified in a number of different TSEs.

At the same time, understanding of the structure of PrP^{C} and PrP^{Sc}, and some of the mechanisms involved in the pathological process in prion diseases was accumulating. A structural basis for the different isotypes was proposed, based on the site of cleavage at the amino terminal of the PrP molecule. This in turn was influenced by the amount of β-sheet in PrP^{Sc}. It has been proposed that protein isotype is a marker of strain of the prion agent.

In addition, it has been shown that the site of amino terminal cleavage is also affected by the codon 129 polymorphism, theoretically explaining the influence that codon 129 has on the disease phenotype.

Parchi et al. found some clinical and pathological correlates in each group, however, their data were amalgamated from different countries with varying surveillance systems. It is possible that many of the cases were not seen in life by a neurologist and data extracted from case notes can be less accurate. This study had the advantage of a dedicated surveillance system in which as many cases as possible were seen by a research fellow. In addition, I reviewed all the EEG and MRI records available myself. Whilst the overall ratio of groups was similar and there were some trends identified that were the same as Parchi, in general the results were less conclusive.

At this stage the clinical correlates in sporadic CJD do not yet support the laboratory models of strain variation. Although there are some trends relating to the 6 genotype/ isotype groups of sporadic CJD, they do not neatly segregate into distinct clinic-pathological entities. In addition, many elements of the formation of PrP^{Sc} from PrP^{C} are still not fully understood and all hypotheses on the structural variation related to prion strain remain theoretical.

ii): Influence of the host

It is possible that as yet there are simply insufficient data to prove the existence of strain. However, there are other factors to consider. The influence of the biochemistry and cell structure within the brain could affect the progression of disease and deposition of PrP^{Sc}. The structural differences seen in protein isotype may be related to the cellular environment, rather than a strain of PrP. Puoti et al have identified a case of sporadic CJD with different PrP^{res} isotypes within the same brain.[398] One iatrogenic case in this study also contained both type 1 and 2A isotypes. They suggested this finding might provide evidence of the mechanism of PrP^{res} formation from PrP^{c}, as it is conceivable that the influence of different areas of the brain might alter the conformation of PrP^{res}.

Piccardo et al. demonstrated variations in glycoform ratio between different cases of GSS with the P102L mutation. They also demonstrated variation within the same

brain.[397] Again, the degree to which the PrP^{Sc} molecule is glycosylated could depend on the biochemical milieu, as with many proteins. In addition age and factors such as the amount of corticosteroid in the body can influence the glycosylation of proteins.

The sites of glycosylation are at Asn 181-183 and 197-199. It is interesting to note that many of the mutations associated with familial CJD and GSS occur around these sites, e.g. D178N and F198S. It has been suggested that a mutation at either site affects glycosylation, and hence the conformation of the protein.[461]

This might support the theory that sporadic CJD is a chance stochastic mutation within PRNP, and therefore can occur at several sites. The different protein isotypes produced may form into β-sheet at different sites producing slightly different patterns of protein deposition within the brain and hence different clinical phenotypes. Type 1 is more common, and may be the preferred, more stable conformation, but certain cell types may predispose to the type 2 conformation. Formation of more of either isotype may then influence the course of the disease.

One of the difficulties of a clinical, rather than an *in vitro* study is removing the influence of other diseases and factors in the host from the influence of the infective agent in the disease process. In human prion diseases codon 129 has a marked influence on the disease phenotype, irrespective of the agent strain. Polymorphisms in sheep also exist, influencing the susceptibility to disease.[462] A possible structural basis for this has already been mentioned but the mechanisms involved are not fully understood. It is not clear why Met homozygosity should be so strongly associated with the type 1 isotype, but this may also be due to a structural alteration in PrP^{Sc} related to the site at codon 129.

The influence of genotype on disease has been studied in other neurological conditions. Alzheimer's disease has a diverse clinical picture and is associated with the deposition of amyloid protein within the brain. In addition a point mutation

within the Presenilin-1 gene[463] and a polymorphism on the dopamine receptor gene[464] have both been associated with different disease phenotypes of Alzheimer's disease. In addition codon 129 of PRNP has also been shown to influence the disease phenotype in Alzheimer's disease.[465]

There is however no evidence that Alzheimer's disease is an infectious disease and no transmissible agent has been identified. The majority of human diseases, whether infectious or not, have a diverse clinical course, determined by many factors such as the health of the individual prior to the illness, the treatment of concurrent infections etc, all of which determine the disease phenotype.

There are other factors that could be affecting the disease phenotype, e.g. the presence of pre-existing disease such as Alzheimer's disease or stroke. Both are far more common in the elderly than sporadic CJD and could easily be co-occurring.

It is possible that other unidentified polymorphisms may also affect the disease phenotype. In recent years the Japanese have identified a polymorphism at codon 219 that they believe influences disease phenotype. They have also demonstrated that heterozygosity at this site may protect against sporadic CJD.[449]

iii): Evidence from vCJD

Does this mean that there is no good evidence for the existence of strain in human prion diseases? The vCJD cases form a distinct group with a relatively consistent clinical and pathological picture. Even allowing for the fact that all the cases to date have been Met homozygotes, they are a more consistent group than that seen in any of the genotype groups in sporadic, familial and iatrogenic cases.

In addition, research in recent years has shown that vCJD is causally related to BSE,[101, 102, 103] probably from the ingestion of contaminated meat products. Transmission studies have shown that the neuropathological lesion profile and

incubation period of vCJD is distinct from scrapie but remarkably similar to BSE.[104] This suggests that vCJD and BSE are a distinct prion strain.

Western blot of vCJD cases by both the NCJDSU and Collinge et al.[20] has shown a distinct pattern. However, the difference is in glycosylation ratio rather than in protein isotype mobility. Collinge et al. have placed more weight on the importance of glycosylation, using this ratio to differentiate a larger number of isotypes than the Parchi group. However, Parchi et al. have found the only difference between the vCJD cases and the type 2 sporadic, iatrogenic and familial cases, and indeed Kuru, is in the glycosylation ratio.[379]

These data would suggest that protein isotype is a marker of strain and that the glycosylation of PrP^{Sc} is dependent on the strain of prion agent rather than the cellular makeup within the brain. An alternative explanation is that the differences are related to the route of infection. Further studies on kuru might support this as this disease is also likely to be passed on through oral ingestion. However, Parchi et al. identified type 2A in Kuru PrP^{res}.[379] In addition, the neuropathology of vCJD and Kuru have been compared and were distinct.[446]

Typing the protein isotype in vCJD has been used as evidence that cases of Val homozygous and heterozygous vCJD have not been missed. It seems likely, based on the study of sporadic, familial and iatrogenic cases, that a Val homozygous or heterozygous case of vCJD will have an atypical clinico-pathological phenotype. It is therefore conceivable that some of the sporadic cases of these genotypes are actually vCJD. The protein isotyping studies suggest this is unlikely, as they have consistently demonstrated the vCJD cases have a type 2 mobility with a distinct glycosylation pattern. This does not of course imply that a different strain is involved; it simply demonstrates the deposition of an alternative protein.

9.10: POSSIBLE RESEARCH IN THE FUTURE

There are still some methodological problems to resolve: Parchi et al.[379] and Collinge et al.[396] have yet to agree on the exact number and typing of the different protein isotypes. Clearly, until this is resolved it is difficult to know how to classify the individual cases. In this study, neither classification could be definitely associated with a distinct clinico-pathological phenotype. The Parchi classification had more potential associations but there were fewer groups and more cases, making statistical analysis somewhat easier.

Two groups have identified both isotypes in the same brain. Parchi et al.[399] noted both isotypes in 10 cases and a further case report by Puoti has noted the same changes. In addition Piccardo et al.[397] have identified different glycoform ratios within the same brain. This is clearly evidence against protein isotype being a marker of strain. This study only analysed one area of the brain in each case but further analysis of the regional variation within the brain of a case series is required in order to prove that protein isotype is not brain region-specific.

In addition, 3 cases in this study were of intermediate mobility. These cases had no distinguishing features and it is not clear if the intermediate cases are a distinct protein isotype or related to methodological problems. The technique of isotyping is relatively new and these results may be due to early methodological difficulties. As familiarity with techniques improve, results may be more consistent.

Prospective analysis of cases, looking for identified features may take away some of the uncertainties of the clinical features attributable to each isotype. However, as has already been shown, the UK surveillance system has been prospectively studying as many cases as possible, using a dedicated research fellow, for 10 years. Despite this data are still lacking due to the difficulties of monitoring such a rare disease. The differences seen between vCJD and sporadic CJD are more compelling andfurther

analysis of the differences in isotype between these two conditions might provide more information about the relationship between isotype and strain.

Further *in vitro* work might allow a greater understanding of the pathological and structural processes involved in prion diseases and the molecular basis of strain variation. Currently, there is insufficient evidence to conclude that protein isotype is indeed a marker of strain, rather than a different conformation of PrP^{Sc} produced in different areas of the brain.

Future research should first of all establish a unified classification of the protein isotypes seen in CJD and establish the significance of identifying both isotypes within the same individual. If this is done, prospective surveillance could look in more detail at the onset of the disease, as this seems to be the most accurate symptom to evaluate. In addition the significance of the duration of illness could be looked in relation to age, PEG feeding and the onset of the disease. (An onset in the brainstem would presumably be of shorter duration.)

Further analysis of investigations in CJD will hopefully expand the diagnostic criteria, possibly to include MRI. 14-3-3 has already been shown to be of importance. The EEG does not appear to be of use in "atypical groups" but serial recordings in all groups would have to be performed to establish this.

The diagnostic criteria have been shown to be helpful even in "atypical groups". However, it may be possible in the future to subdivide the criteria for each subgoup if future analysis shows there are definite differences.

CHAPTER 10: CONCLUSIONS

This study has illustrated the difficulties of a surveillance system for a rare disease. Even with a registrar dedicated to obtaining information on any case of CJD throughout the UK, there are limitations in the data obtained, both in the timing of symptoms and the analysis of whether symptoms or signs have definitely developed during the illness. Additionally, the division of cases into one large group and five small groups makes statistical analysis very difficult.

Allowing for these difficulties, are there any trends that divide the cases into the six distinct groups? As has been illustrated, there is a suggestion that the MM-1 cases are of shorter duration and follow the more classical course of sporadic CJD and that the other groups tend to include the more atypical cases. However, some of the MM-1 cases would be considered atypical and the other groups all include cases that follow a typical course for sporadic CJD.

The most discriminating sign was the presence of visual symptoms at the onset of the illness, but not even this symptom was a truly 100% discriminator as it occurred in at least 1 case other than an MM-1 case. The result did not achieve statistical significance because of the small numbers involved.

As far as investigations are concerned, a typical EEG is almost always a marker of the MM-1 group, but there are exceptions and these factors again cannot be regarded as wholly specific to the MM-1 group. There were insufficient data on the results of 14-3-3 protein analysis and MRI.

The data were analysed to see if the changes could be due to either genotype or isotype alone. Again, a Heidenhain type onset of illness and a typical EEG were the most significant discriminators, both seemed to be associated with Met homozygosity and isotype. However the numbers are too small for this result to

achieve significance. Pathologically, the changes between the groups were more suggestive of differences due to genotype than isotype.

Clinically and pathologically therefore there are some trends in the six groups that may suggest differences in the clinico-pathological phenotype, but there is insufficient evidence to confirm that the groups are distinct clinical entities.

The differences between sporadic CJD and vCJD are more marked and provide the best evidence of strain of the CJD agent. However, there is insufficient evidence at this stage to conclude that protein isotype is a marker of strain, or that the molecular basis for this, proposed from *in vitro* studies of strain, is correct. Further analysis of the regional variation of protein isotype and the clinical and pathological correlates with isotype and genotype are required.

APPENDIX A: DESCRIPTION OF CLINICAL SYMPTOMS/ SIGNS

The following descriptions were included as synonyms of a particular symptom or sign. This is not a definition of each category but a glossary of terms found in case notes which were felt to be sufficiently suggestive of each category:

AGGRESSION: bad-tempered, angry, fighting, argumentative

AKINETIC MUTE: Immobile and mute, immobile or mute alone classified as "don't know"

ALIEN LIMB: limb moving out of control, posturing limb

ANXIETY: panic attacks, tearful, fear, worried

CEREBELLAR: gait/ truncal/ limb ataxia, broad-based gait, poor coordination, as if drunk, finger nose ataxia, heel shin ataxia. "unsteady" and nystagmus not included if no qualification

CORTICAL BLINDNESS: loss of vision due to loss of cortical visual function in which anterior visual structures are intact, often described in notes as absent menace response

DELUSIONS: persecutory, paranoid

DEPRESSION: low mood, withdrawn, loss of interest, anhedonia

DIZZY: giddy, vertigo, light-headed

EYE MOVEMENT DISORDER: reduced upgaze, nystagmus, limited eye movement

EXTRAPYRAMIDAL: parkinsonism, gegenhalten, shuffling gait, cogwheeling

HALLUCINATIONS: visual or auditory, seeing things, talking to someone not there

HEARING PROBLEM: deaf, not hearing properly, buzzing in ears

MOVEMENT DISORDER: athetoid, chorea, writhing movement, restless, tremor if distinct from extrapyramidal or cerebellar, grimacing, blepharospasm, dystonia

MYOCLONUS: jerking, startle, jumpy

PRIMITIVE REFLEXES: frontal release, grasp, rooting, palmomental, pout reflexes

PRODROME: flu like illness, "not him/ herself", headache, tired, mood change, depressed, weight-loss, insomnia, personality change, memory problem, moody, irritable. Combination of above.

PYRAMIDAL: rigidity if associated with brisk reflexes or extensor plantars, brisk reflexes, paratonic rigidity, hemiparesis. "upgoing plantars" or brisk jaw jerk alone not included. Classified "don't know"

RAPIDLY PROGRESSIVE DEMENTIA: memory loss, confusion, disorientation, behavioural abnormality, progressive dysphasia, forgetful. Significant deterioration in cognitive function over a year or less.

SENSORY: cold feelings, dysaesthesia, paraesthesia, pins and needles, limb pain, burning, tingling, numb, loss of sensation, coldness

SWALLOWING: absent gag response, food pooling in mouth, unable to eat, nasogastric or PEG tube sited

VISUAL PROBLEM: diplopia, double vision, blurring of vision, problem with glasses, smearing of vision, script moving

APPENDIX B **CLASSIFICATION OF EEG**

NON-SPECIFIC

Non-specific deterioration in normal background rhythms

Non-specific excessive slow wave activity

Non-specific excessive fast wave activity

SUGGESTIVE

General deterioration in/ loss of normal background

Intermittent bi/ tri -phasic discharges similar to those seen in classical CJD records

BUT

1. Occurring in bursts of only relatively short duration (<15 seconds)

AND either 2 or 3 or both

2. Not being *truly* generalised and synchronous
3. Without *true* periodicity

HIGHLY SUGGESTIVE

General deterioration in/ loss of normal background

Intermittent bi/ tri phasic discharges similar to those seen in classical CJD records, being truly generalised and periodic at times

BUT EITHER 1 or 2

1. Occurring in bursts of only relatively short duration (<15 seconds) and occupying less than a quarter of the record
2. Not being truly generalised and synchronised in *all* portions of the record

TYPICAL

Generalised deterioration in/ loss of normal background

Truly periodic generalised synchronous bi/ tri -phasic discharges

Occurring throughout the whole record or at least one quarter of it and in relatively long segments (15 seconds at a minimum)

APPENDIX C: DIAGNOSTIC CRITERIA FOR SPORADIC CJD

1. OXFORD CRITERIA. Used until 1993

SPORADIC CJD

I Rapidly Progressive Dementia

II
- A Myoclonus
- B Cortical Blindness
- C Pyramidal/Extrapyramidal/Cerebellar Signs
- D Akinetic Mutism
- E Early Onset of Neurogenic Muscle Wasting

III Typical EEG

IV Neuropathology

DEFINITE:	IV
PROBABLE:	I + 2 of II and III
POSSIBLE:	I + 3 of II

2 ROME CRITERIA. Used 1993-1998

CLASSICAL SPORADIC CJD

1. DEFINITE

a) Neuropathologically (histologically) confirmed
and/or
b) Immunocytochemically confirmed PrP positive (or Western blot)
and/or
c) Scrapie associated Fibrils detected

2. PROBABLE

Progressive dementia
and
Typical periodic EEG findings
and at least 2 out of the following 4 clinical features:
a) Myoclonus
b) Visual or Cerebellar problems
c) Pyramidal or Extrapyramidal features
d) Akinetic mutism

3. POSSIBLE

Progressive dementia
and
2 out of the 4 above clinical features
But no EEG or atypical EEG
and
Duration less than 2 years

3. ROTTERDAM CRITERIA. Agreed 1998.

Used for reclassification purposes in this study.

SPORADIC CJD

I Rapidly Progressive Dementia

II A Myoclonus
B Visual or Cerebellar problems
C Pyramidal or Extrapyramidal Features
D Akinetic Mutism

III Typical EEG

DEFINITE: Neuropathological/immunocytochemically confirmed

PROBABLE: I + 2 of II + III
or
Possible + positive 14-3-3

POSSIBLE: I + 2 of II and duration < 2 years.

APPENDIX D: DIAGNOSTIC CRITERIA FOR 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

II
- A - EARLY PSYCHIATRIC SYMPTOMS*
- B - PERSISTENT PAINFUL SENSORY SYMPTOMS**
- C - ATAXIA
- D - MYOCLONUS OR CHOREA OR DYSTONIA
- E - DEMENTIA

III
- A. EEG DOES NOT SHOW THE TYPICAL APPEARANCE OF CLASSICAL CJD*** (OR NO EEG PERFORMED)
- B. BILATERAL PULVINAR HIGH SIGNAL ON MRI SCAN

IV
- A. POSITIVE TONSIL BIOPSY

DEFINITE: IA (PROGRESSIVE NEUROPSYCHIATRIC DISORDER) **and** NEUROPATHOLOGICAL CONFIRMATION OF DIAGNOSIS OF NV CJD****

PROBABLE: I **and** 4/5 OF II **and** III A **and** III B

PROBABLE: I **and** IV A

POSSIBLE: I **and** 4/5 II **and** III A

* depression, anxiety, apathy, withdrawal, delusions

** this includes both frank pain and/ or unpleasant dysaesthesia

*** generalised triphasic complexes at approximately one per second

**** spongiform change and extensive PrP deposition with florid plaques, throughout the cerebrum and cerebellum.

APPENDIX E: LABORATORY TECHNIQUES

1): BRAIN–SPECIFIC PROTEINS

i): 14-3-3 protein

CSF 14-3-3 in the CSF was detected by Western blotting after SDS-PAGE.[180] 50 μL of CSF sample was mixed with an equal volume sample buffer (0.125M tris-CL, containing 20% v/v glycerol, 0.2M dithiothreitol, 4% sodium dodecyl sulphate and a 0.02% bromophenol blue, pH 6.8) and boiled for 4 minutes. Sample proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (4% stacking gel and 10% resolving gel) for 3 hours at 100V. The proteins were transferred by electroblotting to nitrocellulose for 2 hours using a current of 0.8 mA/cm^2. The unbound protein binding sites on the nitrocellulose were blocked using 2% non-fat milk powder in phosphate-buffered saline.

Immunodetection was carried out by incubation with 1:1000 dilution of rabbit ant-14-3-3 gamma (Santa Cruz Biotechnology, Germany) followed by incubation with 1:1000 horseradish peroxidase conjugated swine anti-rabbit Ig (DAKO, Denmark) and visualisation with ethylaminocarbazole. A positive control (CSF from histopathologically confirmed case of sporadic CJD), a negative control (CSF from a patient without histological evidence of CJD) and molecular weight markers were included on each run. The results obtained are qualitative.

ii): S-100b

CSF S-100b was measured using sandwich ELISA.[459] Micro titre plates were prepared by coating overnight with monoclonal anti-S-100b (Sigma Chemical Company, Poole, UK) at a concentration of 9.3 μg/ ml in 0.05M carbonate buffer, pH9.5. The plate was washed with 0.1% bovine serum albumin in phosphate-buffered saline (PBS) containing 0.05% Tween 20 (wash solution), and the unbound ii sites were blocked with 1% bovine serum in PBS for 30 min.

50 µl of CSF sample were added in duplicate to the plate, and 50 µl of 0.06M barbitone buffer, pH 8.6 containing 1mM calcium lactate were then added to each well (incubation buffer). The plate was incubated at 37°C for 3 h. After washing rabbit, anti-S-100 antibody conjugated with horseradish peroxidase diluted 1/1000 with incubation buffer was added. The microtitre plate was incubated for 1 hour at room temperature. The microtitre plate was washed with wash solution and 100 µl of enzyme substrate (1mg/ml o-phenylenediamine in 0.05M acetate buffer containing 0.01% hydrogen peroxide) was added. After incubating the plate in the dark for 30 minutes, the reaction was stopped by adding 1M HCl. The absorbance was read at 492nm with 405nm as the reference wavelength, using an Anthos 2001 Plate reader (Denley Instruments, Sussex, UK).

The upper limit of normal was taken to be 0.38 ng/mL.

2): PATHOLOGY

The tissues were prepared by a standard method. The brains were fixed by immersion in 15% formalin for a minimum of 3 weeks. Tissue blocks were taken from areas of the cortex, deep grey matter, cerebellum and brainstem. Blocks were decontaminated in 96% formic acid for 1 hour prior to routine processing into paraffin wax. From each block, 5 µm serial sections were floated on Vectabond-coated slides and stained using haematoxylin and eosin (H&E). After counterstaining with haematoxylin, sections were dehydrated, cleared in xylene and mounted in Pertex.

3): HISTOPATHOLOGY

10% tissue extracts of samples of frozen frontal cortex were made by homogenisation of <100 mg brain in Tris buffered saline pH 7.6 containing 0.5% Nonidet P40 and 0.5% Sodium deoxycholate. Extracts were cleared by low speed centrifugation and subjected to limited proteolysis by digestion with Proteinase K (BDH, 50 µg/mL, 1hr, 37°C).

Electrophoresis was performed on a 12%T Acrylamide SDS-PAGE mini-gel format (Bio-Rad Laboratories) and proteins transferred to Hybond ECL nitrocellulose or Hybond-P PVDF membranes (Amersham Pharmacia Biotech). The anti-PrP monoclonal antibody 3F4 (Senetek) was used at a 1:10,000 dilution followed by an HRP conjugated secondary antibody (SAPU) and enhanced chemiluminescence.

APPENDIX F: SPORADIC CJD; GROUP B DATA

A: CASE DATA

48 cases were available for classification by Group B. Four cases were classified by Group B only and there was no classification by Group A. These were; MM type 2, MM type 3, VV type 2 and VV type 3. Of the 44 remaining cases, both Group A and B classifications were available for comparison. (See Table F:1)

Table F:1: SPORADIC CJD: CLASSIFICATION OF GROUP B CASES

GROUP A			GROUP B		
Genotype	Isotype	N=	Genotype	Isotype	N=
MM	**1**	**27**	**MM**	**1**	**8**
			MM	**2**	**18**
			MM	**6**	**1**
MM	**2A**	**1**	**MM**	**3**	**1**
VV	**1**	**1**	**VV**	**2**	**1**
VV	**2A**	**6**	**VV**	**2**	**2**
			VV	**3**	**4**
MV	**1**	**3**	**MV**	**2**	**3**
MV	**2A**	**6**	**MV**	**2**	**2**
			MV	**3**	**3**
			MV	**7**	**1**

The subgroups were already small and further subdivision made analysis difficult. Of the 48 cases, the registrar had examined the patient in 40 cases, the relatives were interviewed in 44 cases and the hospital notes were available in 29 cases. All the cases were from the current NCJDSU study. The clinical features of each group are set out in table F:2.

i): Group B Type 1 (B1)

All the group B type 1 cases would also have been Group A type 1 Met homozygotes. There were 8 cases in this group (MM-B1).

ji): Group B Type 2 (B2)

There were a total of 28 type B2 cases. Of these 19 were Met homozygotes, 5 were heterozygotes and 4 were Val homozygotes.

If compared to the Group A classification, 2 were classified only by Group B. (1 Met homozygote and 1 Val homozygote.) 22 cases were type 1 in the Group A criteria (18 MM, 1 VV, 3 MV) and 4 cases were type 2A (2 VV, 2 MV).

iii): Group B Type 3 (B3)

There were 10 type 3 cases. Two of these were classified only by Group B. (1 Met homozygote and 1 Val homozygote.)

There were 2 Met homozygous cases (MM-B3). 1 case would have been MM-2A in the Group A classification. There were 3 heterozygotes (MV-B3). All would have been MV-2A in the Group A classification. There were 5 Val homozygotes (VV-B3). 4 would have been VV-2A in the Group A classification.

iv): Group B type 6 (B6)

There were 2 additional subgroups: 1 case classified MM-1 by Group A was classified type 6 by Group B (MM-B6).

v): Group B type 7 (B7)

The second case was an MV-2A according to Group A analysis but was classified type 7 by Group B (MV-B7). The 48 cases were analysed in the same way as described for the NCJDSU analysis. The results are laid out in tables 8.40 and 8.41.

B: GENDER DISTRBUTION

20 cases were male and 28 female (ratio 0.7:1).

C: AGE AT ONSET

Group	N	Median age of onset	Range
MM-B1	8	64 years	56-79
MM-B2	19	64 years	43-78
MV-B2	5	65 years	15-79
VV-B2	4	53 years	41-79
MM-B3	2	71 years	63-79
MV-B3	3	61 years	61-77
VV-B3	5	58 years	46-65
MM-B6	1	69 years	
MV-B7	1	65 years	

D: DISEASE DURATION

Group	N	Median duration	Range
MM-B1	8	2 months	1-5
MM-B2	19	4 months	1-17
MV-B2	5	3 months	2-54
VV-B2	4	6 months	5-11
MM-B3	2	7 months	3-12
MV-B3	3	8 months	7-14
VV-B3	5	3 months	2-11
MM-B6	1	3 months	
MV-B7	1	13 months	

E: SYMPTOMS AND SIGNS

MM-B1	Onset	Cerebellar	1 case (13%)
		Dementia	1 case (13%)
		Visual	3 cases (38%)

They all went on to develop a rapidly progressive dementia and followed a typical course for sporadic CJD.

MM-B2	Onset	Cerebellar	2 cases (11%)
		Dementia	8 cases (43%)
		Visual	3 cases (16%)

Dementia was an early feature of the illness in all but 5 cases (26%). All but 2 cases developed myoclonus. 1 case had some hemi-sensory neglect and numbness. In general there were no unusual features in any of the cases.

MV-B2

No case had a mono-symptomatic onset. All developed dementia early in the illness. One patient complained of paraesthesia from the onset. Cerebellar features presented early in the illness in 3 cases (60%), but all cases developed cerebellar features at some time. The case who survived for 54 months was NG fed. All cases deteriorated to a dependent state within a relatively short period of time.

VV-B2	Onset	Dementia	3 cases (75%)

One case also developed dementia early in the course of the illness but cerebellar symptoms and signs were one of the first features of the illness. 2 other cases probably had cerebellar signs at some point (1 patient was documented to be unsteady but the reason for this was not stated). All the cases probably developed myoclonus. 2 cases (50%) developed chorea.

MM-B3 Onset Dementia 1 case (50%)

Both patients presented with a dementia. In one case cerebellar symptoms were also present at onset. Both developed visual symptoms after some months. The other case developed cerebellar symptoms some way into the illness.

MV-B3 Onset Cerebellar 1 case (33%)

All the cases probably had cerebellar symptoms and signs at the onset. 1 case had associated dementia from the onset. The other 2 went on to develop dementia. 2 cases developed visual symptoms during the course of the illness.

VV-B3 Onset Dementia 3 cases (60%)
Cerebellar 2 cases (40%)

3 cases had dementia from the onset and another case developed dementia early on. The final case developed dementia some months into the illness. 2 cases had cerebellar symptoms from the onset and another 2 cases developed cerebellar symptoms and signs early on in the illness. 1 case was noted to have generalised muscle wasting and another had fasciculations. 4 of the cases developed visual symptoms midway into the illness. 1 case had paraesthesia from the onset and another developed a painful arm some months into the illness.

MM-B6 Onset Visual failure

Dementia developed rapidly and the patient became unsteady although cerebellar signs were not documented. Occipital blindness was a feature early on. Choreaform movements developed midway into the illness and myoclonus developed late.

MV-B7 Onset Cerebellar

Dementia developed after some months and there was no visual upset. Myoclonus was not noted.

F: INVESTIGATIONS

MM-B1 The EEG was typical or highly suggestive in 5 cases. (68%)
14-3-3 not done.
MRI positive in 0/5 cases. (0%)

MM-B2 The EEG was typical or highly suggestive in 11 cases. (65%)
14-3-3 was positive in the 2 cases tested. (100%)
2 of the 6 available MRIs (33%) were positive.

MV-B2 None of the 3 cases had a typical EEG.
1 case had 14-3-3 tested - negative result.
0/2 positive MRI. (0%)

VV-B2 The EEG was not typical in any of the cases.
14-3-3 not done.
MRI was negative in the 1 case in whom it was performed.

MM-B3 The EEG was suggestive in 1 case and the other was reported as typical. (50%)
MRI and 14-3-3 protein were not performed.

MV-B3 The EEG was negative in 3 cases (0%).
1 case had a negative 14-3-3 protein.
1 case had a positive MRI scan.

VV-B3 The EEG was non-specific or suggestive in all cases. (0%)
1/4 cases had a positive14-3-3 protein.
1 case had an MRI available for analysis. This was strongly positive.

MM-B6 The EEG was classified suggestive.
14-3-3 and MRI not performed.

MV-B7 The EEG was reported as suggestive but was not seen by me
MRI and 14-3-3 protein analysis was not done.

G: PATHOLOGY

The details of the pathological changes in each group are set out in table F:3.

MM-B1 The pathological changes were of spongiform change, predominantly cortical. No additional features.

MM-B2 The pathological features of spongiform change, predominantly cortical. No additional features.

MV-B2 There were no distinguishing features on pathological examination, except that the case of 54 months duration had widespread changes of status spongiosis and many plaque-like deposits.

VV-B2 Spongiform change was most marked in the basal ganglia in 3 of the cases (75%). 2 of the cases (50%) had plaque-like deposits on PrP staining.

MM-B3 The pathology was of mild spongiform change. There were no other distinguishing features.

MV-B3	All the cases demonstrated spongiform change greatest in the basal ganglia. Kuru-type plaques throughout the cerebellum and to a lesser extent in the cortex. PrP staining predominantly reticular, throughout the brain with many plaques and plaque-like structures.
VV-B3	Spongiform change was present throughout the cortex but was more marked in the basal ganglia. PrP staining revealed plaque-like structures in the cortex, basal ganglia, thalamus and cerebellum.
MM-B6	The pathological features of spongiform change, predominantly cortical. No additional features.
MV-B7	Pathological changes were of marked spongiform change in the basal ganglia and of widespread kuru-type plaques.

Table F:2: GROUP B DATA: CLINICAL FEATURES

Genotype	N=	Median Onset Years	Range Years	Median Duration Months	Range Months	Dementia At Onset %	Visual At Onset %	Cerebellar At Onset %	EEG Typical %	14-3-3 Positive	MRI positive
Type 1											
MM	8	64	56-79	2	1-5	13	38	13	68	Not done	0/5
Type 2											
MM	19	64	43-78	4	1-17	43	16	11	65	2/2	2/6
MV	5	65	15-79	3	2-54	0	0	0	0	0/1	0/2
VV	4	53	41-79	6	5-11	75	0	0	0	Not done	0/1
Type 3											
MM	2	71	63-79	7	3-12	50	0	0	50	Not done	Not done
MV	3	61	61-77	8	7-14	0	0	33	0	0/1	1/1
VV	5	58	46-65	3	2-11	60	0	40	0	1/4	1/1
Type 6											
MM	1	69		3		0	100	0	0	Not done	Not done
Type 7											
MV	1	65		13		0	0	100	0	Not done	Not done

Table F:3: GROUP B DATA: PATHOLOGY

Genotype	N=	Spongiform Cortex	Spongiform Basal Ganglia	Spongiform Cerebellum	PrP cortex	PrP Basal Ganglia	PrP Cerebellum	Amyloid Plaques
Type 1								
MM	**8**	**Microvacuolar**	**Patchy**	**Present**	**Synaptic/ perivacuolar**		**Granular**	**No**
Type 2								
MM	**19**	**Microvacuolar**	**Patchy**	**Present**	**Synaptic/ perivacuolar**		**Granular**	**No**
MV	**5**	**Present**	**Present**	**Patchy/present**	**Synaptic/ perivacuolar**			**1/5 plaques**
VV	**4**	**Present**	**Marked**	**Patchy**	**Synaptic/ perivacuolar**		**Granular**	**2/4 plaque-like**
Type 3								
MM	**2**	**Microvacuolar**	**Patchy**	**Patchy**	**Synaptic/ perivacuolar**		**Granular**	**No**
MV	**3**	**Present**	**Marked**	**Present**	**Synaptic**		**Granular**	**Plaques & P-like**
VV	**5**	**Present**	**Marked**	**Patchy**	**Linear**	**Reticular**	**Granular**	**Plaque-like**
Type 6								
MM	**1**	**Present**	**Present**	**Patchy**	**Synaptic/ Perivacuolar**		**Reticular**	**No**
Type 7								
MV	**1**	**Present**	**Marked**	**Patchy**	**Reticular**		**Reticular**	**Plaques++**

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