# GENETIC ASPECTS OF HUMAN PRION DISEASES

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## Declaration of Authorship

I hereby confirm that this thesis was composed by myself, and is the outcome of my own work. Certain parts of this work were undertaken in collaboration with colleagues, and this is acknowledged in the text. Data used in this thesis was originally gathered by the National Creutzfeldt-Jakob Disease Research and Surveillance Unit, and by Professor R. Will, and this is acknowledged in the text. This work has not been submitted for any other degree or professional qualification.

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0.0 Abbreviations

*Table 0.1* *Abbreviations*

| | |
|---|---|
| BASE | Bovine atypical spongiform encephalopathy |
| BSE | Bovine spongiform encephalopathy |
| CJD | Creutzfeldt-Jakob disease |
| CNS | Central nervous system |
| CSF | Cerebrospinal fluid |
| CT | Computed tomography |
| CWD | Chronic wasting disease |
| EEG | Electro-encephalogram |
| DLB | Dementia with Lewy Bodies |
| FFI | Fatal familial insomnia |
| FHx | Family history |
| FLAIR | Fluid attenuated inverse recovery |
| gCJD | Genetic Creutzfeldt-Jakob disease |
| gPD | Genetic prion disease |
| GSSS | Gerstmann-Sträussler-Scheinker syndrome |
| gTSE | Genetic transmissible spongiform encephalopathy |
| iCJD | Iatrogenic Creutzfeldt-Jakob disease |
| IHC | Immunohistochemistry |
| MBM | Meat and bone meal |
| MM | Methionine homozygosity encoded by codon 129 of *PRNP* |
| MRI | Magnetic resonance imaging |
| MV | Methionine / valine heterozygosity encoded by codon 129 of *PRNP* |
| NA | Not applicable |
| NCJDRSU | National Creutzfeldt-Jakob disease research and surveillance unit |
| OPRI | Octapeptide repeat insertion |
| PM | Post-mortem |
| *PRNP* | Prion protein gene |
| $PrP^{c}$ | The normal cellular isoform of the prion protein |
| $PrP^{Res}$ | The protease-resistant core fragment of $PrP^{Sc}$ |
| $PrP^{Sc}$ | The misfolded, pathogenic form of the prion protein |

Continued on the next page

*Table 0.1 (continued)* *Abbreviations*

| | |
|---|---|
| pTau | Phosphorylated tau protein |
| sCJD | Sporadic Creutzfeldt-Jakob disease |
| SFI | Sporadic fatal insomnia |
| SNP | Single nucleotide polymorphism |
| TME | Transmissible mink encephalopathy |
| TSE | Transmissible spongiform encephalopathy |
| vCJD | Variant Creutzfeldt-Jakob disease |
| VPSP | Variably protease sensitive prionopathy |
| VV | Valine homozygosity encoded by codon 129 of *PRNP* |

*Table 0.2* *Definitions*

| **Term** | **Definition** |
|---|---|
| Mutation | A genetic change (insertion or point mutation) not seen in healthy populations (excluding relatives of those with genetic prion disease) and causative of disease. |
| Polymorphism | A genetic locus at which variation is seen in normal individuals; genetic variation not thought to be causative of disease |
| Genotyping or Gene sequencing | Complete sequencing of the *PRNP* gene to seek any genetic polymorphisms or mutations (as opposed to determination of codon 129 only) |

## I.0 Abstract

### *Introduction*

Human prion diseases are progressive, fatal neurological conditions linked to conformational changes in the structure of the prion protein. Prion diseases may be sporadic (sporadic Creutzfeldt-Jakob disease or sCJD, Sporadic Fatal Insomnia), acquired (variant CJD, iatrogenic CJD, kuru) or genetic (genetic prion disease, gPD). gPD is due to a disease-specific point or octapeptide repeat insertion (OPRI) mutation in the prion protein gene (*PRNP*). Numerous different *PRNP* mutations have been described. In some cases of gPD the phenotype may closely resemble that of sCJD, and it can be impossible to distinguish sporadic from genetic cases without genetic screening. The clinico-pathological phenotype of gPD is highly variable, both between different mutations and even within families carrying the same mutation. This variability can be partly explained by a polymorphism at codon 129 of *PRNP*. Codon 129 encodes either methionine or valine, and the status of both the mutated and wild-type alleles may influence disease susceptibility and phenotype.

Codon 129 may also affect the manifestations of sporadic and acquired prion disease. Homozygosity for methionine at codon 129 is over-represented in both sporadic CJD (sCJD) and variant CJD (vCJD); indeed all definite or probable clinical cases of vCJD seen to date have been homozygous for methionine. Other polymorphisms of *PRNP* have been found in a small number of patients with sporadic and variant CJD. The significance of these polymorphisms has not been fully investigated. It is likely that other, as yet unidentified, genetic factors also play a role in influencing susceptibility to prion diseases and the clinico-pathological phenotype. A recent genome wide association study of vCJD patients found codon 129 to be the main genetic risk factor for vCJD, but did identify other candidate loci that may contribute to disease susceptibility. Work is in progress to carry out genomic screens for other, novel polymorphisms in 309 patients with sCJD and 118 patients with vCJD.

## *Aims of this MD Thesis*

The aims of the work described in this MD thesis are:

1) To review all cases of gPD on the database of the National Creutzfeldt-Jakob Disease Research and Surveillance Unit. The clinico-pathological phenotype, investigative findings and family history will be reviewed in detail. The findings will be compared with those cases of gPD previously described, in particular with cases seen in other European countries. The incidence and prevalence of these diseases in the UK will also be assessed.

2) To review cases of sCJD and vCJD with novel *PRNP* polymorphisms of uncertain significance. The clinico-pathological phenotype will be reviewed in detail to attempt to establish if these novel polymorphisms exert any influence over disease susceptibility or phenotype.

## *Results*

159 cases of gPD were identified between 1970 and 2009, representing 7.8% of the prion disease (of any type) cases referred to the NCJDRSU over this time period. 17 different *PRNP* haplotypes were identified: P102L-129M, P105L-129V, A117V-129V, S132I-129M, Y163X, D167G-129M, D178N-129M, D178N-129V, E200K-129M, D202N-129V, V210I-129M, Q212P-129M, 2-OPRI, 4-OPRI, 5-OPRI, 6-OPRI, 7-OPRI. The clinicopathological phenotypes were highly variable and often difficult to distinguish from sCJD. The highest number of cases was caused by the 6-OPRI, most of which belonged to a single kindred.

Several cases in the 4-OPRI group were found to share an additional risk allele, rs1029273C. In may be that this mutation is not pathogenic unless this risk allele is also present. This raises the possibility that other as yet unidentified genetic risk factors exist which influence gPD susceptibility and clinicopathological phenotype.

Overall 61.4% of cases tested had a positive cerebrospinal fluid (CSF) 14-3-3, 90.0% an elevated S100b, 23.1% had Magnetic Resonance Imaging (MRI) of the brain showing basal ganglia or cortical high signal, and 18.1% had an electroencephalogram (EEG) showing triphasic periodic complexes. A positive family history of prion disease was present in 57.9% of cases.

## *Discussion*

The range of point mutations and OPRI seen in the UK is considerable, but the majority of cases were due to 6-OPRI, E200K, or P102L. The UK differs from the rest of the world in that E200K is not the commonest mutation, due to the presence of a large British kindred with the 6-OPRI. Even within the larger kindreds, the clinicopathological phenotype remained very variable. Some distinctive features which may act as pointers towards gPD were found, such as a linear pattern of $PrP^{Sc}$ deposition in the cerebellum seen in E200K-129M cases. Analysing the data in the smaller groups should be done with caution, and further large international studies

are needed in order to truly determine the influence of factors such as codon 129 status.

As with other forms of prion disease, there is an excess of individuals with methionine homozygosity at codon 129. It is unclear whether or not *PRNP* mutations in cis with valine at codon 129 will result in prion disease at an older age or with a different phenotype, or if these are not actually pathogenic in this genetic context. In the case of 4-OPRI, it appears that an additional risk allele is required for the development of disease, and it remains to be seen if other additional genetic factors will be found to influence disease susceptibility and phenotype.

A relatively small percentage of cases had EEGs showing periodic triphasic waves, or basal ganglia or cortical high signal on MRI. CSF S100b was more sensitive than 14-3-3, the reverse of the pattern seen in sCJD. A pattern of a negative 14-3-3 and a very high S100b should lead to suspicions of gPD.

The current diagnostic criteria for gPD are relatively strict, and may exclude some individuals who have neuropathologically confirmed prion disease (without *PRNP* genotyping) and several second degree relatives with gPD. This is a potential problem, especially as the neuropathological appearances cannot be relied upon to distinguish sporadic from genetic disease. Particular attention should be paid to the family history and any subtle unusual neuropathological appearances to try and reduce the risk of gPD cases being missed. In conclusion, gPD remains a difficult condition to diagnose and study. Large systematic collaborative studies are essential to increase our understanding of these rare conditions.

# CHAPTER 1

# INTRODUCTION

## I.I A Historical Overview of Prion Disease

Prion diseases are a group of neurodegenerative diseases affecting various mammalian species. They are also known as Transmissible Spongiform Encephalopathies (TSE). Many prion diseases have now been experimentally transmitted to various animal species but the host range of naturally occurring diseases is more constrained[110]. Species affected outwith the laboratory include humans, sheep, mink, elk, mule deer and, in recent decades, cattle[343,201]. It is now thought that acquired prion diseases are caused by an infectious agent known as a prion (for 'proteinaceous infectious particle')[313] , but the exact nature of prions remains unclear.

Prion diseases are unusual for two reasons. First, prions appear to lack any nucleic acid but are able to replicate, and second prion diseases can be sporadic or hereditary but also retain infectious properties[110]. Prion diseases have been spread by both medical and veterinary procedures, and epidemics have arisen via the cannibalistic consumption of infectious material in both humans and cattle[107,42,378] . Unfortunately in the latter case we have witnessed the ability of prions to cross barriers between species; consumption of meat from cattle infected with Bovine Spongiform Encephalopathy (BSE) gave rise the new zoonosis known as 'variant CJD' (vCJD) in humans[387].

Genetic prion diseases were first described almost a hundred years ago, when neurodegenerative illness characterised by ataxia and dementia was observed to afflict families across multiple generations. It is now known that this was due to mutations of the gene which encodes for the normal cellular isoform of the prion protein (*PRNP*); although typically autosomal dominant, cases of gPD frequently occur in the absence of any history of affected relatives.

Here I will briefly summarise developments in prion disease research from the 18$^{th}$ century to the present day, concentrating on human and in particular genetic forms of disease.

## I.II Scrapie: The Prototypic Prion Disease

In the mid 18th century, descriptions emerged from Britain and continental Europe of a new, fatal disease of sheep, which caused animals to bite and scratch themselves and to develop gait abnormalities. These symptoms were the source of the several different disease names: '*scrapie*' in England; '*tremblante*' ('trembling') in France; '*traberkrankheit*' ('trotting disease') in Germany and '*rida*' ('tremor') in Iceland [334]. Wool was a vital part of the British economy at the time and the financial impact of scrapie was so severe that the topic was debated in the House of Commons[36]. However scrapie was not viewed as a threat to human health. The description of Leopoldt in 1750 neatly summarises the clinical manifestations:

*'Occasionally, some sheep contract scrapie, an illness recognizable in the recumbency of the animal. It nibbles at its claws and feet and scratches its back against posts. It ceases to prosper, looses appetite and finally gets weary. They drag themselves for a long time, are consumed more and more, and in the end, they are doomed to death. Animals that fall ill with this distemper won't recover. Therefore the very best a shepherd can do who has caught sight of an animal that has fallen ill with scrapie, is to cull the animal and slaughter it for the nobleman's servants. Thus, it is advisable for a shepherd to immediately separate such an animal from the healthy life-stock, as this disease is contagious and can cause great damage to the flock.'*

Leopoldt 1750; translated in [334].

Scrapie is primarily a disease of sheep, but may also spread to goats and moufflon[394]. It is highly contagious and once introduced into a flock frequently becomes endemic, resulting in an extensive geographical range[418,85].

## I.III Creutzfeldt and Jakob

The archetypal human prion disease is CJD, named after the European neurologists responsible for the earliest known descriptions of the disease.

Hans Gerhard Creutzfeldt was a German physician with expertise in neuropathology, neurology and psychiatry. He also earned the Iron Cross (First and Second class) for his services as Staff Surgeon of the German Naval Reserve during the First World War. In 1939 he became the professor and director of the Kiel University psychiatric and neurological department, where he continued to work until his retirement in 1953 when he moved to Munich to pursue scientific research; his time there was however most notable for his denunciation of the war criminal Werner Heyde, director of the medical division of the T4 program, who was practicing in Munich under a pseudonym. (Creutzfeldt was probably aware of his true identity for several years but alerted the authorities after becoming embroiled in a dispute with him over a medicolegal forensic report). In 1955 Creutzfeldt became an Honorary Senator of the Christian Albrecht University; in 1959 the Kiel Faculty commemorated the Golden Jubilee of his doctorate. Creutzfeldt died in Munich on the 30$^{th}$ of December 1964 [393].

Alfons Maria Jakob trained in psychiatry under such luminaries as Kraepelin, Nissl and Alzheimer, before habituating in neurology at the University of Hamburg. Jakob contributed extensively to many areas of neurology and neuropathology, producing monologues on the cerebellum and extrapyramidal system. He also studied neurosyphilis and the effects of malaria therapy, a cutting edge treatment at the time. He died at the age of 47 years having developed staphylococcal septicaemia as a consequence of chronic osteomyelitis [332].

## I.IV Creutzfeldt-Jakob Disease

In 1913 Creutzfeldt first met Bertha E, the patient who would be the subject of his seminal 1920 publication. Bertha E. was described as having symptoms of a 'noticeably awkward gait', 'childish and stubborn nature' and motor spasms and hyperalgesia, with onset at age 16 years followed by a progressive decline to a state

of severe dementia accompanied by mutism, muscular twitches, spasticity and seizures. She died in status epilepticus at 22 years of age. On neuropathological examination there was parenchymal disintegration with vascular involvement, neuronophagy and glial and vascular proliferation and diffuse nerve tissue degeneration. There was a family history of mental impairment in 2 siblings. Creutzfeldt concluded that the aetiology of the condition was obscure [76,240].

In 1921 Alfons Jakob published 3 cases of what he termed 'spastic pseudosclerosis'; he subsequently added a further 2 cases to the literature[179,178]. Jakob described a fatal illness characterized by early personality change followed by cognitive decline and hallucinations, accompanied by a progressive decline in mobility, speech impairment and involuntary movements. Pathological changes of 'encephalomyelopathy with disseminated foci of degeneration' were seen[240]. Jakob linked his cases to the earlier description of Bertha E. by Creutzfeldt and the term 'Creutzfeldt-Jakob disease' entered the literature shortly afterwards. However it was to be some decades before the detailed phenotypical and pathological characteristics of CJD were fully delineated.

## I.V Early Descriptions of Familial Prion Diseases

In the early years of the 20th century there was considerable interest in hereditary diseases, in particular familial forms of neurological and psychiatric illness. At the 1912 meeting of the Viennese Neurological and Psychiatric Association a report of a family afflicted by a hereditary neurodegenerative disease was presented. This family was subsequently reported in greater clinico-pathological detail first by Gerstmann in 1928 and then by Gerstmann, Sträussler and Scheinker in 1936[86]. Affected individuals developed a progressive cerebellar syndrome accompanied by cognitive decline, with a mean age of onset of 46 years and a mean disease duration of 6 years. Pathologically degeneration of the spinocerebellar and corticospinal tracts was seen, with 'peculiar inclusions of a foreign substance' described as being roughly sphererical inclusions of a bluish-green colour when viewed after Nissl staining[241]. An eponymous label of Gerstmann-Sträussler-Scheinker syndrome (GSSS) was appended and numerous further case reports

appeared in various countries. As early as 1940 links were made with CJD on clinical grounds although the pathology was recognized as being distinct[241].

Reports of familial CJD also appeared in the literature around the same time. The 'Backer' family of Kirschbaum and Meggendorf is the earliest example[196,258]; the importance of such families as being affected by a disease that is distinct from sCJD was not appreciated for some decades.

It was not until the molecular biology breakthroughs of Watson and Crick[375] and the subsequent explosion of knowledge regarding the intricacies of genetics and *PRNP* in particular, that GSSS and familial CJD were linked to genetic mutations. Whether or not these mutations are actually pathogenic in nature or give rise to a susceptibility to an external agent remains highly controversial.

## I.VI The Transmissible Nature of Prion Diseases

In the early 1930s brain tissue from sheep with scrapie was shown to be infectious; this was demonstrated both intentionally in the laboratory and unintentionally in the field. A vaccine for the ovine disease louping-ill was developed in the 1930s using the brain, spinal cord and spleen of donor sheep. After 4 years of trials the vaccine was released for general use; unfortunately 2 and a half years later animals which had received one particular batch began to develop scrapie. In some cases scrapie appeared in breeds of sheep which had hitherto never appeared to be susceptible to the disease. Subsequent investigations revealed that scrapie had also appeared in flocks from which donor sheep had been taken; it was concluded that some donor animals had been incubating scrapie during vaccine production. This was then confirmed experimentally, with both brain and spinal cord being shown to be infectious, with long incubation times. Intracerebral inoculation was a more efficient and faster route of transmission that the subcutaneous one. The agent was noted to be small in size and resistant to formalin[140]. At the same time Cuille and Chelle had been conducting the experimental inoculation of sheep with central nervous system (CNS) tissue from animals with scrapie and successfully transmitted the disease[78,77,79]. The louping-ill incident demonstrated the formalin resistance of the

infectious agent and is the first known case of 'iatrogenic' transmission of prion disease.

## I.VII Defining A Disease

During the first half of the 20th century the debate over the definition and indeed the very existence of CJD continued. Various competing terminologies were in use at the time, and many of the early case reports of CJD would not be classified as such using modern criteria. Jakob himself used the term 'spastic pseudo-sclerosis' , whilst others favoured 'disseminated encephalomyelopathy' or 'cortico-pallido-spinal degeneration' [332,82,240]. There was considerable debate as to whether or not CJD was 'a pathological entity, or … a heterogeneous collection of presenile cerebral degenerations' [43]. The classic clinical triad of dementia, myoclonus and increasing immobility and the neuropathological triad of spongiform change, neuronal loss and astrocytosis were first described by Nevin et al. in 1960[275]. The term 'sporadic CJD' is used to describe cases of CJD occurring spontaneously, with no underlying iatrogenic or genetic cause. The distinctive clinical subtypes of sCJD are eponymously known by their earliest descriptors, Heidenhain, Brownell and Oppenheimer. Heidenhain's syndrome refers to those presenting with cortical blindness [263,158] whilst Brownell-Oppenheimer syndrome describes those with a predominantly cerebellar onset[43] .

## I.VIII Kuru: The Missing Link?

The description of kuru on the island of Papua New Guinea in 1957 by Gajdusek and Zigas heralded a resurgence of interest in the spongiform encephalopathies[107,112,7,6]. Kuru is characterised by progressive cerebellar ataxia, with dementia being conspicuous by its late onset or absence[337,336,112,108]. Amyloid plaques containing abnormal prion protein ($PrP^{Sc}$) are seen in the cerebellum, accompanied by cerebellar atrophy, generalised astrocytosis and neuronal degeneration, with relatively mild spongiform change [200,372].

Kuru affected the Fore tribe, who believed that the soul of a deceased person could only pass to the afterlife if the body was entirely consumed by female relatives (with male children occasionally and adult males rarely participating)[381]. These mortuary feasts resulted in a prion disease epidemic, thought to have originated in an individual who died of sCJD; this theory is supported by molecular and biological similarities between kuru and sCJD[372]. The incidence of kuru rapidly declined after the outlawing of mortuary feasts in 1950. The last known patient died in 2005[287].

Polymorphisms of *PRNP* have been shown to exert an influence on kuru susceptibility. Women who participated in multiple mortuary feasts but did not develop kuru are usually heterozygotes at codon 129[255]; as were most of the patients who developed kuru after incubation periods of several decades[70]. Recently another *PRNP* locus, codon 127, has also been shown to influence susceptibility.

In 1959 similarities in the neuropathological appearances of kuru and scrapie were identified[147,148], leading to increased efforts to transmit kuru to experimental animals, with researchers crucially allowing for much longer incubation times. In 1966 the first successful transmission of kuru to chimpanzees was reported[109,111,22]. This was quickly followed by the transmission of sCJD, gCJD and GSSS to primates[129,110,128]. These findings contributed a new, defining characteristic to CJD, GSSS, kuru and scrapie: not only are they spongiform encephalopathies but they are potentially transmissible, clearly differentiating them from other neurodegenerative diseases.

## I.IX Emerging Animal Prion Diseases

Transmissible mink encephalopathy (TME) first appeared in fur farms in Wisconsin and Minnesota in 1947[45,154 154,239]. It has now been reported in Canada, Finland, Germany and Russia[395]. Animals display an insidious onset of behavioural change with increased aggression and hyperaesthesia, followed by hind limb ataxia, self-mutilation, terminal blindness and coma[154,239]. TME is highly contagious and an outbreak usually results in the death of all the animals on a farm. Neuronal degeneration and astrocytosis occur, accompanied by extensive neuropil vacuolation[91,343]. The origins and routes of transmission of TME are unclear; feed

containing prion-contaminated animal offal is considered to be the most likely culprit[45,238,343,239,321]. Disease control concentrates on the avoidance of high-risk offal; given the paucity of recent outbreaks this approach appears to be effective.

Chronic wasting disease (CWD) is a fatal disease of farmed and wild Cervidae first identified in Colorado in 1967[390,342,391]. Behavioural change occurs followed by weight loss, teeth grinding, excessive salivation, polydipsia and polyuria. Widespread spongiform change is seen accompanied by astrocytosis, neuronal degeneration and intracytoplasmic vacuolation, with lesion distribution varying between species[144,145].Extensive $PrP^{Sc}$ is found in the CNS and extraneural tissues; the distribution differs between species, as does the influence of various codons of *PRNP*[342]. Transmission routes are unclear, but CWD is certainly contagious and impossible to eradicate even if all animals in an affected area are culled and the land decontaminated[264]. There has been no evidence of transmission to humans [247] but research continues to assess CWD's zoonotic potential[1,204].

Prion disease in cattle is discussed later in the introduction.

## I.X The Unconventional Nature of the Infectious Agent

Although Leopoldt referred to scrapie as being a contagious disease in 1750 the infectious nature of scrapie was not widely accepted by the scientific establishment until the transmission studies of Gordon and Chelle[140,80]. They noted the infectious agent to be small, filterable and also resistant to treatment with formalin. It was concluded at the time that a virus was responsible; later the concept of a 'slow virus' was introduced to highlight the long incubation periods. This term was then modified to 'unconventional virus' to reflect the agent's extreme resistance to radiation, heat and formalin[140,4,5,130]. The fact that infectivity persisted following procedures which would be expected to degrade nucleic acid led to further speculation regarding the nature of the infectious agent.

## I.XI Purifying Prions

Early work attempting to isolate and characterize the infectious agent was hampered by difficulties in purification and the long incubation periods encountered when transmitting scrapie to mice. The use of Syrian Golden hamsters proved to be a significant development because of the shorter incubation periods of scrapie in them. Prusiner and Bolton speeded up laboratory experiments even further by developing a incubation time assay which greatly reduced the number of experimental animals needed for a single experiment[32]. This paved the way for the purification of a protein associated with scrapie infectivity of 27,000 to 30,000 Daltons molecular weight, which was present only in the brains of animals infected with scrapie and was resistant to digestion with proteinase K[32,315].

## I.XII The Prion Protein Hypothesis

Prusiner hypothesised that the scrapie agent was not a virus but was a 'novel infectious entity' based on its aforementioned resistance to procedures which inactivate nucleic acids and its small size. He coined the acronym 'prion' to demonstrate the *pro*teinaceous and *in*fectious nature of the agent[313]. The prion protein was subsequently found to be encoded by a host cellular gene[283], on the short arm of chromosome 20 in humans (*PRNP*)[347]. Genetic variation at *PRNP* is very important. In this thesis the term 'polymorphism' is used to refer to a genetic locus at which normal individuals display genetic variation, which may influence disease susceptibility or phenotype, but does not cause disease. The term 'mutation' is used to refer to genetic abnormalities which are thought to be causative of genetic disease, and not found in healthy populations.

The primary structure of the prion protein is believed to remain unchanged in health and disease; its pathogenic properties arise from a change in the three dimensional structure. The normal cellular isoform of the prion protein ($PrP^{C}$) is mostly an alpha-helical structure. This undergoes a conformational change to a structure rich in beta-sheets (the pathogenic prion protein $PrP^{Sc}$) which is deposited extracellularly as amyloid plaques or concentrated intracellularly[290,350]. This

transition from α-helical to β-sheet appears to be a central event in prion propagation and pathogenesis. The exact mechanism by which it occurs remains obscure. There is still controversy as to whether prions are purely proteins, or whether they include some hitherto undetectable nucleic acid. Similarly the pathogenesis of gPD causes argument; are these solely due to additional mutations of *PRNP* or are there more complex gene-environmental factors?

## I.XIII Sporadic CJD

The majority of cases of human prion disease fall into the category of sCJD, which has a worldwide annual mortality of 0.5 to 1.5 cases per million population per year[395]. Despite large scale epidemiological studies the aetiology remains obscure. The encoding of methionine or valine at codon 129 of *PRNP* appears to have a major influence on disease susceptibility and phenotype.

The mean age at onset is 65 years, with an extensive range from 14 to 92 years. From symptom onset the median disease duration is short, 4.5 months, with a mean of 8 months, although 4 percent of patients live for more than 2 years[395].

sCJD is typified by a triad of rapidly progressive dementia, myoclonus and periodic triphasic complexes on the electro-encephalogram (EEG). The commonest presenting complaint is of cognitive decline. Less frequently there is a purely cerebellar[43] or visual onset[158], or, rarely, presentation with other features such as stroke-like illness. Occasionally there are non-specific prodromal symptoms. Personality and behavioural change are often seen, as are psychiatric features such as depression and anxiety. Visual symptoms include visual blurring or loss, diplopia or field defects, and may progress to cortical blindness. Visual hallucinations and misperceptions are common. As the disease progresses dementia becomes a universal finding, and 80% develop myoclonus[395].

Frequent examination findings are pyramidal, extrapyramidal and cerebellar signs, paratonic rigidity, primitive reflexes and cortical blindness. Myoclonus and a startle response may occur in response to auditory, tactile or visual stimuli. Terminally the patient becomes akinetic and mute and often cortically blind.

Aspiration pneumonia due to dysphagia is a common occurrence. Cheyne-Stokes respiration may be seen.

The diagnosis of prion disease is frequently difficult, especially in the early stages of the illness. The differential is wide and includes conditions ranging from Alzheimer's disease and vascular dementia to metabolic encephalopathies. The most useful tests when investigating a potential case of sCJD are brain magnetic resonance imaging (MRI), EEG and cerebrospinal fluid (CSF) analysis.

sCJD is classically associated with an EEG finding of periodic triphasic complexes with a frequency of 1Hz, but these may not develop until late on in the disease. Their sensitivity and specificity are approximately 66 and 74% respectively[415]. Periodic complexes are seen in a number of other conditions, some of which may clinically resemble sCJD. Whilst it is estimated that between 60 to 80% of patients will develop these EEG changes at some point, sometimes only non-specific slow wave abnormalities are recorded[395].

MRI findings of bilateral, symmetric high signal intensities in the basal ganglia are highly sensitive and specific for sCJD. Typically the putamen and caudate are involved[121,97,335], less often the corpus striatum, anterior putamen, cerebral or cerebellar cortex are affected.

Routine CSF analysis is usually normal; the total protein may be modestly elevated but is rarely greater than 1 gram per litre. Neuronal proteins such as 14-3-3, S100b and tau have been found to be present at elevated levels in the CSF of patients with prion disease. These are non-specific markers of neuronal damage and may be present in conditions such as recent infarction or seizure, so some interpretive caution is required. However in the context of a patient who is suspected of having sCJD on clinical grounds they have a high sensitivity and specificity[63,301].

sCJD cases can show considerable clinical and pathological heterogeneity. Work is on-going to try to sub-classify patients at the molecular level, and tie this in to distinct clinicopathological phenotypes. Two major techniques used are the identification of the prion protein type, and the pattern of $PrP^{Sc}$ glycosylation. Partial digestion of $PrP^{Sc}$ by protease K results in 2 types fragments of different molecular weights. Type 1 has a relative electrophoretic mobility of 21 kilodaltons, and Type 2 is 19 kilodaltons. It was originally thought that the majority of patients have only

Type 1 or Type 2 $PrP^{Sc}$, but recent work involving the sampling of multiple brain regions suggests that many individuals may have both types present[409,156]. It also appears that there may be further sub-types of $PrP^{Sc}$ distinguishable by highly sensitive gel electrophoresis, and also truncated prion protein fragments in various forms have been reported by several authors.

An additional method for analysing differences between PrP subtypes is by looking at the glycosylation at residues 181 and 197. At these points PrP may undergo post-translational modification by glycosylation. Thus $PrP^{Sc}$ (and $PrP^{c}$) may be unglycosylated, monoglycosylated or diglycosylated. The relative prevalence of the different forms is termed the glycoform ratio[153]. For convenience a predominance of monoglycosylated $PrP^{Sc}$ is known as type A, whilst if diglycosylation prevails this is termed type B. This classification is probably an over-simplification, as variable glycosylation ratios have been reported in the different brain regions in the same individual. Some types of mutant PrP associated with *PRNP* mutations may also show distinct glycosylation ratios, which may be helpful in the neuropathological diagnosis[53].

The codon 129 status is also used to classify cases into those with methionine homozygosity (MM); valine homozygosity (VV); or heterozygosity (MV) at this locus. For example, the 'classical' sCJD phenotype of rapidly progressive dementia with myoclonus and ataxia is seen in MM or MV individuals with $PrP^{Res}$ type 1 (sCJD-MM1 or sCJD-MV1), whilst sCJD-VV2 patients have a cerebellar variant[293]. The neuropathological features provide additional classification criteria, as to whether findings affect predominantly cortical or thalamic areas, or display plaques similar to those seen in kuru.

At neuropathology, spongiform change, astrocytosis and neuronal loss are seen, with amyloid plaques present in a minority. Typically the cerebral cortex, putamen, caudate nucleus, thalamus and molecular layer of the cerebellar cortex are involved, but the severity and distribution of neuropathological changes is highly variable.

Overall the molecular classification of sCJD (and other forms of prion disease) is very much still evolving, and it is likely that the current system will be expanded and become more complex over time. The following tables summarise the

key subtypes and their clinical and neuropathological characteristics. The mechanisms underlying links between particular molecular and clinical findings also remain to be elucidated.

## I.XIV The Molecular Classification of Sporadic CJD

(adapted from Parchi 2011[296])

*Table 1.1 Molecular subtypes of sCJD: disease duration and age at onset*

| sCJD Subtype | Percentage of Cases | Age at Onset (years) | Disease Duration (months) |
|---|---|---|---|
| **MM1 or MV1** | 40 | 70.1 (48-86) | 4 (1-24) |
| **VV2** | 15 | 64.5 (45-83) | 6.3 (3-18) |
| **MV 2 kuru** | 8 | 65.4 (48-81) | 15.8 (5-48) |
| **MM/MV 2 cortical** | 1 (approximately) | 67.8 (61-75) | 20 (12-36) |
| **MM2 – thalamic** | 1 (approximately) | 52.3 (36-71) | 15.5 (8-24) |
| **VV 1** | 1 (approximately) | 39.3 (24-49) | 15.3 (14-16) |
| **MM/MV 1 + 2 cortical** | 28 | 68.6 (42-89) | 4.0 (1-26) |
| **MV2 kuru + 2 cortical** | 3 (approximately) | NA | NA |
| **VV 2 + 1** | 3 (approximately) | 69.3 (59-85) | 6.5 (3.5-13) |

*Table 1.2 Clinical features of the sCJD subtypes*

| sCJD Subtype | Clinical features |
|---|---|
| **MM1 or MV1** | Rapidly progressive dementia and myoclonus. Ataxia at onset in half and visual impairment in a third. |
| **VV2** | Often ataxia present at onset with dementia appearing later. |
| **MV 2 Kuru** | Dementia and ataxia. Duration may exceed 2 years. |
| **MM/MV 2 cortical** | Dementia, myoclonus and pyramidal signs. Ataxia uncommon. |
| **MM2 – thalamic** | Usually insomnia and psychomotor hyperactivity, accompanied by ataxia and other motor signs (Sporadic Fatal Insomnia) |
| **VV 1** | Progressive dementia followed by myoclonus and pyramidal signs. |
| **MM/MV 1 + 2 cortical** | Usually similar to MM/MV1; influenced by the relative predominance of $PrP^{Sc}$ type 1 or 2. |
| **MV2 Kuru + 2 cortical** | NA |
| **VV 2 + 1** | Similar to VV2 |

*Table 1.3* *Neuropathological features of the subtypes of sCJD*

| sCJD Subtype | Neuropathological features |
|---|---|
| **MM1 or MV1** | Spongiform change of the neocortex (particularly the occipital cortex), striatum, thalamus. Sparing of hippocampus and brainstem. Focal changes in the molecular layer of the cerebellum. Synaptic and sometimes punctate $PrP^{Sc}$ deposition. |
| **VV2** | Microvacuolar spongiform change in the deep layers of the neocortex. Involvement of the subcortex and hippocampus and cerebellum. Plaque-like and perineuronal $PrP^{Sc}$ deposits. |
| **MV 2 Kuru** | Kuru type plaques in the cerebellum. Plaque-like deposits (similar to VV2). |
| **MM/MV 2 cortical** | Cerebral cortex severely affected with widespread, often confluent vacuoles. Basal ganglia and thalamus less severely affected; hippocampus and cerebellum usually spared. |
| **MM2 – thalamic** | Marked atrophy of the thalamus and inferior olive. Focal spongiform change, usually limited to the cerebral cortex. |
| **VV 1** | Severe spongiform change in the cerebral cortex and striatum. Sparing of the brain stem and cerebellum. Faint synaptic $PrP^{Sc}$ deposition. |
| **MM/MV 1 + 2 cortical** | Similar to MM/MV 1. Also similar findings to MM 2 cortical (large confluent vacuoles, perivacuolar and focal $PrP^{Sc}$ staining). |
| **MV2 kuru + 2 cortical** | Similar to MV 2 kuru with additional large confluent vacuoles. Mixed kuru plaques and cortical $PrP^{Sc}$ deposition. Perivacuolar and coarse focal $PrP^{Sc}$. |
| **VV 2 + 1** | Similar to VV2 |

## I.XV Iatrogenic Human Prion Diseases

The earliest report of iatrogenic transmission of CJD came from the USA in 1974, when a 55 year old woman died of neuropathologically confirmed CJD 18 months after a corneal transplant; upon review of the donor's records it was found that she too had died of neuropathologically confirmed CJD[90]. 3 years later CJD was transmitted by depth electrodes, which were first implanted into the brain of a woman who subsequently died of neuropathologically confirmed CJD[28]. The electrodes were cleaned with benzene, ethanol and formaldehyde and reused twice;

both patients subsequently died of CJD. 28 months after the original event the electrodes were experimentally implanted into the frontal lobe of a chimpanzee; the animal also contracted CJD[126].

The majority of cases of iCJD have occurred following exposure to either cadaveric derived dura mater grafts or growth hormone. Nearly 200 cases of dura mater graft related CJD had been reported, 142 of which occurred in Japan, where the use of such grafts during neurosurgery was particularly common. In most cases the Lyodura brand was implicated, probably due to the practice of pooling dura from multiple donors[14,37].

The use of cadaveric growth hormone to treat growth hormone deficiency was widespread from the late 1950s to the 1980s, with little or no screening of donors and the pooling of up to two thousand glands during hormone extraction[202 310]. In Australia cadaveric gonadotrophin was used as fertility treatment, and this too has been linked with CJD transmission, albeit in only a handful of cases[65,37].

Three cases of secondary transmission of symptomatic vCJD via blood transfusion have occurred[234,162,300]. In one additional case blood from a donor who later developed vCJD was transfused into a man who later died of an aneurysm; at post-mortem (PM) $PrP^{Sc}$ was found in his spleen, but not in the CNS. This is considered to be a case of sub-clinical vCJD, and it is unclear if this *PRNP* codon 129 heterozygous individual would have developed a clinical illness if he had lived longer. One further pre-symptomatic individual was identified as part of a study of neurologically asymptomatic patients with haemophilia who were thought to be at high risk of vCJD[299]. This individual had severe haemophilia and as a result has received large amounts of Factor VIII, 2 batches of which included a donation from a single donor who subsequently died of vCJD. The recipient died of a thrombosed iliac artery aneurysm, with no clinical evidence of neurological illness. $PrP^{Sc}$ (with a glycoform ratio consistent with vCJD) was found in his spleen but not in other tissues (including brain) tested[9].

As with other types of human prion disease the *PRNP* codon 129 status may influence the risk of developing certain forms of iCJD, with homozygous individuals being at increased risk. There are now rigorous mechanisms in place to minimise the

risks of iatrogenic transmission via known routes; however the spectre of further cases transmitted via a novel route remains.

## I.XVI Bovine Spongiform Encephalopathy

On the twenty second of December, 1984, a veterinary surgeon was called to a farm in Sussex to examine a cow with unexplained weight loss and an arched back[236]; this was the first known case of BSE[236,378,384,383]. BSE is characterized by behavioural change, hyperaesthesia to touch and sound, ataxia, tremors, decreased milk yield and progressive weight loss. Illness duration is usually between one and two months[382,18]. There is spongiform change of the grey matter with neuronal vacuolation, neuronal loss and astrocytosis. $PrP^{Sc}$ is widely found, and scrapie associated fibrils are also seen[380,165].

Epidemiological evidence points towards the epidemic being due to an extended common source, with the onset of exposure being 1980 or 1981[384,383]. It was quickly recognized that the most likely source of the epidemic was the use of meat and bone meal (MBM) as cattle feed[407, 384,383]. The original source of the infectious material is has been postulated to be scrapie sheep, but the possibility of a novel bovine prion disease having arisen spontaneously cannot be excluded[98,168]. The recycling of animals which died of BSE into the food chain may have helped to lower the 'species barrier' and favoured the propagation of the disease strain with the highest level of infectivity and shortest incubation time[194].

The compulsory notification and slaughter of cases of BSE, and a ban on the use of MBM were effective in controlling the epidemic, which peaked in January 1993[236,379]. Novel prion diseases later appeared in felines and exotic zoological animals, probably due to infected bovine material being used as animal feed[195,357,397,379,162]. In all nearly two hundred thousand cases of BSE have occurred in the UK to date[405,395]. Atypical forms of BSE have subsequently been identified during active surveillance, which appear to occur sporadically in animals over 8 years of age, which may show little or no signs of disease[73,54,29]. These can be further subdivided based on the molecular mass of the $PrP^{Res}$ fragment seen on Western blot after partial protease digestion – those with the lower molecular mass are termed L-

type and those with a higher weight H-type[177]. It has been proposed that the classical BSE may have arisen due to animals with sporadic spongiform encephalopathy being rendered into MBM, but this is controversial.

## I.XVII Variant CJD

Although it was first thought that BSE did not pose a threat to human health[127], the potential for prion strains to alter their characteristics after cross-species passaging was recognised, and led to a robust system of surveillance being put in place in the UK. Some years later several cases of young adults affected by prion disease were identified[387,35,21]. Not only were these patients much younger than those typically affected by sCJD, but their presentation, disease duration and neuropathological findings were also distinct. From the outset this 'new variant' of CJD (vCJD) and BSE were thought to be causally linked, and there is now strong evidence to support this view[228].

The mean age at onset in vCJD is 28 years (range 12 to 74 years) with a median disease duration of 14 months. At onset there are typically psychiatric or behavioural symptoms, and many patients also experience painful sensory symptoms[412]. Psychiatric features are usually the sole manifestation for several months; later clinical features such as cognitive decline, myoclonus, cerebellar, pyramidal and extrapyramidal signs appear. Terminally there is akinetic mutism[414].

To date with only one exception all definite or probable cases have been methionine homozygous at *PRNP* codon 129. Routine investigations (such as full blood count, metabolic and vasculitis screens, autoantibodies and CT brain imaging) are usually normal. The EEG typically shows non-specifically slow-wave abnormalities or is normal[413]. Routine CSF analysis may show a modestly elevated CSF protein. CSF 14-3-3 analysis is of less utility in vCJD, being less sensitive. MRI characteristically shows symmetrical hyperintensity of the pulvinar nucleus of the thalamus, changes which in the correct clinical context are highly sensitive and specific for vCJD.

On neuropathological examination widespread florid amyloid plaques are found, typically surrounded by an area of spongiform change. The cerebral and

cerebellar grey matter are particularly severely affected. Clusters of multiple smaller plaques lacking a halo of spongiform change are also found in the cerebral and cerebellar cortex. $PrP^{Sc}$ is detectable within these plaques, and also around neurons and blood vessels in the cerebral and cerebellar cortex. A distinct isoform of the prion protein is found, with a glycosylation ratio not typically seen in sCJD[173]. $PrP^{Res}$ can be detected outwith the CNS, with lymphoid tissues such as the tonsils, lymph nodes, appendix and spleen being involved.

The current statistics record 176 cases of vCJD in the UK, and an additional 47 cases elsewhere. The yearly incidence of vCJD in the UK seems to be falling; there are concerns over the possibility of a 'second wave' of cases occurring, but calculations indicate that this is unlikely to affect a large number of individuals[119].

## I.XIII Sporadic Fatal Insomnia

Cases of Sporadic Fatal Insomnia (SFI) have a phenotype indistinguishable from that of the gPD Fatal Familial Insomnia (FFI) but there are no detectable *PRNP* mutations. To date only 13 cases have been described, all with methionine homozygosity at codon 129[292,245,333,303,260,50,311,193].

Most patients initially developed decreased vigilance, altered sleep-wake cycles and behavioural change, followed by pyramidal signs, dysarthria, dysmetria, impaired short-term memory and decreased spontaneous speech. As with FFI, dysautonomia and a loss of normal regulation of cortisol and norepinephrine are seen, whereas dementia is not. The EEG shows abnormal slow wave activity, and neuroimaging shows atrophy. Polysomnography has demonstrated severe disruption to normal sleep patterns, with loss of REM sleep, lack of sleep spindles and slow wave sleep[333].

The pathological changes are comparable to those of FFI, with spongiform change of the thalamus, caudate nucleus and inferior olivary nucleus. Mild spongiosis of the cerebral cortex with mild neuronal loss and gliosis has been seen in some cases, as has cell loss in the cerebellum. $PrP^{Sc}$ deposition is most marked in the basal ganglia, with a finely granular, synaptic pattern seen. Type 2 $PrP^{Sc}$ is usually

seen, with the unglycosylated form being under-represented. This last finding distinguishes SFI from FFI.

Although the SFI phenotype tends to be consistent between cases, there are several exceptions. The patient described by Priano et al. had similar clinical and pathological features but with the presence of type 1 $PrP^{Sc}$, which the authors speculated could possibly be related to neurosurgery 21 years prior to onset[311]. Of interest is a case report of an individual with SFI who was related to a confirmed FFI kindred. This individual's *PRNP* genotype was normal, as was her father's. However there were several paternal relatives with confirmed FFI due to the D178N–129M mutation[50]. Non-paternity was considered but excluded, and the proband was lacking in the unglycosylated form of $PrP^{Res}$ on immunohistochemistry (IHC), a finding typical of SFI. The only explanations are coincidence (the authors calculated the probability of an SFI case occurring in an FFI family by chance to be 0.000078 to 0.000156) or that somatic mosacism was in some way involved. Another case exists with pathological and genetic findings consistent with SFI, but with a cerebellar onset[260]. SFI is now classified as being part of the spectrum of sCJD (sCJD MM thalamic), and there is evidence from transmission studies that SFI and FFI share the same prion strain[296].

## I.XIX Variably Protease Sensitive Prionopathy

Recently a small number of cases of a novel human prion disease have been reported[114,157], with low levels of variably protease sensitive $PrP^{Sc}$ (VPSP). Whilst the first reported cases were all valine homozygous at *PRNP* codon 129, further affected individuals with heterozygosity or methionine homozygosity have now been reported[155,117]. Whilst VPSP is described as being a sporadic disease and none have been found to have mutations of *PRNP*, a significant number of the cases identified to date had a strong family history of dementia[419]. This suggests that the condition could be linked to genetic factors other than *PRNP*.

## I.XX *PRNP* & the Significance of Codon 129

The prevalence and characteristics of human and animal prion diseases are known to be intricately linked to host and donor genetic factors. In humans a particular polymorphic residue of *PRNP* at codon 129 has a major impact upon disease susceptibility and phenotype[69,115]. Individuals may be homozygous for methionine or valine, or heterozygous, at codon 129. In the British population the majority (47%) are heterozygous, with the remainder being homozygous for either methionine (42%) or valine (11%)[281]. However in those afflicted by prion disease, these proportions change. Methionine homozygosity appears to confer a greater risk of developing sporadic and variant CJD[296]. Codon 129 status is also a major determinant of clinical phenotype amongst sCJD patients[293]. In iCJD homozygosity at codon 129 (either valine or methionine) is over-represented. There are interactions between codon 129 and mutations of *PRNP*; for example FFI occurs when an individual has both the D178N mutation and methionine at codon 129 of the mutated allele; if the mutated allele carries valine the disease phenotype is radically altered to one resembling gPD[116].

In non-Caucasian populations the normal distribution of codon 129 differs, with Far Eastern groups having a predominance of methionine homozygotes. However, this does not seem to lead to differences in the incidence of prion disease. For example in Japan the distribution of codon 129 in the normal population is MM 92%, MV 8% and VV 0%, but the incidence of sCJD is the same as that of Caucasian populations[281].

In animals, equivalent genes to *PRNP* have been found and again these play an important role[89,316]. The genetics of sheep in this regard are far more complex than those of people, with different breeds of animals having multiple different risk alleles, with some of these behaving differently in different breeds[92,93]. When disease is transmitted from one species to another, genetic factors influence the disease characteristics seen[99]. It is possible that BSE only came into existence because a cow of a particular genotype was exposed to a particular strain of scrapie in a particular breed of sheep of a particular genotype.

It has been speculated that genetic factors other than *PRNP* codon 129 status may influence human susceptibility to prion disease. Modern genome wide association techniques allow this hypothesis to be tested, by comparing the genomes of large groups of individuals affected by sporadic, iatrogenic or variant CJD or kuru with control groups. In those with vCJD a genome wide association study found (as expected) a strong association with *PRNP* codon 129, and also several promising new SNPs which may be related to diseases susceptibility[254]. In particular a polymorphism of the cathepsin D gene (*CTSD*) has been found to be associated with an increased risk of vCJD[30]. However it does not appear to influence the risk of developing sCJD or gPD[211]. When kuru was investigated, those who lived through the epidemic and participated in mortuary feasts but did not develop kuru were found to be highly likely to be heterozygous at codon 127 of *PRNP* (G127V). This haplotype was not seen in samples from kuru victims or from control individuals living in areas of Papua New Guinea which were not affected by kuru[257].

## I.XXI Polymorphisms of *PRNP*

*PRNP* has been extensively studied in human populations, with a bias in studies towards Caucasian subjects. At least 65 pathogenic mutations and 23 polymorphisms have been identified, with interactions between mutations and polymorphisms occurring in some instances. In some cases there is uncertainty as to whether or not a mutation is pathogenic or not. Not all polymorphisms result in a change of the amino acid encoded for. A summary is given in table 1.4.

*Table 1.4. Polymorphisms of PRNP*

| Codon | Original amino-acid | Final amino-acid | Reference |
|---|---|---|---|
| **P39P** | Proline | Proline | Beck 2010[25] |
| **G54S** | Glycine | Serine | Beck 2010[25] |
| **P68P** | Proline | Proline | Windl 1999[392] |
| **A117A** | Alanine | Alanine | Hsiao 1989[169] |
| **G124G** | Glycine | Glycine | Prusiner 1997[314] |
| **Y128Y** | Tyrosine | Tyrosine | Beck 2010[25] |
| **G127V** | Glycine | Valine | Beck 2010[25] |
| **M129V** | Methionine | Valine | Owen 1990[284] |
| **I138M** | Isoleucine | Methionine | http://www.mad-cow.org/prion_point_mutations |
| **G142S** | Glycine | Serine | http://www.mad-cow.org/prion_point_mutations |
| **Y150Y** | Tyrosine | Tyrosine | Beck 2010[25] |
| **V161V** | Valine | Valine | Prusiner 1997[314] |
| **N171S** | Asparagine | Serine | Samaia 1997[331] |
| **N173N** | Asparagine | Asparagine | http://www.mad-cow.org/prion_point_mutations |
| **H177H** | Histidine | Histidine | Ripoll 1993[320] |
| **T188T** | Threonine | Threonine | http://www.mad-cow.org/prion_point_mutations |
| **D202D** | Aspartic acid | Aspartic acid | http://www.mad-cow.org/prion_point_mutations |
| **R208R** | Arginine | Arginine | http://www.mad-cow.org/prion_point_mutations |
| **Q212Q** | Glutamine | Glutamine | Windl 1999[392] |
| **E219K** | Glutamic acid | Lysine | Furukawa 1995[104] |
| **R228R** | Arginine | Arginine | Windl 1999[392] |
| **S230S** | Serine | Serine | Windl 1999[392] |
| **1-Octapeptide Repeat Insertion** | | | Yu 2009[408] |

## I.XXII Prion Diseases Associated with Mutations of *PRNP*

As previously discussed, the occurrence of familial human prion diseases was recognised at the beginning of the 20th century.

The first mutation of the *PRNP* gene to be discovered was the 6 octapeptide repeat insertion (6-OPRI) [286]. This was rapidly followed by the identification of numerous other insertional and point mutations as familial groups were genotyped. New mutations are still being found, and to date the total comprises 65 pathogenic *PRNP* mutations, with additional 'silent' polymorphisms However only a handful of mutations are responsible for the majority of gPD cases.

The clinicopathological phenotype varies considerably, and can differ even between patients from the same family with the same mutation. As with sCJD, *PRNP* codon 129 may exert a major influence upon the manifestations of the disease, which can result in a distinct disease phenotype arising. A strict division into gCJD and GSSS phenotypes is not always possible (or indeed meaningful) and therefore inherited prion diseases are now often classified according to the haplotype of the pathogenic mutation and the codon 129 status of the mutated allele. GSSS may also be diagnosed by its characteristic neuropathological appearances. In addition there are several instances of novel *PRNP* mutations associated with dementia or psychiatric illness, without any patients having confirmed prion disease. The current list of known pathogenic mutations and a brief résumé of their geographical range and clinical manifestations are given in tables 1.5 and 1.6.

The incidence of gCJD, FFI and GSSS varies across the world; given the difficulties in diagnosing prion disease, the similarities between some genetic and sporadic cases, and the ethical issues surrounding genotyping it is not surprising that comprehensive figures can be difficult to obtain. It is once again a case of 'you find what you look for', and it would be wise to assume that a lack of reported cases does not necessarily equate to a lack of disease, and may in fact indicate a lack of surveillance. Where adequate surveillance is performed, considerable variation in the incidence of different mutations is found. In some cases this is due to a mutation arising in an isolated population and being perpetuated by inbreeding, and in others the statistics for an entire country are skewed by the presence of one large (well

studied) family. The EuroCJD study findings are summarised in table 1.7; overall an incidence of 0.17 cases per million population per year was found, with 10.2 % of all cases of human prion disease having a genetic origin [210].

*Table 1.5. All known PRNP point mutations*

| Haplotype | Epidemiology |
|---|---|
| S97–129M | China; single case |
| P102L-129M | Extensive range worldwide |
| P102L–129M, 219K | Japan; single family |
| P102L-129V | USA/Italian; 2 cases |
| P105L-129V | Japan; rare |
| P105S-129V | N. America; single family |
| P105T-129M | E. India; 2 families |
| P105T-129V | 1 family |
| G114V-129M | Uruguay, China, S. India |
| A117V–129V | N. Europe & N. America |
| G131V–129M | America, Holland; 2 cases |
| Y145X–129M | Japan; single case |
| R148H–129M | Germany, N. America; 2 cases |
| Q160X–129M | Austria, N. America; 2 families |
| D167N-129M | Turkey; single case |
| N171S–129V | Brazil; single family |
| D178N–129M | Europe, N. America, Australia |
| D178N–129V | Europe, N. America, Israel |
| D178N-129V & N171S-129V | Single African-American family |
| V180I–129M | Japan, S. Korea, France, N. America |
| V180I–129 & M232R-129M | Japan; single case |
| T183A–129M | Germany, Brazil; 2 families |
| H187R–129V | N. America; single family |
| T188A–129M | Australia; single case |
| T188K–129M | Austria, Germany; 4 cases |
| T188R–129V | Germany, N. America; 2 cases |
| T193I–129M | Greece; single case |
| E196K–129M | Germany; 3 cases |
| E196K-129V | Germany; 1 case |
| P198S–129V | N. America; large kindred |
| F198V–129M | China; single case |
| E200K–129M | Commonest cause of gPD worldwide |
| E200K–129V | Europe; rare |
| D202G–129V | Germany; single case |
| D202N-129V | Canada, 1 family; England, 1 case |
| V203I–129M | Italy; 2 cases |
| V203I-129V | Korea; single case |
| R208H–129M | China, Europe, N.America; 4 cases |
| R208H–129V | France; single case |
| R208C–129M | China; single case |

*Continued on next page*

*Table 1.5 (continued) All Known PRNP Point Mutations*

| Haplotype | Epidemiology |
|---|---|
| V210I–129M | China, Europe, Japan, N.America |
| E211Q–129M | France, Italy |
| Q212P–129M | Ireland, N. America; 2 cases |
| Q217R–129V | N. America |
| Y218N | Spain; single case |
| M232R–129M | China, Japan, Korea |
| M232T | Poland; single case |
| Y226X-129V | Holland; single case |
| Q227X-129V | Holland; single case |
| P238S | Germany; single case |

*Table 1.6 PRNP deletions & insertions*

| Haplotype | Epidemiology |
|---|---|
| del 24 base pair 129M | Europe; 2 cases |
| 24 nucleotide insertion | Canada; 1 case |
| 1-OPRI 129M | France, Italy; 3 cases |
| 2-OPRI 129V | N. America, Holland; 2 families |
| 4-OPRI 129M | Britain, Italy, Japan |
| 4-OPRI 129V | France; single case |
| 5-OPRI 129M | Britain, N. America, Ukraine, Germany |
| 5-OPRI 129V | N. America, Germany |
| 6-OPRI 129M | Europe, Japan, N. America |
| 6-OPRI 129V | France; single case |
| 7-OPRI 129M | N. America (single family) & Japan (single case) |
| 7-OPRI 129V | Holland |
| 8-OPRI 129M | France; single family |
| 8-OPRI 129V | France, Holland |
| 9-OPRI 129M | England, Germany, 2 families |
| 12-OPRI | N. America; single family |

## I.XXIII The Worldwide Incidence of Genetic Prion Disease

*Table 1.7 The worldwide incidence of genetic prion disease*
*Adapted from Kovacs et al. 2005[210] with additional information from other sources.*

| Country | Genetic prion disease cases (% of all prion disease cases) | Incidence (per million population per year) |
|---|---|---|
| **Australia** [210] | 10.2 | 0.14 |
| **Austria** [210] | 14.4 | 0.28 |
| **Canada** [210] | 8.5 | 0.12 |
| **China**[118] | 7.8 | - |
| **France** [210] | 9.0 | 0.18 |
| **Germany** [210] | 7.6 | 0.13 |
| **Italy** [210] | 17.4 | 0.30 |
| **Netherlands** [210] | 2.1 | 0.02 |
| **Slovakia** [210] | 69.5 | 1.07 |
| **Spain** [210] | 10.3 | 0.23 |
| **Switzerland** [210] | 1.2 | 0.04 |
| **UK** [210] | 6.6 | 0.07 |
| **Hungary**[210] | 25.60 | 0.27 |
| **Libyan Jews in Israel** [417] | - | 44.14 |
| **Japan** [270] | 15.90 | - |
| **Chile** [113] | 45.00 | - |
| **Belgium** [289] | 4.00 | - |
| **Sweden** [235] | 0.92 | - |
| **Finland** [214] | 27.27 | - |
| **Total**[210] | 10.2 | 0.17 |

Reports from those undertaking prion disease surveillance in Kenya[2], Mexico[367], and India[259] are negative for genetic cases; however in these countries no *PRNP* genotyping has been carried out so there is an absence of evidence rather than evidence of absence. Surveys found a case of GSSS in Poland due to the novel T232T mutation[34], whilst in Ireland a case of FFI due to D178N 129M was detected

between 1980 and 2002[167]; in Sweden 1 case of GSSS was found between 1985 and 1996[235]. All of the reported cases of gPD in Finland are from the same kindred and attributed to the D178N mutation[214].

## I.XXIV Rationale for the Current Study

The clinicopathological phenotype of different human gPD is still not fully understood, largely due to the rarity of these conditions and the ethical difficulties surrounding genetic testing for untreatable, fatal disorders. The primary aim of this thesis therefore, is to systematically review cases of gPD in the UK over a substantial time period in order to gain insights into the manifestations of these mutations, and how their distribution in the UK compares with that reported elsewhere.

The *PRNP* codon 129 polymorphism is thought to play a major role in determining disease susceptibility and phenotype, but the effect of other polymorphisms is less clear. A second aim is to investigate the influence exerted by other *PRNP* polymorphisms found in cases of sporadic and variant CJD.

# CHAPTER TWO

# LITERATURE REVIEW OF INDIVIDUAL *PRNP* MUTATIONS

## 2.I Introduction to Literature Review

A large number of *PRNP* mutations and polymorphisms have now been described worldwide. All are rare, and some have only been reported in one individual. Fully characterising their clinicopathological phenotype is therefore difficult, compounded by interactions with other genetic factors such as the polymorphic residue at codon 129 of *PRNP*. The purpose of this literature review is to summarise the status quo of the gPD literature, and to provide a basis for comparisons with the finding of the present study of gPD in the UK. Figure 1 shows the mutations reviewed in this chapter. The prion protein gene consists of 2 exons with the open reading frame being within the second exon. There are 253 amino acids. Normally the nucleotide sequence from codons 51 to 91 codes for a nonapeptide followed in tandem by four identical octapeptide repeats which are also almost identical to the nonapeptide (except for the omission of a glycine). This area is shown in red in the figure. Figure 2 shows non-pathogenic polymorphisms of *PRNP*.

*Figure 2.1. PRNP mutations*

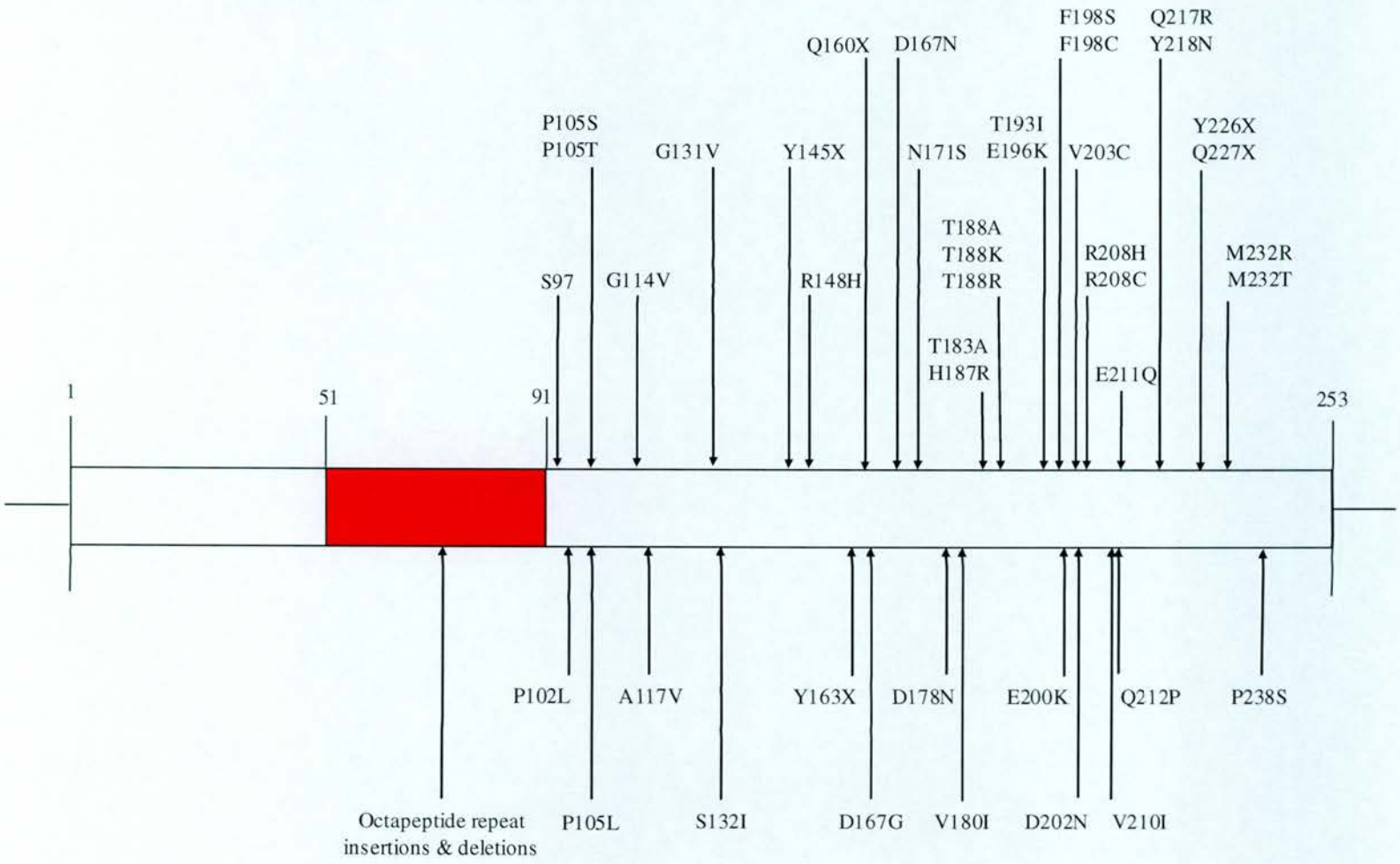
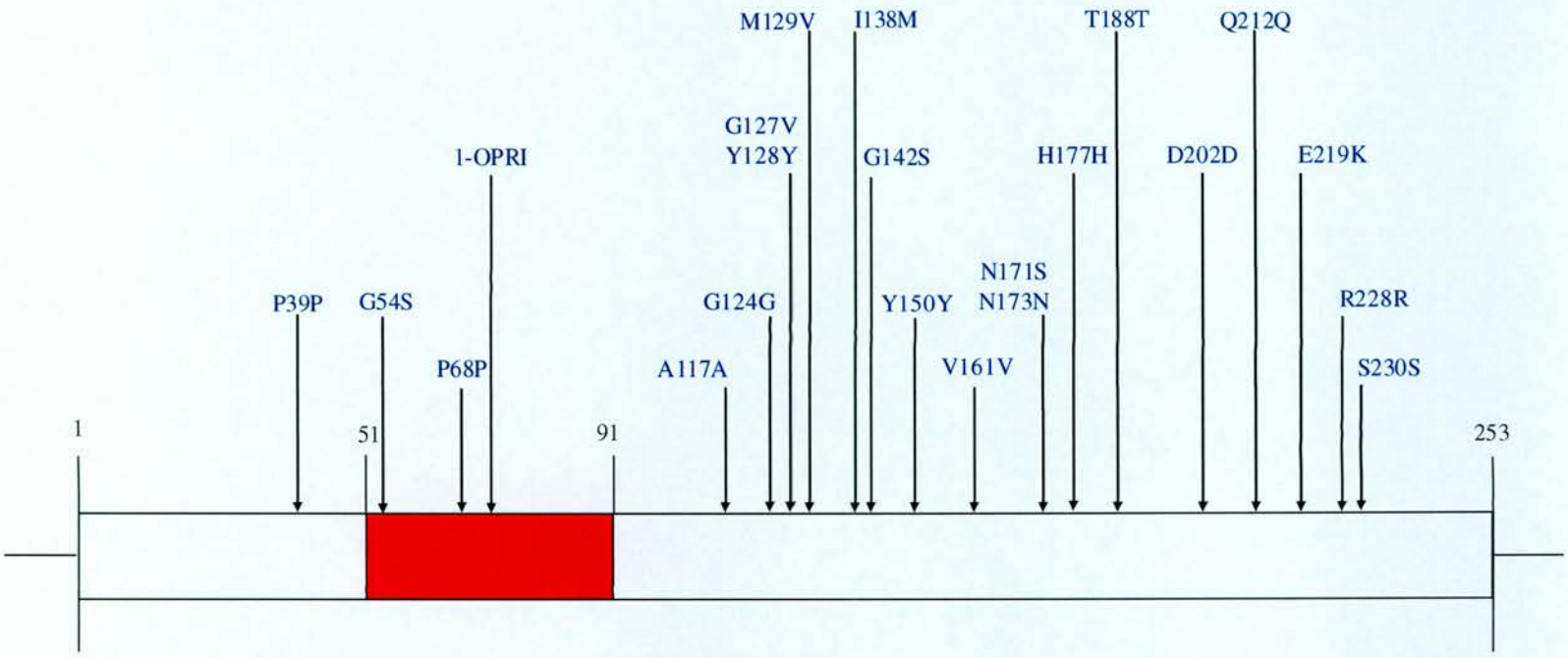


*Figure 2.2* *Polymorphisms of PRNP*



## 2.II Literature Review Methodology

A comprehensive search of Medline and Pubmed databases was undertaken, using multiple relevant keywords selected to be as inclusive as possible. Keywords used included: 'prion disease'; 'genetic prion disease'; 'familial prion disease'; 'hereditary prion disease'; 'transmissible spongiform encephalopathy'; 'genetic transmissible spongiform encephalopathy'; 'FFI'; 'fatal familial insomnia'; 'Indiana kindred'. Multiple variants on these terms were also used as search terms, as were the individual mutations and halotypes. Key authors in the field were identified and searched for to obtain a full list of publications by them. The abstracts of publications identified were reviewed to assess their relevance. Any potentially useful publications were obtained and reviewed in full. A small number of references were not available in full (primarily those not published in English). The references cited in these papers were also reviewed for relevance. Further references were obtained from existing reviews such Kong et al. and Kovacs et al. This process was repeated and results cross-referenced until no further relevant publications were found.

Data regarding age at onset, clinical features and investigative findings, disease duration, neuropathological and immunohistochemical analysis were extracted. Details of the ethnic origin of individuals and their families, the pattern of inheritance and genetic studies were recorded.

Where possible the available data was summarised into tables. These were sub-divided as appropriate, in order to collate patients or families of the same haplotype, and to identify those with additional *PRNP* polymorphisms which could influence the phenotype. In the OPRI cases, those with a known insertion sequence are sub-divided according to the exact sequence. This information was not always available, and this is stated in the text.

## 2.III P102L–129 M

The P102L–129 M mutation is the commonest cause of GSSS worldwide, and has been identified in at least 31 families[328,88,138,169,218,139,225,149,406,83,220,374,132,297,62]. The mutation has been reported in Austria, Canada, China, Denmark, France, Germany, Hungary, Israel, Italy, Japan, Korea, Mexico, Poland, the UK and the USA. Linkage between the mutation and the disease has been demonstrated[348]. There is evidence that the mutation has occurred on multiple occasions, and it has a high penetrance[376]. There is considerable phenotypic heterogeneity both between and within families. Several different clinical phenotypes have been described, which are not completely explained by differences at codon 129. The mean age at onset has been found to be younger in methionine homozygous patients, although the disease duration is not affected[376]. $PrP^{Res}$ of a type similar to type 1 has been described, but the glycoform ratio is distinct, with a predominance of the diglycosylated form of $PrP^{Res}$ [294]. There is speculation that differences in the propagation of wild-type and mutant prion protein may explain some of the phenotypic heterogeneity[371]. P102L was one of the earliest prion diseases to be transmitted to experimental animals, where it often closely resembles experimentally transmitted sCJD and lacks the amyloid deposition seen in humans[241,19].

*Table 2.1* *P102L-129M Clinical phenotype, investigative and neuropathological findings*

<table>
<tr><th>Codon 129</th><th colspan="2">Mean age at onset (years; SD)[376]</th><th>Mean duration (months; SD)[376]</th></tr>
<tr><td>MM</td><td colspan="2">47.3 +/- 10.2</td><td rowspan="2">49 +/- 26</td></tr>
<tr><td>MV</td><td colspan="2">54.0 +/- 5.7</td></tr>
<tr><th colspan="3">Clinical features</th><th>FHx</th></tr>
<tr><td colspan="3">Slowly progressive cerebellar ataxia & late onset dementia; occasional myoclonus<br>Prominent cognitive & psychiatric features; few other neurological features<br>Clinical features may be similar to sCJD</td><td>Positive</td></tr>
<tr><th>EEG</th><th>Neuroimaging</th><th>14-3-3</th></tr>
<tr><td>Rarely periodic</td><td>Atrophy<br>(basal ganglia high signal reported rarely)[62]</td><td>Occasionally positive</td></tr>
<tr><th colspan="3">Neuropathological findings</th></tr>
<tr><td colspan="3">Multicentric $PrP^{Sc}$ positive plaques<br>Fibrillar & non-fibrillar $PrP^{Sc}$ in the cerebral & cerebellar parenchyma<br>Variable spongiform change; more severe if disease duration shorter</td></tr>
</table>

## 2.IV P102L–129M, 219K

One Japanese family has been described with the P102L mutation and lysine at residue 219, in conjunction with methionine at codon 129. E219K is a normal polymorphism and was not found on the mutated allele of 20 other (unrelated) P102L cases screened. Of the 4 family members reported, 2 had dementia without other neurological features, 1 had cerebellar signs alone, and 1 had a cerebellar onset with the later appearance of dysarthria, dystonia and apraxia. These findings are in contrast to the typical P102L–129M phenotype of a cerebellar onset followed by late cognitive decline. The pathological findings were also distinct (but this finding should be interpreted with caution as only 1 individual with P102L-129M, 219K has undergone neuropathological examination).

*Table 2.2 P102L–129M, 219K Clinical phenotype, investigative and neuropathological findings*

<table>
<tr><th>Codon 129</th><th colspan="2">Codon 219</th><th>Onset (mean; years)</th><th>Duration (years)</th></tr>
<tr><td>MM</td><td colspan="2">Lysine (segregating with the P102L mutated allele)</td><td>44</td><td>4 to > 5</td></tr>
<tr><th colspan="3">Clinical features</th><th colspan="2">FHx</th></tr>
<tr><td colspan="3">Cerebellar signs without dementia; or dementia alone</td><td colspan="2">Positive</td></tr>
<tr><th>EEG</th><th>Neuroimaging</th><th colspan="3">14-3-3</th></tr>
<tr><td>Not typical</td><td>Atrophy</td><td colspan="3">Not reported</td></tr>
<tr><th colspan="5">Neuropathological findings</th></tr>
<tr><td colspan="5">A few $PrP^{Sc}$ positive plaques in the cerebrum, cerebellar cortex & basal ganglia</td></tr>
</table>

## 2.V P102L–129V

Two cases of GSSS due to the P102L mutation coupled with valine at codon 129 have been reported[361,405]. Brain homogenate from the first reported case was used to successfully transmit disease to humanised transgenic mice; however, no details are available other than that this individual was valine homozygous at codon 129 and of North American origin[358]. Details of other case are given below.

*Table 2.3 P102L-129V Clinical phenotype, investigative and neuropathological findings*

| **Sex** | **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|---|
| M | Italian American | 33 | 12 | VV |
| **Onset** | **Clinical features** | | | **FHx** |
| Seizures | Progressive sensory loss & weakness, gait disturbance, bilateral hearing loss, dysarthria, dysphagia, epilepsy | | | Positive |
| **Neuropathological findings** | | | | |
| $PrP^{Sc}$ positive amyloid plaques in the cerebral & cerebellar cortex, basal ganglia & hippocampus<br>Degeneration of the corticospinal, spinocerebellar & gracile tracts<br>Punctate $PrP^{Sc}$ deposition in the substantia gelatinosa<br>No evidence of spongiform change | | | | |

The clinical and pathological findings were distinct from those typically seen with P102L–129M. The disease duration was longer, with seizures being a prominent feature, and no evidence of dementia. The spinal cord was affected and there was a lack of spongiform change. A maternal cousin of the proband was also thought to be affected, but no further data regarding this individual is available.

Five unrelated Japanese families have been reported with prion disease due to the P105L (CCA to CTA) mutation, coupled with valine on the mutated allele[13,362,198,398,273,399]. The majority of patients had an illness characterised by a spastic paraparesis and dementia, however in 2 cases gait apraxia was present rather than spasticity. The neuropathological findings have also been variable, with 2 cases showing numerous neurofibrillary tangles. This finding was absent from the other reported cases. All cases have had a positive family history, with an apparently autosomal dominant pattern of inheritance. Overall the clinicopathological phenotype resembles that of GSSS.

*Table 2.4* *P105L-129V Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|
| Japan | 5th decade (38 to 57 years) | 5 to 12 years | MV |
| **Clinical features** | | | **FHx** |
| Dementia, dysarthria, spastic paraparesis, pyramidal signs, ataxia. Tremor in 2 cases | | | Positive |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| No periodic complexes | Atrophy | | Not reported |
| **Neuropathological findings** | | | |
| Atrophy, neuronal loss & gliosis<br>$PrP^{Sc}$ positive amyloid plaques, predominantly in the cerebral cortex<br>Pyramidal tract degeneration. Sparing of the cerebellum<br>Neurofibrillary tangles in 2 cases | | | |

## 2.VII P105S–129V

One family with prion disease due to the P105S mutation has been described. Details of the proband are given below[364]. The mutation was also found in a parent, who was described as being unaffected. There was a limited FHx, but no clear history of neurological illness in any other members.

*Table 2.5 P105S-129V Clinical phenotype, investigative and neuropathological findings*

| Sex | Origin | Mean Onset (years) | Duration (years) | Codon 129 |
|---|---|---|---|---|
| F | N. America | 30 | 10 | MV* |
| **Onset** | | **Clinical features** | | **FHx** |
| Cognitive decline, behavioural change & dysphasia | | Frontal type dementia, paratonia, extrapyramidal signs, mutism | | Negative |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Slow | | High signal in the caudate & putamen; cortical high signal | | Not done |
| **Neuropathological Findings** | | | | |
| Marked atrophy<br>Spongiform change, neuronal loss, astrocytosis<br>Multicentric & punctate plaques | | | | |

* The P105S segregated with valine at codon 129. The other allele carried a 1-OPRI deletion, a normal polymorphism.

The glycosylation ratio was also analysed, and the abnormal prion protein was found to contain unglycosylated and monoglycosylated fragments, with the monoglycosylated being predominant. This pattern is unusual for GSSS, and resembled that seen in prion disease due to T183A and V180I, although it remained distinct from these.

## 2.VIII P105T-129M

This haplotype has been described in 2 families; only very limited information is available about the first family, which was reported on the website http://www.mad-cow.org/prion_point_mutations.html. The father apparently died of CJD at the age of 42 years, and his son subsequently developed symptoms at the age of 30 years. The P105T mutation was found in the son, with heterozygosity at codon 129. Details of the other family[326] are shown below.

*Table 2.6 P105T-129M Clinical phenotype, investigative and neuropathological findings of the family reported by Rogaeva[326]*

| **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|
| East India | 13 to 40 | Approximately 2 to 3 | MM & MV |
| **Onset** | **Clinical features** | | **FHx** |
| Psychiatric symptoms or behavioural and gait problems | Spasticity, hyper-reflexia, paratonia, tremor, dystonia, ataxia, dementia | | Positive |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| Normal or diffuse slowing | White matter high signal (1 case) | | Negative |

The clinical phenotype seen in the family reported by Roagaeva et al. was variable, with some individuals primarily having dementia, pyramidal signs and ataxia in their 30s, whilst the proband initially developed psychiatric features at the age of 13 years followed by pyramidal and cerebellar signs but without dementia. These differences may be related to the proband being heterozygous at codon 129 whilst other family members were homozygous for methionine.

## 2.IX P105T-129V

One family has been described with the P105T mutation in conjunction with valine at codon 129V[308]. Three members were affected, all of whom developed progressive cognitive decline followed by a cerebellar syndrome with gait ataxia. The clinical details are given below.

*Table 2.7 P105T-129V Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Onset (years; mean)** | **Duration (mean)** | **Codon 129** |
|---|---|---|---|
| Not stated | 41 | 4 years and 8 months | VV |
| **Onset** | **Clinical features** | | **FHx** |
| Cognitive disturbance | Dementia, cerebellar ataxia, saccadic dysmetria, myoclonus | | Positive |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| Normal or diffuse slowing | Cortical ribboning and thalamic high signal (1 case) | | Negative (1 case) |
| **Neuropathological Findings** | | | |
| Spongiform change and neuronal loss throughout cerebral cortex (cerebellum mildly affected)<br>Synaptic $PrP^{Sc}$ deposition in all cortical layers<br>Some plaque-like deposits and unicentric $PrP^{Sc}$ plaques | | | |

## 2.X G114V–129M

Two families have been reported with prion disease due to a glycine to valine change at codon 114 of the *PRNP* gene, with an autosomal dominant pattern of inheritance[233,322,403,339]. In addition 1 affected South Indian male and a female Turkish patient have been described[25]. In the small number of cases reported the codon 129 status of the wild type allele did not obviously influence the clinical phenotype. In the families reported by Rodriguez et al.[322] and Liu et al.[233] methionine was present at codon 129 of the mutated allele. Unaffected mutation carriers who were older than the affected individuals were found in both families, implying incomplete penetrance. The mutation has not been found in control populations.

*Table 2.8 G114V clinical phenotype (1)*

| Reference | Sex | Origin | Onset (years) | Duration | Codon 129 |
|---|---|---|---|---|---|
| Rodriguez[322] | M & F | Uruguay | mean 23.4 (range 18 to 28) | 2.6 years (1 to 4) | MM & MV |
| Liu[233] | M & F | China | 32 to 45 | 2 to 3 years | MM |
| Beck[25] | M | S. India | 75 | 6 months | MV |

*Table 2.9 G114V clinical phenotype (2)*

| Reference | Onset | Clinical features | FHx |
|---|---|---|---|
| Rodriguez[322] | Personality & behavioural change | Dementia, extrapyramidal & pyramidal signs, myoclonus, mild cerebellar features. | Positive |
| Liu[233] | Cognitive decline, occasional insomnia | Pyramidal and extra-pyramidal signs, hallucinations | Positive |
| Beck[25] | Fatigue, hemiparesis, sensory disturbance | Asymmetrical akinetic rigid syndrome, myoclonus, mild apraxia, well preserved cognition | Negative |

*Table 2.10 G114V investigative findings*

| Reference | EEG | Neuroimaging | 14-3-3 |
|---|---|---|---|
| Rodriguez[322] | Encephalopathic changes | Atrophy | Not done |
| Liu[233] | Non-specific slowing | Bilateral caudate nuclei and putaminal high signal & atrophy (1 case) | Negative (1 case) |
| Beck[25] | Non-specific slowing | Basal ganglia high signal | Negative |

*Table 2.11 G114V neuropathological findings*

| Reference | Neuropathological Findings |
|---|---|
| Rodriguez[322]* | Spongiosis, gliosis & neuronal loss (2 cases)<br>Amyloid plaques & synaptic $PrP^{Sc}$ deposition (1 case) |
| Liu[233] | Neuronal loss, spongiform change and astrocytosis in the cortex.<br>Synaptic $PrP^{Sc}$ deposition. Type 1 $PrP^{Res}$ |
| Beck[25] | Not done |

* The neuropathological data comes from frontal brain biopsies of 2 cases.

## 2.XI A117V–129V

Nine families have been reported with prion disease due to this mutation; there are 2 base changes at codon 117, firstly an A to G substitution at the third base (a normal finding in 10% of the population), and secondly a C to T substitution at the second base. The second substitution causes a coding change from alanine to valine. The affected families are variously of French, German, British, Irish and North American origin[88,280,171,363,243,160,237,209]. The pattern of inheritance is autosomal dominant, with high penetrance.

The clinical phenotype varies both between and within reported families. Dementia may be the dominant problem, or there may be dementia and a cerebellar syndrome. Other cases have marked extra-pyramidal and pyramidal features and myoclonus, or present with neuropsychiatric problems. 1 case with prominent lower motor neurone signs and spinocerebellar tract degeneration has been described[209]. Patients frequently have a background of behavioural and personality change prior to the onset of overt neurological problems, often being reported as being aggressive and antisocial.

There are usually widespread $PrP^{Sc}$ amyloid plaques in the cerebral cortex, hippocampus, basal ganglia and thalamus. The cerebellum is usually spared, however in a minority it is severely affected. Spongiform change, neuronal loss and neurofibrillary tangles have also been found in some cases.

Valine homozygous and heterozygous individuals have been described; it is not clear if the codon 129 status significantly affects the clinico-pathological phenotype. Valine homozygosity may possibly be associated with a younger age at onset[237].

A transgenic mouse model of A117V has been created. These animals develop a disease with a longer duration than is seen in mouse models of sCJD, and have more prominent ataxia[402]. Neuropathologically there were $PrP^{Sc}$ positive plaques present. As with the human cases, the neuropathological features seen varied between individuals, with differences in plaques distribution.

*Table 2.12* *A117V-129V Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|
| Various | Second to seventh decade | 1 to 11 | MV & VV |
| **EEG** | | **Neuroimaging** | **14-3-3** |
| Slow or non-specifically abnormal | | Atrophy | Not reported |
| **Clinical features** | | | **FHx** |
| Dementia in isolation or associated with a cerebellar syndrome; possibly other findings such as pyramidal or extrapyramidal signs | | | Positive |
| **Neuropathological findings** | | | **$PrP^{Sc}$ type** |
| Classically widespread $PrP^{Sc}$ amyloid plaques; the cerebellum is often spared. Possibly spongiform change; usually focal in nature | | | Unknown |

## 2.XII G131V-129M

G131V-129M is a rare mutation which has been reported in association with a GSSS-type phenotype. Both of the published cases had a similar age at onset, clinical and pathological features although one was codon 129 heterozygous and the other homozygous. Neurofibrillary tangles were seen in the Australian case and tau positivity in the Dutch patient. This pattern of findings has been seen with other pathogenic *PRNP* mutations, and raises questions regarding whether these findings are causally linked or coincidence.

*Table 2.13 G131V-129M clinicopathological phenotype of the patient reported by Panegyres*[291]

| **Origin** | **Sex** | **Onset (years)** | **Duration** | **Codon 129** |
|---|---|---|---|---|
| Australia | Male | 42 | 9 years | MM |
| **Clinical features** | | | | **FHx** |
| Behavioural change, dementia, anxiety, apraxia, gegenhalten, hyper-reflexia | | | | Negative |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| No periodic complexes | | Generalised atrophy | | Unknown |
| **Neuropathological Findings** | | | | |
| Abundant $PrP^{Sc}$ positive amyloid plaques in the cerebellum.<br>Molecular layer particularly affected<br>Occasional neurofibrillary tangles<br>Protease resistant PrP. No spongiform change. | | | | |

*Table 2.14* *G131V-129M clinicopathological phenotype of the patient reported by Jansen*[181]

| Origin | Sex | Onset (years) | Duration | Codon 129 |
|---|---|---|---|---|
| Holland | Male | 36 | Not reported | MV |
| **Onset** | | **Clinical features** | | **FHx** |
| Slowly progressive dementia | | Ataxia, parkinsonism | | Positive |
| **Neuropathological Findings** | | | | |
| Numerous $PrP^{Sc}$ amyloid plaques in the cerebellum<br>Tau positive amyloid deposits in the cerebral cortex, striatum, hippocampus & midbrain<br>2 distinct PrP types: 8 kDa unglycosylated fragment & a detergent-insoluble but protease-sensitive form of $PrP^{Sc}$. No spongiform change | | | | |

## 2.XIII Y145X–129M

One Japanese patient with a stop mutation at codon 145 has been described. This patient died after a prolonged illness, and on PM did not have spongiform change. No transmission studies have been published from this patient, and her illness is currently termed 'PrP cerebral amyloid angiopathy'. The authors report that the neuropathological changes seen resembled the findings in scrapie[124,199].

*Table 2.15 Y145X-129M Clinical phenotype, investigative and neuropathological findings*

| **Sex** | **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|---|
| F | Japan | 38 | 21 | MM |
| **Onset** | | **Clinical features** | | **FHx** |
| Memory problems & disorientation | | Dementia | | Negative |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Normal | | Severe atrophy | | Not done |
| **Neuropathological findings** | | | | |
| Cerebral atrophy<br>Extensive $PrP^{Sc}$ amyloid deposition in cerebral blood vessels<br>Neurofibrillary lesions in cerebral gray matter<br>Severe neuronal loss and gliosis | | | | |

## 2.XIV R148H–129M

Two patients with prion disease due to a histidine to arginine substitution (CGT to CAT) at codon 148 of the *PRNP* gene have been described[298,217]. The clinical features are summarised below.

*Table 2.16 R148H-129M Clinical phenotype, investigative and neuropathological findings of the family reported by Krebs[217]*

| **Sex** | **Origin** | **Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|---|
| F | German | 82 | 6 | MM |
| **Onset** | | **Clinical features** | | **FHx** |
| Depression & anxiety | | Dementia, mutism, pyramidal signs, myoclonus, paratonia | | Negative |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Periodic complexes | | Small subcortical & peri-ventricular high signal lesions | | Positive<br>Tau elevated |
| **Neuropathological findings** | | | | **$PrP^{Res}$ Type** |
| Slight atrophy<br>Moderate spongiform change, neuronal loss & astrocytosis<br>Synaptic $PrP^{Sc}$ staining | | | | 1 |

*Table 2.17* *R148H-129M Clinical phenotype, investigative and neuropathological findings of the family reported by Pastore*[298]

| **Sex** | **Origin** | **Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|---|
| F | N. America | 62 | 18 | MV |
| **Onset** | | **Clinical features** | | **FHx** |
| Hearing loss & difficulty driving | | Fatigue, sleep disturbance, balance & gait problems, dizziness, dementia, delusions, hallucinations | | Negative* |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Normal | | Unknown | | Negative |
| **Neuropathological findings** | | | | **$PrP^{Res}$ Type** |
| Spongiform change, neuronal loss & gliosis<br>Kuru plaques in the granular & Purkinje layers of the cerebellum<br>$PrP^{Sc}$ positivity in plaques & in a synaptic fashion | | | | 2 |

* The mutation was also found in the proband's 2 sisters, who were well at the ages of 66 and 70 years.

The methionine homozygous case closely resembled the clinicopathological phenotype of sCJDMM2, whilst the heterozygous case resembled sCJDMV2.

## 2.XV Q160X–129M

Two families with probable prion disease and the Q160X–129M mutation have been identified. Only limited clinical information is available for the first family[95,96]. In the second family a mother and daughter were affected. Both had an Alzheimer's type presentation and neuropathology, but with $PrP^{Sc}$ positivity[186]. However there was no spongiform change at PM so the possibility remains that these individuals were affected by familial Alzheimer's disease rather than their illness being linked to the Q160X mutation. There is also speculation that *PRNP* stop mutations as a group may tend to give rise to neurofibrillary tangles and neuritic plaques.

*Table 2.18 Q160X-129M Clinical phenotype, investigative and neuropathological findings of the family reported by Finck[96]*

| Sex | Origin | Mean Onset (years) | Clinical features | Codon 129 | Family History |
|---|---|---|---|---|---|
| M | Austria | 32 | Dementia | MM | Positive |
| M | Austria | 48 | Dementia | MV | Positive |

*Table 2.19 Q160X-129M Clinical phenotype, investigative and neuropathological findings of the family reported by Jayadev[186]*

| Sex | Origin | Onset (years) | Duration (years) | Codon 129 |
|---|---|---|---|---|
| F | N. America | 39 & 59 years | 8 | MV & MM |

| Onset | Clinical features | FHx |
|---|---|---|
| Cognitive decline | Slowly progressive dementia | Positive |

| EEG | Neuroimaging |
|---|---|
| 'unremarkable' (sic) | 'unremarkable' (sic) |

| Neuropathological findings |
|---|
| Extensive neurofibrillary tangles and neuritic plaques. Tau and $PrP^{Sc}$ positive |

## 2.XVI D167N-129M

One individual with a D167N mutation has been reported; it is unclear if this mutation is pathogenic or not, especially as the patient's mother is a carrier, and reported to be unaffected at the age of 65 years. [25]Clinical details of the proband are given below. It has not been identified in normal populations.

*Table 2.20 D167N-129M Clinical phenotype and investigative findings*

| **Sex** | **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|---|
| M | Turkey | 33 | 2 | MM |
| **Onset** | | **Clinical features** | | **FHx** |
| Forgetful, emotional lability, aggression | | Pyramidal signs, gait disturbance, startle response, primitive reflexes | | Negative |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Generalised slowing | | Moderate frontotemporal cortical atrophy | | Not done |

## 2.XVII N171S–129V

One Brazilian family has been identified with an adenine to guanine substitution at codon 171, resulting in an asparagine to serine change. This mutation was found in 6 family members, 4 of whom had a history of psychiatric illness, with auditory hallucinations, persecutory delusions, depression and aggression. 1 affected relative had a 7 year history of behavioural change, gait disturbance and dementia, and a further relative carried the mutation but was asymptomatic in their 5$^{th}$ decade. The proband had a 10 year history of psychiatric illness, whilst his mother had a 35 year history of psychiatric illness, and now has dementia at the age of 76 years. No investigative findings or pathological data are available. It is therefore unclear if this family is affected by prion disease or not; of note is the fact that a sibling of the proband had bipolar disorder but had no mutations of *PRNP*[331].

## 2.XVIII D178N

The D178N mutation is one of the commonest causes of gPD worldwide, and is one of the most intriguing mutations. Two distinct clinicopathological phenotypes can arise, depending on the codon 129 status of the mutated allele. If methionine is encoded, then FFI occurs; if valine is present then a sCJD-like phenotype is seen [137]. The phenotype is further modified by codon 129 of the wild-type allele[271]. These rules are not absolute, with a sCJD-like phenotype being reported in individuals with D178N-129M[411]. However no cases of FFI due to the D178N-129V haplotype have been reported. The mechanisms responsible for the different phenotypes are not well understood. D178N-129M is much more widely reported than D178N-codon 129V, and haplotype analysis indicates that many European FFI cases can be linked to one of two common founders[323]. Of particular interest is a recently reported patient with somatic mosacism of D178N-129V; this was a de novo mutation (not being present in either parent) and presumed to be post-zygotic as wild type D178-129M, D178-129V and mutated D178N-129V were all detectable in blood and brain tissue[12].

The existence of SFI further confuses the issue; as previously discussed, SFI has an almost identical phenotype to FFI. The only difference (excepting the lack of

a mutation on *PRNP* genotyping) is the glycosylation ratio[292]. This raises interesting speculation as to whether or not mutations elsewhere in the genome could be contributing to these diseases, or if SFI in fact belongs to the sCJD phenotypic spectrum.

## 2.XIX D178N–129M (FFI)

FFI has been reported in at multiple families and individuals from various European countries (including Germany, Austria, Spain, Italy, the UK, France and Finland), Australia, N. America, Japan, China and Korea[312]. Haplotype analysis of European cases suggests that that two historical independent mutational events may be responsible for many current cases[323]. The codon 129 status of the wild type allele influences the disease phenotype; there is a tendency for the age at onset to be younger in homozygous patients, but this is not statistically significant, whereas the mean disease duration does significantly differ between homozygous and heterozygous cases[271]. Patients do not seem to develop dementia, but display progressive confusion with decreased vigilance, working memory and attention. Details of the phenotype are given below.

*Table 2.21* *D178N-129MM Clinical phenotype, investigative and neuropathological findings*

| **Onset (years)** | | **Duration (months)** |
|---|---|---|
| 49 (range 20 to 72) | | 11 +/- 4 (range 5 to 21) |
| **Onset** | **Clinical features** | **FHx** |
| Sleep-wake cycle disturbance & decreased vigilance | Insomnia, autonomic dysfunction, confusion, motor symptoms, terminal akinetic mutism | Positive |
| **EEG** | **Neuroimaging** | **14-3-3** |
| Non-specific slow wave activity | Hypometabolism in the thalamus, basal ganglia & cingulate cortex; mild atrophy | Positive in around 50% |
| **Neuropathological findings** | | **Immunohistochemistry** |
| Severe neuronal loss & astrogliosis of the thalamus<br>Minor spongiform change in the cerebral cortex* | | Often negative<br>Occasional $PrP^{Sc}$ deposition in the molecular layer of the cerebellum;<br>Type 2 $PrP^{Res}$ |

* Tau deposition reported in one case[183]

*Table 2.22* *D178N-129MV Clinical phenotype, investigative and neuropathological findings*

| **Onset (years)** | | **Duration (months)** |
|---|---|---|
| 49 (range 20 to 72) | | 23 +/- 18 (range 7 to 84) |
| **Onset** | **Clinical features** | **FHx** |
| Ataxia & dysarthria | Later onset of sleep-wake disturbance; more motor symptoms, terminal akinetic mutism | Positive |
| **EEG** | **Neuroimaging** | **14-3-3** |
| Transient periodic complexes in some long duration cases | Mild atrophy<br>More widespread hypometabolism | Positive in around 50% |
| **Neuropathological findings** | | **Immunohistochemistry** |
| Similar to D178N-129MM but more severe cerebral cortical involvement | | Often negative<br>Occasional $PrP^{Sc}$ deposition in the molecular layer of the cerebellum;<br>Type 2 $PrP^{Res}$ |

The distinction between homozygous and heterozygous cases does not always hold; homozygous cases with normal sleep patterns and a sCJD-like clinical phenotype have been reported[330,411,354]. An increasing number of reports describe considerable phenotypic variation within families, and some authors postulate that there is a spectrum of disease ranging from classical FFI to a sCJDMM1-like phenotype [143]. In one case an ataxic phenotype resembling GSSS was seen[352]. Variations in the amount and type of abnormal prion protein found may possibly correlate with the phenotypic type. The disease has been transmitted by the inoculation of humanised transgenic mice, which go on to display prominent thalamic pathology. However unlike the donor cases, the mice display $PrP^{Res}$ type 1; the reason for this difference between donor and recipient is not fully understood[359]. Animals genetically modified to have a *PRNP* mutation homologous to D178N–129M also develop spontaneous prion disease, and like their human counterparts they display abnormalities of the sleep-wake cycle and EEG[176,89].

## 2.XX D178N–129V

The D178N mutation with valine on the mutated allele gives rise to a sCJD-like clinicopathological phenotype; no cases with this mutation and features akin to FFI have been reported. Both codon 129 heterozygous and valine homozygous individuals have been described, which appears to significantly influence the age at onset and disease duration; however the clinical features and pathological appearances are homologous in the 2 groups (although the number of cases reported in detail with known codon 129 status is somewhat limited). Twelve families have been described from N. America, France, Finland, Germany (the original Backer family first described in 1920), and Israel[312]. Of the N. American kindreds one is of Hungarian-Romanian, one of Dutch and one of French-Canadian origin. Brain tissue has been used to successfully transmit disease to squirrel monkeys but not to transgenic mice[39,359].

*Table 2.23* *D178N-129V Clinical phenotype, investigative and neuropathological findings*

| **Codon 129** | **Mean Onset (years; range)*** | **Mean Duration (months; range) ** ** |
|---|---|---|
| VV | 39 +/- 8 (26 to 47) | 14 +/- 4 (9 to 18) |
| MV | 49 +/- 4 (45 to 56) | 27 +/- 14 (7 to 51) |

| **Onset** | **Clinical features** | **FHx** |
|---|---|---|
| Cognitive decline, behavioural change & psychiatric features | Ataxia, dysarthria, aphasia, tremor, myoclonus | Positive |

| **EEG** | **Neuroimaging #** | **14-3-3 #** |
|---|---|---|
| Slow | CT normal<br>SPECT: hypoperfusion | Positive |

| **Neuropathological findings** |
|---|
| Spongiosis, severe gliosis, variable neuronal loss. Possibly ballooned or enlarged neurons<br>Widespread changes especially in the frontal & occipital cortex, putamen & caudate nucleus, subiculum & entorhinal cortex. Cerebellum spared |

| **Immunohistochemistry** |
|---|
| Punctate $PrP^{Sc}$ deposition (associated with spongiosis)<br>Minimal $PrP^{Sc}$ in cerebellum<br>Type 1 $PrP^{Res}$; unglycosylated form under represented |

* $p < 0.01$; ** $p < 0.05$; figures from Kong et al in Prion Biology and Diseases[312] .

# Neuroimaging results and CSF analysis findings have only been reported for a very small number of cases.

## 2.XXI D178N-129V & N171S-129V

An African-American family has recently been reported with both the D178N mutation and the N171S mutation, with valine on the mutated allele[16]. N171S has been linked to psychiatric disturbance, and all of the affected family members with D178N and N171S presented with depression and anxiety. Further details of the proband are given below. The later clinical features were similar to those of FFI.

*Table 2.24 D178N-129V & N171S-129V Clinical phenotype and investigative findings[16]*

| **Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|
| 52 | 52 | MV |
| **Onset** | **Clinical features** | **FHx** |
| Psychiatric disturbance | Insomnia, pyramidal, cerebellar and visual disturbance, involuntary movements (chorea, tremor & myoclonus), dementia, autonomic failure | Positive |
| **EEG** | **Neuroimaging** | **14-3-3** |
| Slow | Basal ganglia & cortical high signal | Not done |

## 2.XXII V180I–129M

The V180I mutation has been reported in a small number of Japanese patients and individuals from the USA, France and South Korea. In addition 1 Japanese patient was found to have both the V180I and the M232R mutation (discussed in 'Single cases' section)[401,404,164,197,351,189]. In codon 129 MV cases amyloid angiopathy and neurofibrillary tangles were found alongside severe spongiform change[351]. Five patients were methionine homozygous and 6 heterozygous at codon 129 (the status of the American patient was not reported). The age at onset was slightly younger in the homozygous cases (mean age at onset 70.3 years vs. 74.8 years). Only very subtle $PrP^{Sc}$ deposition was found, although there was widespread spongiform change. In the codon 129 heterozygous cases kuru plaques were also found[351,60,279].

*Table 2.25 V180I-129M Clinical phenotype, investigative and neuropathological findings of the Japanese cases*

| **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|
| 66 to 81 | 1 to 2 | MM & MV cases |
| **Onset** | **Clinical features** | **FHx** |
| Cognitive decline | Dementia, extrapyramidal signs, myoclonus, akinetic mutism | Negative |
| **EEG** | **Neuroimaging** | **14-3-3** |
| Slow; no periodic complexes | Extensive cortical high signal | Raised (2 out of 3 cases) |
| **Neuropathological findings** | | |
| MM cases | Widespread spongiform change, astrogliosis & neuronal loss<br>Subtle synaptic $PrP^{Sc}$ deposition<br>Cerebellum relatively spared | |
| MV case | Spongiform change & kuru plaques in the cerebral cortex | |

*Table 2.26* *V180I-129M Clinical phenotype, investigative and neuropathological findings of the French case*

| **Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|
| 66 | 54 | MM |
| **Onset** | **Clinical features** | **FHx** |
| Cognitive decline & dysphasia | Mutism, myoclonus, rigidity, dystonia | Negative |
| **Neuropathological findings** | | |
| Severe spongiosis & gliosis of the cerebral cortex, striatum & entorhinal cortex<br>Moderate neuronal loss & gliosis of the thalamus<br>Synaptic $PrP^{Sc}$ deposition<br>Cerebellum spared; no plaques in any brain region<br>Western blotting demonstrated mono- and unglycosylated $PrP^{Res}$ | | |

## 2.XXIII T183A–129M

Two families with prion disease due to the T183A–129M mutation have been described[278,277,95,141]. In the Brazilian family[278] individuals with methionine homozygosity and heterozygosity at codon 129 were identified; this possibly influenced the disease duration. The proband was heterozygous at codon 129 and had a disease duration of 9 years, whereas his mother was methionine homozygous and died within 2 years of onset. However the age at onset did not seem to be affected by the codon 129 status.

*Table 2.27* *T183A-129M Clinical phenotype, investigative and neuropathological findings of the family reported by Nitrini*[278]

| **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|
| Brazil | 44.8 | 4.2 | MM & MV |
| **Clinical features** | | | **FHx** |
| Personality change, frontotemporal features, dementia | | | Positive |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| Slow | Severe atrophy or striatal & cortical high signal | | Negative |
| **Neuropathological findings** | | | |
| Severe spongiform change & neuronal loss in the deep cortical layers & the putamen, with minimal gliosis. $PrP^{Sc}$ immunoreactivity in the putamen & cerebellum only. | | | |

*Table 2.28* *T183A-129M Clinical phenotype, investigative and neuropathological findings of the family reported by Grasbon-Frodl*[141]

| **Origin** | **Onset (years)** | **Duration (months)** |
|---|---|---|
| Germany | 40 | 4 |
| **Clinical features** | | **FHx** |
| Dementia, cerebellar ataxia | | Positive |
| **EEG** | **Neuroimaging** | **14-3-3** |
| Normal | Global atrophy | NA |
| **Neuropathological findings** | | |
| Severe spongiform change, particularly in the molecular layer of the cerebellum. Neuronal loss Faint $PrP^{Sc}$ positivity; intra-neuronal $PrP^{Sc}$ & small plaque-like $PrP^{Sc}$ deposits Predominantly monoglycosylated $PrP^{Res}$ | | |

## 2.XXIV H187R

The H187R mutation has been described in 2 large multi-generational kindreds, both in North America[151,46,56,72]. The codon 129 status is only known for one family, where the mutation was in sinc with valine[46]. Both MV and VV individuals have been reported, and the codon 129 status of the wild type allele may influence the neuropathological manifestations. The clinical phenotype in this family was one of dementia, ataxia, myoclonus and seizures, with a median age at onset of 42 years and disease duration of 12 years. 9 affected individuals of both sexes have been identified, with an autosomal dominant pattern of inheritance. A possibly unique type of 'curly' PrP deposition was seen in one VV patient[72]. Further details are given in the tables below.

The family reported by Hall et al. was also large, with 12 affected individuals over 4 generations[151]. A high level of childhood psychiatric disorders was reported in affected individuals, one of whom had neurological problems from birth with language and motor development. Further details of the clinico-pathological phenotype are given in the table; overall the clinical features after the onset of dementia were similar to those reported by Butefisch et al, as was the lack of spongiform change, plaques or significant astrocytosis. However the 'curly' PrP found by Butefisch et al. was not seen.

Interpreting the significance of early psychiatric problems is difficult. In this particular pedigree, 2 individuals in one generation had early psychiatric problems, and each subsequently had children with early psychiatric problems. Does this indicate neurodevelopmental problems caused by the *PRNP* mutation? Were these symptoms actually the onset of clinical prion disease? Or are they coincidental and due to environmental or independent genetic factors? Transgenic mice models of this mutation would be helpful, as this would allow the study of early behaviour in a controlled social and genetic environment, and for the serial examination of neuropathological tissue at different ages to determine if behavioural changes were linked to pathological prion disease.

*Table 2.29* *H187R–129V Clinical phenotype, investigative and neuropathological findings of the MV cases reported by Butefisch*[46]

| **Mean Onset (years)*** | **Mean Duration (months)*** | |
|---|---|---|
| 36 | 13 | |
| **Onset** | **Clinical features** | |
| Dementia, behavioural change | Dementia, gait disturbance, dysarthria, pyramidal signs, myoclonus, seizures, cerebellar signs | |
| **EEG** | **Neuroimaging** | **14-3-3** |
| Slow | Diffuse atrophy | Negative |

*Table 2.30* *H187R–129V Clinical phenotype, investigative and neuropathological findings of the VV cases reported by Butefisch*[46]

| **Mean Onset (years)*** | **Mean Duration (months)*** | |
|---|---|---|
| 48 | 13 | |
| **Onset** | **Clinical features** | |
| Gait disturbance, dementia, headache | Dementia, gait disturbance, pyramidal signs, myoclonus, seizures. | |
| **EEG** | **Neuroimaging** | **14-3-3** |
| Slow | Diffuse atrophy | Negative |
| **Neuropathological findings*** | | |
| 'curly' prion protein deposits with a laminar distribution in the cerebellar cortex.<br>Minimal astrocytosis<br>No plaques or spongiosis | | |

*The only neuropathology details known are from a brain biopsy performed on a VV individual.

*Table 2.31* *H187R Clinical phenotype, investigative and neuropathological findings of the cases reported by Hall*[151]

| **Mean Onset of Dementia (years)*** | **Disease duration (years)** |
|---|---|
| 32 | 9 to 28 |
| **Onset** | **Clinical features** |
| Childhood psychiatric disorders in 4 patients. Later onset of dementia | Dementia in all cases. Extra-pyramidal & cerebellar signs common, seizures in 50% of cases |
| **EEG** | **Neuroimaging** |
| Bursts of sharp, slow activity or generalized slowing. | Mild to moderate diffuse cortical atrophy |
| **Neuropathological findings*** | |
| Moderate to severe atrophy in frontal, temporal and parietal regions. Mild gliosis and reactive astrocytosis. No spongiform change or plaques. Scattered tau positive staining in 1 case. | |
| **Immunohistochemistry** | |
| 2 cases tested: negative in 1, positive in the other with intermediate resistance to protease K digestion | |

## 2.XXV T188K–129M

Four women have been reported with prion disease due to the T188K mutation, 3 from Germany and 1 from Austria[96,95,324]. None were known to be related but the 3 German cases lived in relatively close proximity to each other. In addition to the CSF being positive for 14-3-3, in 1 case the tau was elevated and in another the NSE was significantly raised[324]. The mutation was also found in the healthy 51 year old brother and 79 year old father of 1 case. However it was not found in German controls. Overall the phenotype resembles that of sCJD-MM1.

*Table 2.32 T188K-129M Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|
| Austria & Germany | 57 to 76 | 5 to > 12 | MM |
| **Onset** | **Clinical features** | | **FHx** |
| Variable; gait disturbance & incoordination or cognitive decline & dizziness; or personality change & visual loss | Dementia, myoclonus & other involuntary movements, cerebellar & pyramidal signs, akinetic mutism | | Negative (2 cases) Positive (2 cases) |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| Periodic complexes | Basal ganglia & cortical high signal in some cases | | Positive |
| **Neuropathological findings** | | | |
| Spongiform change, astrocytosis & gliosis. Moderate atrophy. $PrP^{Sc}$ positive (Details available for 1 case only[355]) | | | |

## 2.XXVI T188R–129V

Only 2 individuals with the T188R mutation have been reported, both of whom had an illness consistent with prion disease but only 1 of whom had neuropathological confirmation[355,392,324]. Both had valine encoded at codon 129 of the mutated allele. In the family of the individual reported by Tartaglia et al. there were several individuals with early onset dementia (none of whom underwent *PRNP* genotyping), and 2 asymptomatic carriers of the mutation. One carrier was the patient's father, who was in his 80s, suggesting that the mutation has low penetrance.

*Table 2.33 T188R-129V Clinical phenotype and investigative findings of the patient reported by Roeber*[292,324]

| **Origin** | **Sex** | **Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|---|
| Germany | Female | 66 | 16 | VV |
| **Onset** | | **Clinical features** | | **FHx** |
| Visual loss | | Dementia, mild ataxia | | Negative |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Periodic complexes | | 2 small white matter lesions | | Positive |

*Table 2.34* *T188R-129V Clinical phenotype, investigative and neuropathological findings of the patient reported by Tartaglia*[355]

| Origin | Sex | Onset (years) | Duration (months) | Codon 129 |
|---|---|---|---|---|
| Mexican-American | Male | 55 | 14 | MV* |
| **Onset** | | **Clinical features** | | **FHx** |
| Personality & behavioural change | | Dementia, myoclonus, seizures, mutism | | Positive |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Diffuse slowing; focal slowing in temporal lobe | | Mild cortical atrophy. Subtle basal ganglia and cortical high signal | | 'inconclusive' |
| **Neuropathological findings** | | | | |
| Spongiform change in numerous brain regions. Status spongiosus in the inferior temporal gyrus. Synaptic & plaque-like $PrP^{Sc}$ deposition. Type 1 $PrP^{Res}$ | | | | |

*Valine on the mutated allele

## 2.XXVII E196K

The E196K mutation is rare, and until recently there was doubt as to whether it was truly pathogenic[338]. However 4 cases with the mutation and neuropathologically confirmed prion disease have now been reported; in addition 1 of these cases had a first degree relative with probable gPD and the same mutation[92]. Interestingly, the mutation has been seen in individuals with methionine encoded for at codon 129 of the mutated allele, and in a valine homozygous patient. The mean age at onset reported (72 years) is older than is usually seen with gPD, and the duration (4 months) shorter. Coupled with the lack of a family history in most cases, this makes coming to a correct diagnosis even harder than is usual in prion disease.

*Table 2.35* *E196K-129M Clinical phenotype, investigative and neuropathological findings*

| Origin | Sex | Onset (years) | Duration (months) | Codon 129 |
|---|---|---|---|---|
| Germany | Male & female | 69 to 77 | 2 to 8 | MM & MV cases |

| Onset | Clinical features | FHx |
|---|---|---|
| Dementia; gait problems; tremor | Ataxia, dysarthria, pyramidal signs, akinetic mutism | Positive in 1 case |

| EEG | Neuroimaging | 14-3-3 |
|---|---|---|
| Periodic complexes (2 of 3 cases) | White matter lesions (2 of 3 cases) | Positive (2 of 3 cases) |

| Neuropathological findings |
|---|
| Similar findings in the 2 MM and 1 MV case described |
| Spongiform change, astrocytic gliosis, neuronal loss |
| Cerebral cortex, basal ganglia, thalamus, brainstem nuclei and cerebellum particularly affected |
| Hippocampus mildly affected |
| Synaptic PrP deposition in 2 cases; both had $PrP^{Res}$ type 1 |
| Synaptic PrP & plaque-like deposits in the cortex and cerebellum in 1 case, in whom $PrR^{Res}$ type 1 and 2 were seen. |
| Under-representation of non-glycosylated form of $PrP^{Res}$ seen in all cases |

*Table 2.36* *E196K-129V Clinical phenotype, investigative and neuropathological findings*

| Origin | Sex | Onset (years) | Duration (months) | Codon 129 |
|---|---|---|---|---|
| Germany | Male | 72 | 2.5 | VV |

| Onset | Clinical features |
|---|---|
| Dementia | Ataxia, visual hallucinations, dysarthria, rigor, akinetic mutism |

| EEG | Neuroimaging | 14-3-3 |
|---|---|---|
| Periodic complexes | Mild cerebellar & parietal atrophy | Positive |

| Neuropathological findings |
|---|
| Spongiform change, astrocytic gliosis, neuronal loss |
| Basal ganglia, thalamus, cerebellum & brainstem nuclei involved; hippocampus severely affected |
| Synaptic & peri-neuronal $PrP^{Sc}$ deposition & plaque-like deposits in cerebellum |
| Type 2 $PrP^{Res}$ (under-representation of non-glycosylated form) |

2.XXVIII F198S–129V

Three N. American families with prion disease due to a phenylalanine to serine substitution at codon 198 of the *PRNP* gene are known, although only 2 have been published[93,94,125,305,312]. Both of the 2 published families had a strong FHx of neurodegenerative disease. The vast majority of published cases belong to a large kindred in Indiana. The mutation is coupled with valine at codon 129; both valine homozygous and heterozygous affected individuals have been reported. Homozygous patients have a younger mean age at onset (44.7 years) than heterozygous patients (60.3 years); however the numbers of individuals in this analysis was very small[87]. The investigative findings have not been reported. Transmission to hamsters, mice and marmosets has been attempted, thus far unsuccessfully.

*Table 2.37 F198S-129V Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|
| N. American Caucasian | 40 to 71 | 2 to 12 | VV & MV |
| **Onset** | **Clinical features** | | **FHx** |
| Gait disturbance & cognitive decline | Extrapyramidal syndrome, dementia; possibly psychotic depression | | Positive |
| **Neuropathological findings** | | | |
| Uni- & multi-centric $PrP^{Sc}$ positive amyloid plaques in the gray matter of the cerebrum, cerebellum & mid-brain<br>Astrocytosis, atrophy & neuronal los<br>Neurofibrillary tangles in the neocortex & subcortical gray matter<br>Abnormal neuritis (similar to those of Alzheimer's disease) | | | |

## 2.XXIX E200K-129M

In the 1960s and 1970s clusters of CJD cases were identified in rural Slovakia[248,249,269] and in Libyan immigrants to Israel[190,26]. Initially various environmental sources of infection were postulated, such as consuming sheep brain and eyeball[8,9,161,27,10]. However it soon became apparent that these clusters had an underlying genetic cause[274,268,267]. The E200K mutation was first identified in a Polish family[138,136], and shortly afterwards in Israeli patients[136]. An A to G transition at codon 200 of the coding region of *PRNP* results in a change from glycine (E) to leucine (K)[138]. The majority of cases of inherited prion disease in the world are due to this substitution, which has been found in populations from England, Austria, Chile, France, Germany, Greece, Italy, Japan, Libya, North American, Slovakia, and Tunisia[136,135,206,68,175]. E200K has been shown to be causally linked to the occurrence of prion disease[105] and transmission to primates has been performed successfully[59]. Slovakia and Israel remain the epicentre of two major clusters, and have rates of gPD greatly in excess of the rest of the world.

The incidence of E200K-129M in Libyan Jews in Israel is 43 cases per million inhabitants per year[417]. The origins of this population can be traced back to Spain in the 15$^{th}$ century; in 1492 the monarchs of Spain, Ferdinand and Isabella, issued a decree ordering the expulsion of every Spanish Jew who refused to convert to Christianity. This resulted in the forced migration of two hundred thousand Sephardic refugees (Sephardic: a Jewish person of Spanish of Portuguese descent) to North Africa, Turkey, France, Greece, Yugoslavia, Syria, Palestine and Italy. North African Sephardim later emigrated to France and Libya. For the next 5 centuries the Jewish community in Libya lived in relative isolation, resulting in decreased genetic diversity. Many Libyan Jews emigrated to Israel when it was founded in 1948; others left Libya and Tunisia when these countries gained independence in the 1950s and migrated to France and Italy. High rates of E200K-129M are seen in North African immigrants to France[55], and also in Chile[38,113]. The Chilean focus also has a Spanish origin, with the majority of affected families able to trace back their ancestry to the Conquistadors[135]. The Conquistadors in question were probably originally Jewish;

many Spanish Jews who converted to Christianity found their position in Spain to be precarious, and therefore emigrated to South America.

In Slovakia the clusters of gPD were originally reported from two isolated rural areas, with a stable indigenous population and little immigration for some centuries. In one of the cluster areas records of births, marriages and deaths extending back to 1634 are available. These have provided genealogical evidence that seemingly unrelated patients in fact share a common ancestor in the 17th or 18th century[266]. However the records have not shown evidence of ancestors dying of neurological or psychiatric causes, as would have been expected. This may reflect the vagaries of historical records and the short life expectancy of pre-20th century rural populations, but is nevertheless of considerable interest.

Modern genetic techniques allow us to directly elucidate the ancestral origin of the E200K mutation. Libyan, Tunisian, Italian, Chilean and Spanish families share a major haplotype on chromosome 20, suggesting that they also share a common founder[230], confirming the theories outlined above. However the E200K mutation appears to have arisen independently on several occasions, as the eastern European cases tested showed major differences to the Mediterranean and South American cases. German, Sicilian and Austrian patients also had a unique haplotype, as did a Japanese family.

### *Penetrance*

The early identification of elderly pre-symptomatic mutation carriers initially lead to a mistaken belief that the penetrance of disease was around 0.56 [135,136]; subsequent work demonstrated that penetrance is in fact dependent upon both age and nationality. Amongst Libyan Jews the penetrance is high, rising to 0.96 is those over 80 years of age[349] . In the Italian Calabrian cluster the penetrance was found to be lower, 50% at 60 years of age and 61% at 70 years[81], with Slovakian cases having a similar penetrance to the Italians.

### *Pre-symptomatic Mutation Carriers*

Increased levels of anxiety have been found amongst apparently healthy carriers of the E200K mutation, and in older subjects poorer performance on some neuropsychological tasks such as object learning and recognition[131] . Changes in the thalamus may also be detectable on neuroimaging prior to symptom onset[229].

### *The effect of codon 129*

The influence of the codon 129 status of the wild-type allele is unclear; conflicting reports have emerged from studies in the Libyan Jewish and Slovakian populations. In the Libyan Jewish cases no effect on age of onset or disease duration was found[106], whilst in the Slovakian group a significantly shorter disease duration was found in the methionine homozygous patients, who also had different $PrP^{Sc}$ deposition patterns to the heterozygous patients[265]. In the methionine homozygous group a synaptic pattern of staining was seen on immunohistochemical analysis, whilst in the heterozygous group granules and plaque like structures were seen.

### *Homozygosity for E200K-129M*

A small number of cases of homozygosity for the E200K mutation have appeared; these patients have a slightly younger age at onset, but otherwise appear to be indistinguishable from heterozygous cases[344,58]. The authors also found a non-significant increase in disease duration; this could well simply be due to the younger age at onset.

*Table 2.38* *E200K-129M Clinical phenotype, investigative and neuropathological findings*

| Origin | Mean age at onset (range) | Mean Duration (range) | Codon 129 |
|---|---|---|---|
| Europe, N. America, Japan | 58 years[115,261,251] (33 to 84 years) | 6 months[115,261,251] (2 to 41 months) | MM or MV |

| Clinical features | FHx |
|---|---|
| Similar to sCJDMM1; cognitive, psychiatric, cerebellar or visual problems or myoclonus at onset<br>Later dementia in all cases, cerebellar signs in 79%, myoclonus in 73%, seizures in 40%, sensory & cranial nerve involvement in 24%[191,40] | Positive or Negative |

| EEG | Neuroimaging | 14-3-3 |
|---|---|---|
| Periodic complexes reported[191,115,40] | High signal in the putamen & caudate reported[102,101,205, 58,15, | Positive in some cases[327,318] |

| Neuropathological findings |
|---|
| Similar to sCJDMM1[295,115] |

| Immunohistochemistry |
|---|
| Synaptic pattern of $PrP^{Sc}$ deposition<br>Type 1 $PrP^{Res}$; unglycosylated form under-represented[115]<br>Stripe-like $PrP^{Sc}$ deposition in the cerebellum[185]<br>Cases with associated tau deposition, neurofibrillary tangles and amyloid angiopathy reported[212] |

The coupling of the E200K mutation and valine at codon 129 has only been observed in a handful of patients.

Hainfellner et al.[150] reported a 66 year old woman, with an extensive family history of dementia and depression (in relatives in their 7th decade). She developed rapidly progressive dementia and pyramidal signs, and died 3 and a half months later. There was a prodrome of a 2 and a half year history of slight memory problems. An EEG showed slowing, with right frontal triphasic spikes, whilst CT and MRI showed evidence of ventricular enlargement. Neuropathological examination revealed atrophy and neurofibrillary degeneration (which may account for her prodromal, mild cognitive decline). Diffuse spongiform degeneration, astrogliosis and neuronal loss occurred in the cerebral and cerebellar cortices and basal ganglia. $PrP^{Res}$ type 2 was present in a synaptic pattern in the cerebral cortex, basal ganglia, brainstem and cerebellar cortex. In addition there were plaque-like deposits in the cerebellar granular layer and subcortical white matter. Little unglycosylated $PrP^{Res}$ was found. The patient was valine homozygous at codon 129.

The female patient described by Puoti et al.[317] developed ataxia at the age of 67 years, followed by dementia and myoclonus, dying 6 months after symptom onset. The EEG showed slowing only. There was no known FHx. Genotyping demonstrated the E200K mutation with valine on the mutated allele and methionine on the wild-type allele. PM analysis demonstrated type 2 $PrP^{Res}$ in most brain regions, with the exception of the cerebellum, where a double band was found on Western blot. The nationality of the patient was not given, but the case report originated in Italy.

It would appear that the phenotype of E200K-129V does differ from that of E200K-129M, but there is insufficient data to categorise it further. It is unclear to what extent the codon 129 of the wild-type allele influences the presentation. The difference in $PrP^{Res}$ type is striking.

## 2.XXXI D202N–129V

One English individual and 1 (unpublished) Canadian family are known with prion disease due to this mutation[304,312]. The salient features of the British case are given below; very few details of the Canadian family are available. Investigative findings for the British case have not been reported.

*Table 2.39 D202N-129V Clinical phenotype, investigative and neuropathological findings*

| Sex | Origin | Onset (years) | Duration (years) | Codon 129 |
|---|---|---|---|---|
| F | England | 73 | 6 | VV |

| Onset | Clinical features | FHx |
|---|---|---|
| Cognitive decline | Dementia, cerebellar features | Unknown |

| Neuropathological findings | Immunohistochemistry |
|---|---|
| Abundant $PrP^{Sc}$ amyloid deposition in the cerebrum & cerebellum<br>Neurofibrillary tangles in the cerebral cortex<br>No spongiform degeneration | Absence of 21-30kDa band<br>Similar pattern to F198S |

## 2.XXXII R208H–129M

Only 4 patients have been reported with neuropathologically confirmed prion disease and the R208H–129M (arginine to histidine) mutation have been described[244,325,49,61]. In all cases the family history was negative, although in the Chinese family several unaffected mutation carriers were found. Overall the phenotype closely resembles that of sCJD MM1, however the case reported by Roeber et al.[325] had unusual features, namely tau positivity and a 17kDa fragment on Western blotting. This fragment was more abundant in the cerebellum.

*Table 2.40 R208H–129M Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Age at onset (range)** | **Duration (range)** | **Codon 129** | **FHx** |
|---|---|---|---|---|
| N.America, China, Italy, Germany | 58 to 69 | 4 to 12 | MM | Negative |

| **Onset** | **Clinical features** |
|---|---|
| Cognitive decline; gait disturbance involuntary movement | Dementia, cerebellar syndrome, involuntary movements, psychiatric symptoms |

| **EEG** | **Neuroimaging** | **14-3-3** |
|---|---|---|
| Positive in 3 of 4 tested | Atrophy in one case; high signal in cortex or basal ganglia not reported | Positive in 1 of 3 cases tested |

| **Neuropathological findings** |
|---|
| Atrophy, severe spongiosis, neuronal loss & astrocytosis<br>Synaptic PrP deposition reported in 2 cases<br>Occasional neurofibrillary tangles & tau positive in 1 case |
| **Immunohistochemistry** |
| $PrP^{Res}$ type 1<br>17kDa fragment in 1 case |

## 2.XXXIII R208H–129V

One patient with definite prion disease and the R208H mutation coupled with valine at codon 129 has been described[20]. In contrast to those cases due to R208H–129M, this case had a long duration, and neuropathological changes more in keeping with GSSS.

*Table 2.41 R208H–129V Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Onset (years, mean)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|
| France | 58 | 36 | VV |
| **Onset** | | **Clinical features** | **FHx** |
| Cognitive & behavioural decline | | Cerebellar ataxia, primitive reflexes, pyramidal signs, gaze paresis, nystagmus, demyelinating peripheral neuropathy, akinetic mutism | Negative |
| **EEG** | | **Neuroimaging** | **14-3-3** |
| Slow | | Normal | Negative |
| **Neuropathological findings** | | | **Immunohistochemistry** |
| Severe spongiform change & gliosis.<br>Kuru plaques in the cerebellum, especially in the molecular layer.<br>Synaptic, granular, perineuronal & perivacuolar $PrP^{Sc}$ | | | $PrP^{Res}$ type 2 |

## 2.XXXIV V210I–129M

The V210I mutation has been described in patients of European, N. American, Chinese and Japanese origin[307,320,103,341,53,272,392,172,242,320,307]. Within Europe it is particularly seen in the Campania and Umbria regions of Italy[223]. Most cases have negative family histories, and the mutation has been demonstrated in elderly, asymptomatic family members. This suggests that the penetrance is low. A summary of the reported clinical features is given below.

*Table 2.42 V210I-129M Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Mean Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|
| France, Italy, Germany, America, Japan, China | 59 (46 to 80) | 6 (2 to 24) | MM & MV |

| **Onset** | **Clinical features** | **FHx** |
|---|---|---|
| Memory or gait problems, dystonia, dysarthria, sensory symptoms | Dementia, cerebellar signs, myoclonus, akinetic mutism | Usually negative |

| **EEG** | **Neuroimaging** | **14-3-3** |
|---|---|---|
| Periodic complexes | Atrophy or cortical & basal ganglia high signal | Positive |

| **Neuropathological findings** | **Immunohistochemistry** |
|---|---|
| Fine spongiform degeneration & gliosis of the grey matter | $PrP^{Res}$ type 1A |

Overall the clinico-pathological features strongly resemble those seen in sCJD, and in several cases the patient was originally diagnosed with sCJD prior to genotyping being performed. Transmission to transgenic mice and bank voles has been successful[242,3]. Both studies found $PrP^{Sc}$ type 1, and neuropathological changes resembling those of animals inoculated with sCJD MM1 or MV1. The mutation has not been found in control subjects.

## 2.XXXV E211Q–129M

Two apparently unrelated families with prion disease due to the E2111Q (glutamic acid to glutamine) mutation have been reported[222,302]. The French family consisted of a proband with neuropathologically confirmed disease, and 3 siblings with similar illnesses but no neuropathological examination. The proband was found to have both the E211Q mutation and the rare silent polymorphism G124G on the mutated allele. The Italian family consisted of a proband with probable prion disease and 3 siblings, 2 with probable and 1 with possible prion disease.

*Table 2.43 E211Q–129M Clinical phenotype, investigative and neuropathological findings of the family reported by Peoc'h*[302]

| **Origin** | **Mean Onset (years)** | **Duration (months)** | **Codon 129** |
|---|---|---|---|
| France | 64 | 6 | MM |
| **Onset** | | **Clinical features** | **FHx** |
| Behavioural change & cerebellar ataxia | | Dementia, myoclonus | Positive |
| **EEG** | | **Neuroimaging** | **14-3-3** |
| Periodic complexes | | Unknown | Unknown |
| **Neuropathological findings** | | | |
| CJD (sic) | | | |

*Table 2.44* *E211Q–129M Clinical phenotype, investigative and neuropathological findings of the family reported by Ladogana*[222]

| Origin | Mean Onset (years) | Duration (months) | Codon 129 |
|---|---|---|---|
| Italy | 68 | 5 | MM |
| **Onset** | | **Clinical features** | **FHx** |
| Cognitive decline & ataxia | | Behavioural change, myoclonus & other involuntary movements | Positive |
| **EEG** | | **Neuroimaging** | **14-3-3** |
| Periodic complexes | | Atrophy | Positive |

## 2.XXXVI Q212P-129M

The rare Q212P mutation has only been described in 2 individuals, 1 of whom was homozygous for the mutation. It is unclear if this is a pathogenic mutation with a low penetrance, or possibly a non-pathogenic polymorphism. In the Irish pedigree investigated by Beck et al, the proband was homozygous of Q212P-129M, and several elderly relatives were heterozygous for it and unaffected[25]. The only positive family history comes from a maternal grandmother with dementia in old age. However the mutation has not been found in control populations, and the affected American individual had neuropathologically confirmed prion disease, increasing the likelihood that it is a pathogenic finding.

*Table 2.45* *Q212P–129M Clinical phenotype, investigative and neuropathological findings of the individual reported by Young* [407]

| Origin | Onset (years) | Duration | Codon 129 |
|---|---|---|---|
| USA | 60 | 8 years | MM |
| **Onset** | | **Clinical features** | **FHx** |
| Incoordination, slurred speech, difficulty swallowing | | Ataxia, preserved cognition | Negative |
| **EEG** | | **Neuroimaging** | **14-3-3** |
| Not done | | MRI normal | Not done |
| **Neuropathological Findings** | | | |
| Mild amyloid deposition, particularly in the cerebellum. PrP positive on IHC<br>Axonal degeneration in the anterior and lateral corticospinal tracts | | | |

*Table 2.46* *Q212P–129M Clinical phenotype, investigative and neuropathological findings of the individual reported by Beck* [25]

| Origin | Onset (years) | Duration | Codon 129 |
|---|---|---|---|
| Ireland | 36 | Alive 4 years after onset | MM |
| **Onset** | | **Clinical features** | **FHx** |
| Balance problems, slurred speech | | Cerebellar & pyramidal signs; dementia | Positive |
| **EEG** | | **Neuroimaging** | **14-3-3** |
| Diffuse slowing | | Moderate cerebral atrophy | Not done |

## 2.XXXVII Q217R-129V

Two N. American families and 1 Swedish family have been described with this mutation[122,123,170,396], which causes an arginine for glutamine substitution. Thus far attempts to transmit the disease to experimental animals have failed[170]. One patient from the Swedish family was reported to be heterozygous at codon 129; a further individual from a different family was reported to be valine homozygous [396]. Further details are given in the tables below.

*Table 2.47 Q217R-129V Clinical phenotype, investigative and neuropathological findings[122]*

| **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|
| N. America, Sweden | <63 to 65 | >4 to 6 | MV |
| **Onset** | **Clinical features** | | **FHx** |
| Cognitive decline | Dementia, gait ataxia, dysphagia, parkinsonism | | Positive |
| **Neuropathological findings** | | | |
| PrP positive amyloid plaques<br>Numerous $PrP^{Sc}$ deposits in the cerebral cortex, basal ganglia, thalamus, cerebellum & midbrain<br>Neurofibrillary tangles & neuropil threads in the cerebral cortex, basal ganglia, thalamus & hippocampus | | | |

*Table 2.48 Q217R-129VV Clinical phenotype, investigative and neuropathological findings*[396]

| **Origin** | **Onset (years)** | **Duration (years)** | **Codon 129** |
|---|---|---|---|
| Canada | 45 | 13 | VV |
| **Onset** | **Clinical features** | | **FHx** |
| Cognitive decline | Dementia, dysarthria, tremor, extrapyramidal signs | | Positive |
| **EEG** | | | |
| Periodic frontal slowing | | | |
| **Neuropathological findings** | | | |
| Abundant PrP positive large amyloid plaques<br>Smaller multi-centric plaques. Neurofibrillary tangles.<br>Minor focal spongiosis. Neuronal loss and astrocytic gliosis | | | |
| **Immunohistochemistry** | | | |
| Bands at 18 to 19 kDa and 27 to 29kDa following digestion with protease K | | | |

## 2.XXXVIII M232R–129M

The M232R–129M mutation has been identified in unrelated Japanese, Chinese and Korean individuals[166,197,416,64], none whom had positive family histories. It is the fourth commonest mutation associated with gPD in Japanese patients[353]. One patient was identified with the mutation and pathological evidence of DLB[203]. It has also been identified in conjunction with the V180I mutation (on the other allele) in a single case[164]. Brain homogenate from patients with M232R–129M has been successfully used to transmit disease to animals[166]. Some authors divide cases into a rapidly progressive group and a slowly progressive group[353,152], which may have differences in $PrP^{Sc}$ type and staining pattern. The numbers reported are small and it is difficult to know how clear a divide there is between these groups. The mutation has not been found in Japanese or Chinese controls[166,416].

*Table 2.49 M232R-129M Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Age at onset (range)** | **Duration (range)** | **Codon 129** |
|---|---|---|---|
| Japan & China | 44 to 70 years | 18 months (4 to 24) | MM* |
| **Clinical features** | | | **FHx** |
| Dementia, gait disturbance, pyramidal & cerebellar signs.<br>Myoclonus occasionally reported | | | Negative |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| Periodic complexes or slow | Atrophy or cortical & basal ganglia high signal | | Positive* |
| **Neuropathological findings** | | | |
| Spongiform change, neuronal loss, severe astrocytosis<br>Diffuse $PrP^{Sc}$ deposition in the grey matter. No plaques.<br>Type 1 $PrP^{Res}$ in a synaptic distribution in the 'slow' cases<br>Type 2 or type 1 and 2 $PrP^{Res}$ in a synaptic and perivacuolar distribution in the 'rapid' cases[353] | | | |

* In addition there is an unpublished case which was heterozygous at codon 129[312].

*Table 2.50 M232R-129M & V180I-129M Clinical phenotype, investigative and neuropathological findings*

| **Origin** | **Mean age at onset (range)** | **Mean Duration (range)** | **Codon 129** |
|---|---|---|---|
| Japan | 84 | 12 | MM |
| **Clinical features** | | | **FHx** |
| Dementia, altered taste, myoclonus (mild), startle response, akinetic mutism. | | | Negative |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| Diffuse slowing | Cerebral & basal ganglia atrophy; cortical high signal | | Not done |
| **Neuropathological findings** | | | |
| Cortical spongiform change<br>$PrP^{Res}$ present with an unusual glycoform ratio | | | |

## 2.XXXIX *PRNP* Mutations Reported as Single Cases

A number of *PRNP* mutations have been reported as occurring in single individuals only. The pathogenenicity of such mutations may therefore be uncertain, as in most cases the family history is negative or unknown. Details are given in the tables below.

*Table 2.51 Single cases clinical phenotype (1)*

| Haplotype | Sex | Origin | Onset (years) | Duration | Codon 129 |
|---|---|---|---|---|---|
| **S97–129M**[416] | F | China | 74 | Alive at 77 years | MM |
| **V180I & M232R**[197] | ? | Japan | 84 | 1 year | MM |
| **T188A-129M**[71] | F | Australia | 82 | 4 months | MM |
| **T193I-129M**[208] | F | Greece | 70 | 9 months | MM |
| **F198V–129M**[416] | M | China | 56 | Alive at 60 years | MM |
| **D202G-129V**[159] | M | Germany | 55 | 16 years | MV |
| **V203I-129M**[302] | M | Italy | 69 | 1 months | MM |
| **V203I-129V**[187] | F | Korea | 66 | 2 months | MV |
| **R208C–129M**[416] | M | China | 81 | Unknown | MM |
| **Y218N**[11] | ? | Spain | Unknown | Unknown | Unknown |
| **M232T**[232,33] | ? | Poland | 44 | 6 years | MV |
| **Y226X-129V**[182] | F | Holland | 55 | 27 months | MV |
| **Q227X-129V**[182] | F | Holland | 39 | 72 months | MV |
| **P238S**[392] | ? | Germany | Unknown | Unknown | Unknown |
| **24 nucleotide insertion**[163] | M | Canada | 31 | Unknown | MV* |

* The insertion was in cis with methionine at codon 129.

*Table 2.52 Single cases clinical phenotype (2)*

| Haplotype | Clinical features | FHx |
|---|---|---|
| **S97–129M**[416] | Dementia, hallucinations, delusions, visual loss, pyramidal signs. Clinical diagnosis of Alzheimer's disease | Negative |
| **V180I & M232R**[197] | Dementia, myoclonus, akinetic mutism | Negative |
| **T188A-129M**[71] | Dementia, myoclonus, hyper-reflexia, extensor plantars, frontal release signs, visual hallucinations, cortical blindness | Negative |
| **T193I-129M**[208] | Dementia, behavioural change, visual hallucinations, hyper-reflexia, gait problems, myoclonus & startle response | Negative |
| **E196K-129M**[302] | Behavioural change, dementia, chorea-athetosis & mutism | Positive |
| **F198V–129M**[416] | Behavioural change, dementia, visual hallucinations, myoclonus, extrapyramidal signs | Negative |
| **D202G-129V**[159] | Dementia & hypersomnolence, marked cerebellar syndrome, pyramidal & extrapyramidal signs, akinetic mutism, mild autonomic dysfunction | Positive |
| **V203I-129M**[302] | Diplopia & dizziness followed by dementia, visual hallucinations, ataxia, nystagmus, myoclonus | Negative |
| **V203I-129V**[187] | Gait disturbance and dementia followed by involuntary movements (including myoclonus) and extrapyramidal signs | Negative |
| **R208C–129M**[416] | Dementia | Negative |
| **Y218N**[11] | Dementia (consistent with Alzheimer's disease or frontotemporal dementia) | Positive |
| **M232T**[232,33] | Cerebellar syndrome, dementia, spastic paraparesis | Negative |
| **Y226X-129V**[182] | Cognitive impairment, pyramidal signs, myoclonus, akinetic mutism | Positive |
| **Q227X-129V**[182] | Cognitive decline, extra-pyramidal signs, seizures, mutism | Positive |
| **P238S**[392] | 'Possible prion disease' (no further details reported) | Unknown |
| **24 nucleotide insertion**[163] | Cognitive decline, epilepsy, gait disturbance, slurred speech, paratonia, axial rigidity, myoclonus | Negative |

*Table 2.53 Single cases investigative findings*

| Haplotype | EEG | Neuroimaging | 14-3-3 |
|---|---|---|---|
| **S97–129M**[416] | Slow | Atrophy | Unknown |
| **V180I & M232R**[197] | No periodic complexes | Unknown | Unknown |
| **T188A-129M**[71] | Periodic complexes | Atrophy (normal for age) | Positive |
| **T193I-129M**[208] | Periodic complexes | Atrophy (normal for age) | Positive |
| **E196K-129M**[302] | No periodic complexes | Unknown | Unknown |
| **F198V–129M**[416] | Slow | Atrophy | Unknown |
| **D202G-129V**[159] | Slow | Cerebellar atrophy | Positive (S100b >30ng/ml) |
| **V203I-129M**[302] | Periodic complexes | Unknown | Unknown |
| **V203I-129V**[187] | Not done | Cortical high signal | Positive |
| **R208C–129M**[416] | NA | Atrophy | Unknown |
| **Y218N**[11] | Unknown | Unknown | Unknown |
| **M232T**[232,33] | Unremarkable | Unremarkable | Unknown |
| **Y226X-129V**[182] | Periodic complexes | White matter high signal | Positive |
| **Q227X-129V**[182] | Not done | Moderate atrophy | Not done |
| **P238S**[392] | Unknown | Unknown | Unknown |
| **24 nucleotide insertion**[163] | Encephalopathic & epileptiform activity | Diffuse cortical & cerebellar vermis atrophy | Positive |

*Table 2.54 Single cases neuropathological findings*

| Haplotype | Neuropathological findings |
|---|---|
| **S97–129M**[416] | Not done |
| **V180I & M232R**[197] | Typical spongiform change in the cerebral cortex, basal ganglia & thalamus.<br>Neuronal loss & gliosis in the cortex<br>Weak $PrP^{Sc}$ staining. Highest molecular weight $PrP^{Res}$ band absent |
| **T188A-129M**[71] | Spongiform change (most severe in occipital cortex);<br>PrP IHC negative. Amyloid plaques ('normal for age') |
| **T193I-129M**[208] | Not done |
| **E196K-129M**[302] | CJD (sic) & protease resistant PrP |
| **F198V–129M**[416] | Not done |
| **D202G-129V**[159] | Unknown |
| **V203I-129M**[302] | CJD (sic) & protease resistant PrP |
| **V203I-129V**[187] | Moderate spongiform degeneration and vacuoles in the<br>frontal cortex with synaptic PrP. Type 1 $PrP^{Res}$<br>Granular $PrP^{Sc}$ deposition in the granular layer of the cerebellum |
| **R208C–129M**[416] | Not done |
| **Y218N**[11] | $PrP^{Res}$ deposition. Widespread tau pathology<br>Mutant ubiquitin in neurofibrillary tangles and dystrophic neurites.<br>Multiple $PrP^{Res}$ bands ranging from 10kd to 80kd. |
| **M232T**[232,33] | Severe atrophy<br>Multiple $PrP^{Sc}$ positive, kuru-type & multi-centric plaques in the molecular<br>layer of the cerebellum, cerebral cortex and hippocampus |
| **Y226X-129V**[182] | Diffuse severe amyloid angiopathy, strongly $PrP^{Sc}$ positive<br>Faint synaptic $PrP^{Sc}$ deposition. Focal tau deposition |
| **Q227X-129V**[182] | Scattered status spongiosus<br>Numerous multicentric and unicentric plaques<br>Neurofibrillary tangles and neuropil deposition (Braak & Braak stage VI) |
| **P238S**[163] | Unknown |
| **24 nucleotide insertion**[163] | Mild neuronal loss, microspongiosis, multi-centric plaques<br>(Right frontal gyrus brain biopsy specimen) |

In addition an I138M mutation has been reported in a French patient described as having 'frontal dementia, not CJD'. A G142S mutation has been reported in a N. African male with multiple sclerosis and a Mali female with viral

meningoencephalitis. However G142S has been found in healthy control populations, and it may well be a normal polymorphism[25,312,25] . A number of other polymorphisms have been identified in both control populations and prion disease patients, which are thought to most likely be non-pathogenic[25].

## 2.XL Insertional and Deletional Mutations

*PRNP* normally has a repeating oligopeptide coding sequence between codons 51 and 91. These can distinguished by their nucleotide code and as classified as : R1 – R2 – R2 – R3 – R4 [24,51]. Families with 1, 2, and 4 to 9 and 12 extra repeat insertions have been described with prion disease. Pathogenicity due to 3 extra repeats has not been seen. Possible and definite CJD linked to a 2 repeat deletion has also been described[219]. The clinicopathological phenotype seen varies according to the number of repeats present; broadly speaking those with 4 or less repeat insertions resemble CJD, and those with higher number of repeats have a GSSS-like disease. The codon 129 status may also influence the phenotype seen.

## 2.XLI Two-Octapeptide Repeat Deletion

Two cases of prion disease due to a deletion of 2-octapeptide repeats have been reported[24,51].

*Table 2.55 2-octapeptide repeat deletion clinical phenotype, investigative & neuropathological findings of the family described by Beck[24]*

| Sex | Origin | Onset (years) | Duration (months) | Codon 129 |
|---|---|---|---|---|
| F | N. Europe | 85 | 23 | MM |
| **Onset** | **Clinical features** | | | **FHx** |
| Confusion | Dementia, pyramidal, extrapyramidal & cerebellar signs | | | Negative |
| **EEG** | **Neuroimaging** | | | **14-3-3** |
| Non-specifically abnormal | White matter high signal & cerebral atrophy | | | Not done |
| **Octapeptide repeat sequence** | | | | |
| R1-2-4 (deletion of R2-3) | | | | |

*Table 2.56 2-octapeptide repeat deletion clinical phenotype, investigative & neuropathological findings of the family described by Capellari et al.[51]*

| Sex | Origin | Onset (years) | Duration (months) | Codon 129 |
|---|---|---|---|---|
| M | Italy | 62 | 18 | MM |
| **Onset** | | **Clinical features** | | **FHx** |
| Personality & behavioural change, dizziness | | Dementia, myoclonus, balance problems | | Positive |
| **EEG** | | **Neuroimaging** | | **14-3-3** |
| Periodic complexes | | Normal | | Positive |
| **Octapeptide repeat sequence** | | | | |
| R1-3-4 (deletion of R2-2) | | | | |

The deletions seen in the 2 cases were different; the first case had a diagnosis of possible CJD, and the second had neuropathologically confirmed prion disease,

with fine spongiosis and gliosis seen, accompanied by severe neuronal loss. The thalamus and pulvinar were relatively spared. Type 1 $PrP^{Res}$ was seen, with a synaptic pattern of deposition. Capellari et al. concluded that their case resembled sCJD MM1, although the duration was significantly longer than is typically seen in sCJD. The only FHx was of the patient's mother having died at the age of 80 years with a diagnosis of Alzheimer's disease. The significance of this is unclear. The 2-repeat deletion has not been found in normal controls, and is therefore likely to have been pathogenic in these 2 cases.

## 2.XLII One-Octapeptide Repeat Insertion

Three male cases of CJD due to 1 extra octapeptide repeat have been described (Case $1^{226}$ Cases 2 and $3^{306}$). A summary of the clinical features and investigative findings is given in the tables.

*Table 2.57 1-OPRI clinical phenotype (1)*

| Case | Sex | Origin | Onset (years) | Duration (months) | Codon 129 | Repeat |
|---|---|---|---|---|---|---|
| $1^{226}$ | M | France | 73 | 4 | MM | R1 – R2 - R2 - **R2** - R3 – R4 |
| $2^{306}$ | M | Italy | 58 | 5 | MM | R1 – R2 - R2 - **R3g** - R3 – R4 |
| $3^{306}$ | M | Italy | 64 | 6 | MV* | R1 – R2 - R2 - **R3g** – R3 – R4 |

* The extra repeat was on the M allele.

*Table 2.58 1-OPRI clinical phenotype (2)*

| Case | Onset | Clinical features | FHx |
|---|---|---|---|
| $1^{226}$ | Dizziness | Visual agnosis & later cortical blindness, cerebellar ataxia, dementia, myoclonus, akinetic mutism | Positive |
| $2^{306}$ | Visual loss & paranoia | Gait ataxia, myoclonus, drowsiness | Negative |
| $3^{306}$ | Paraesthesia | Behavioural change, dementia, aphasia, gait difficulties, hallucinations, myoclonus, Babinski positive, akinetic mutism | Negative |

*Table 2.59 1-OPRI investigative findings*

| Case | EEG | Neuroimaging | 14-3-3 |
|---|---|---|---|
| 1[226] | Positive | Normal | NA |
| 2[306] | Positive | Normal | Positive |
| 3[306] | Positive | Atrophy | Positive |

Only case 1 had any significant family history, with the patient's father having died of an unknown neurological illness at the age of seventy years.

Only case 3 underwent a PM. Gliosis and spongiosis of the cortex were observed, with a diffuse, synaptic pattern of $PrP^{Sc}$ staining in the cortex and sub-cortical grey matter. A granular and thread-like pattern of $PrP^{Sc}$ deposition was found in the thalamus, basal ganglia, hypothalamus and amygdala. The amygdala also had florid plaque-like lesions. In the cerebellum an irregular pattern of $PrP^{Sc}$ deposition was observed. Both type 1 and type 2 $PrP^{Res}$ were present, with the former being in the cortex and the latter in the thalamus, hypothalamus, striatum, pallidum and cerebellum.

## 2.XLIII Two-Octapeptide Repeat Insertion

Two families have been described with gPD due to a 2-OPRI mutation. The family described by Goldfarb et al.[133] had a history of dementia affecting the proband, her mother and maternal grandmother. The proband and her mother both had the same *PRNP* insertion; however the proband died at the age of 58 years after a 3 month long illness characterised by a rapidly progressive dementia, whereas her mother had onset at the age of 75 years and was still alive with severe dementia at the age of 88 years.

The Dutch family described by van Harten et al.[366] comprised the proband, who died of dementia at the age of 68 years, and did not have a PM. There was a history of dementia in both parents and also her brother; however her brother did not have any mutation of the *PRNP* gene. The proband had a different insertional mutation (to the American family) and also had substitution of R2 for the normal R3. It is therefore unconfirmed if the proband's illness was actually due to prion disease or not; of significance are the marked white matter abnormalities seen on neuroimaging, the cause of which is not known.

*Table 2.60 2-OPRI clinical phenotype, investigative and neuropathological findings of the family described by Goldfarb[133]*

| **Sex** | **Origin** | **Mean Onset (years)** | **Duration (range)** | **Codon 129** |
|---|---|---|---|---|
| F | N. America | 71 (58 to 80) | 3 months to 15 years | Not reported |

| **Clinical features** | **FHx** | **Octapeptide repeat sequence** |
|---|---|---|
| Progressive dementia; mutism | Positive | R1-R2-R2-R3-**R2a-R2a**-R4 |

| **EEG** | **Neuroimaging** | **14-3-3** |
|---|---|---|
| Periodic activity | Incidental benign meningioma | Not reported |

| **Neuropathological Findings** |
|---|
| Spongiform change, gliosis & neuronal loss |

*Table 2.61* *2-OPRI clinical phenotype, investigative and neuropathological findings of the family described by van Harten*[366]

| Sex | Origin | Onset (years) | Duration (months) | Codon 129 |
|---|---|---|---|---|
| F | Holland | 61 | 7 years | VV |

| Clinical features | FHx | Octapeptide repeat sequence |
|---|---|---|
| Dementia & gait ataxia | Positive | R1-2-**2a**-2-**2a**-**2a**-4 |

| EEG | Neuroimaging | 14-3-3 |
|---|---|---|
| Not done | Severe cortical atrophy; frontal leukoencephalopathy | Not done |

## 2.XLIV Three-Octapeptide Repeat Insertion

No cases of neurological illness due to a 3-OPRI have been identified. During large-scale screening of the *PRNP* gene in Chinese individuals a 3-OPRI was been identified in an 11 year old Chinese girl; she was healthy at the time and had no family history of neurological illness[408].

## 2.XLV Four-Octapeptide Repeat Insertion

Four different extra octapeptide repeats have been associated with prion disease in 5 cases[47,226,174,400,329]. In 1 further case the mutation was found in an individual with no clinical or neuropathological evidence of prion disease[134]. The 59 year old sister of one of the affected cases also carried the mutation but was unaffected[329]. The clinicopathological phenotype and investigative findings are summarised in the tables. In 3 families the mutation was on an allele encoding methionine at codon 129 and in 1 family valine; the codon 129 status in one further family was unknown.

*Table 2.62 4-OPRI clinical phenotype (1)*

| Family | Sex | Origin | Onset (years) | Duration | Codon 129 | Repeat |
|---|---|---|---|---|---|---|
| Campbell[47] | M | Britain | 56 | 8 weeks | MM | R1-2-2-**2-2-2-2**-3-4 |
| Laplanche[226] | F | France | 82 | 4 months | VV | R1-2-2-**3-2-2-2**-3-4 |
| Rossi[329] | M | Italy | 65 | 6 months | MM | R1-2-2-**3g-2-3g-2**-3-4 |
| Isozaki[174] | M | Japan | 62 | 7 years | Unknown | Unknown |
| Yangihara[400] | M | Japan | 56 | 5 months | MM | R1-2-2-**2-3-2-2**-3-4 |

*Table 2.63 4-OPRI clinical phenotype (2)*

| Family | Onset | Clinical features | FHx |
|---|---|---|---|
| Campbell[47] | Dementia, gait problems, myoclonus | Dementia, cerebellar signs, myoclonus, primitive reflexes, terminal akinetic mutism | Negative |
| Laplanche[226] | Dizziness | Ataxia, visual agnosis, dementia, cortical blindness, myoclonus, akinetic mutism | Positive |
| Rossi[329] | Speech & gait problems | Dementia, gait ataxia, pyramidal signs, myoclonus, terminal akinetic mutism | Negative |
| Isozaki[174] | Not reported | Dementia & extrapyramidal signs | Unknown |
| Yangihara[400] | Cerebellar ataxia & myoclonus | Visual loss, gait problems, dysarthria, ataxia, dementia, myoclonus, increased tone, hyper-reflexia | Negative |

*Table 2.64 4-OPRI investigative and neuropathological findings*

| Family | EEG | Neuroimaging | Neuropathological Findings |
|---|---|---|---|
| Campbell[47] | Positive | Mild global atrophy | Spongiform change, astrocytosis & neuronal loss; $PrP^{Sc}$ deposition in the molecular layer of cerebellum |
| Laplanche[226] | Positive | Normal | Not done |
| Rossi[329] | Positive | High signal in striatum & thalamus | Spongiosis, neuronal loss, gliosis, diffuse $PrP^{Sc}$ deposition in a synaptic pattern |
| Isozaki[174] | Not done | Not done | Spongiform change |
| Yangihara[400] | Positive | Cerebellar atrophy | Not done |

In addition CSF analysis in one case was positive for 14-3-3[329]. In one case an unusual pattern of $PrP^{Sc}$ deposition in the molecular layer of the cerebellum was seen, with the $PrP^{Sc}$ being perpendicular to the leptomeningeal surface[47].

## 2.XLVI Five-Octapeptide Repeat Insertion

Eleven different five-octapeptide insertions have been reported; the findings are summarised below.

*Table 2.65 5-OPRI genotype and clinical phenotype (1)*

| Reference | Repeat | Mutated allele | Origin | No. patients reported |
|---|---|---|---|---|
| 'Kel' family[134] | R 1-2-**2-3-2-3g-2**-2-3-4 | Unknown | N. American | 2 |
| Cervenakova[57] | R1-2-**2-2a-2-2a-2**-2-3-4 | M | N. American | 1 |
| Cochran[66] | R1-2-**2-3-2-2-2**-2-3-4 | M | Ukrainian | 10 |
| Windl[392] | R1-2-2-3-**2-2-2a-2-2**-4 | V | German | 2 |
| Skworc[345] | R1-2-2-**3g-3g-3g-2-2**-3-4 | M | German | 3 |
| Cervenakova[57] | R1-2-2-**3-2-2-2a-2**-3-4 | M | American | 1 |
| Beck[23] | R1-2-**2a-2-2-3g-2**-2-3-4 | M | Japanese | 1 |
| Mead (1)[256] | R1-2-3-**2-3-2-3g-2**-3-4 | M | S. African | 5 |
| Mead (2)[256] | R1-2-2-**3g-2-2-2-2**-3-4 | M | English | 3 |
| Mead (3)[256] | R1-2-2-**3-2-3g-2-2**-3-4 | M | N. Irish | 1 |
| Jansen[180] | R1-2-**2-2-2-2-2**-3-4 | M | Holland | 2 |

*Table 2.66 5-OPRI clinical phenotype (2)*

| Reference | Onset (years) | Duration | Codon 129 | FHx |
|---|---|---|---|---|
| 'Kel' family[134] | 36 | 10 years | Unknown | Positive |
| Cervenakova[57] | Not reported | | M* | Positive |
| Cervenakova | Not reported | | M* | Positive |
| Cochran[66] | 49 | 9 months to 16 years | MM | Positive |
| Windl[392] | Not reported | | MV** | Unknown |
| Skworc[345] | 54 | 9 months to 8 years | MV | Positive |
| Beck[23] | 45 | > 4 years (alive) | MM | Positive |
| Mead (1)[256] | 46 | 18 months to 7 years | MM | Positive |
| Mead (2)[256] | 45 | 9 years | MV & MM | Negative |
| Mead (3)[256] | 63 | 10 months | MM | Positive |
| Jansen[180] | 35 | 92 months | MM | Positive |

* The codon 129 status of the wild type allele was not reported; no clinical details have been published for these families; both were documented as having pathological evidence of prion disease but no further details are available.

** No further details are available regarding these cases.

*Table 2.67 5-OPRI clinical phenotype (3)*

| Reference | Onset | Clinical features |
|---|---|---|
| 'Kel' family[134] | Not reported | Dementia, cerebellar signs, tremor, rigidity, myoclonus, hyper-reflexia |
| Cochran[66] | Cognitive & psychiatric problems | Dementia, myoclonus, pyramidal signs, gait ataxia, rigidity, chorea |
| Skworc[345] | Gait problems; personality change | Gait ataxia, pyramidal signs, cerebellar signs, dementia, increased tone, seizures, myoclonus |
| Beck[23] | Cognitive decline | Slowly progressive cognitive problems & cerebellar ataxia |
| Mead (1)[256] | Personality change | Dementia, ataxia, myoclonus, akinetic mutism |
| Mead (2)[256] | Cognitive & balance problems | Dementia, rigidity, paratonia, primitive reflexes, ataxia |
| Mead (3)[256] | Visual disturbance | Dementia, visual hallucinations, ataxia, myoclonus, hypertonia, pyramidal signs, akinetic mutism |
| Jansen[180] | Memory loss, dysgraphia | Dementia, parkinsonism, anxiety, vertical gaze palsy |

*Table 2.68 5-OPRI investigative findings*

| Reference | EEG | Neuroimaging | CSF 14-3-3 |
|---|---|---|---|
| 'Kel' family[134] | Slow | Not reported | Not reported |
| Cochran[66] | Non-specifically abnormal | Atrophy | Not analysed |
| Skworc[345] | Slow (1 case)<br>Periodic complexes (1 case) | Atrophy | Negative |
| Beck[23] | Slow | Atrophy | Not analysed |
| Mead (1)[256] | Spike & wave complexes but not periodic | Atrophy | Not analysed |
| Mead (2)[256] | Non-specifically abnormal | Atrophy | Not analysed |
| Mead (3)[256] | Periodic sharp waves | Cerebral atrophy | Positive |
| Jansen[180] | Not reported | Atrophy | Negative |

*Table 2.69 5-OPRI neuropathological findings*

| Reference | Neuropathological Findings |
|---|---|
| 'Kel' family[134] | Spongiosis, gliosis & neuronal loss |
| Cervenakova[57] | Prion disease (no further details given) |
| Cervenakova[57] | Prion disease (no further details given) |
| Cochran[66] | Atrophy, spongiosis, neuronal loss, gliosis |
| Skworc[345] | Spongiosis, gliosis, neuronal loss.<br>Most Purkinje cells in molecular layer; $PrP^{Sc}$ plaques in the cerebellum |
| Beck[23] | No details available |
| Mead (1)[256] | Atrophy, severe spongiosis, neuronal loss & astrocytosis. Severe Purkinje cell loss in granular layer of cerebellum. $PrP^{Sc}$ deposition in a synaptic pattern & plaques |
| Mead (2)[256] | Spongiosis, neuronal loss, astrocytosis. $PrP^{Sc}$ deposition in the cerebellar molecular layer, perpendicular to the glial surface |
| Mead (3)[256] | No PM performed |
| Jansen[180] | Mild spongiosis & astrocytic gliosis;<br>frontal, temporal & parietal lobes worst affected<br>Moderate gliosis of the cerebellar molecular layer;<br>unusual localisation of Purkinje cells<br>Faint synaptic $PrP^{Sc}$ deposition; $PrP^{Res}$ types 1 and 2 |

In the family reported by Skworc et al.[345] there was no evidence of anticipation, but there was a history of other family members requiring treatment for psychiatric problems, and 1 affected family member had a history of a long-standing personality disorder. The cerebellar appearances found in the family reported by Skrowc et al.[345] varied between individuals, but all had unusual findings in the cerebellum, with most Purkinje cells being localised to the molecular layer, and PrP positive plaques being present in the molecular and / or granular layers.

The family reported by Cochran et al.[66] had a strong history of psychiatric problems, and 1 affected member had a long-standing personality disorder. Only very limited details were available regarding the families described by Windl et al.[392] and Cervenakova et al.[57]

14-3-3 analysis was performed in 2 cases and was negative in one[66] and positive in the other[256].

Of note is the fact that the insertion differed between the families, except in the case of the 'Kel' family[134] and the Northern Irish patient described by Mead et al.[256]

The patient described by Jansen et al.[180] had an unusual pattern of $PrP^{Res}$ deposition, with both Type 1 and 2 being present in the frontal cortex and cingulate gyrus; type 1 only in the parietal cortex; type 1 and a trace of type 2 in the occipital cortex and type 2 only in the cerebellar cortex and caudate nucleus.

Overall most patients had symptom onset in middle age, with the main features being cognitive decline, gait problems, cerebellar and pyramidal signs and myoclonus. Usually the duration was around a decade, with only a few individuals dying within a year of symptom onset. In most cases there was a positive FHx of prion disease, and several families there were findings of background long-term psychiatric or behavioural problems, even in individuals who tested negative for any *PRNP* mutation. The pathological findings were similarly to sCJD-MM1, with additional features in the cerebellum of unusual PrP deposition or abnormalities of Purkinje cells. In summary the patients described resemble sCJDMM1, albeit with a longer duration and some unusual pathological features.

## 2.XLVII Six-Octapeptide Repeat Insertion

Seven families and one individual with a 6-OPRI in the *PRNP* gene are known; 2 have not been published in detail. Each has a different insertion.

*Table 2.70 6-OPRI genotype and clinical phenotype (1)*

| Family | Insertion | Codon 129 (of mutated allele) | Origin | No. cases reported |
|---|---|---|---|---|
| Poulter[309] | R1 -2-2-**2-3-2-3g-2-2**-3-4 | M | Britain | 86 |
| Nicholl[276] | R1-2-2-**3-2-3g-2-3g-2**-3-4 | M | Britain | > 2 |
| Oda[282] | R1-2-2-**3g-2-2-3g-2-2**-3-4 | M | Japan | 6 |
| Capellari[52] | R1-2-2-**2-2-2-2-2-2**-3-4 | M | Spain | 3 |
| Cervenakova[57] | R1-2-2-**2-2-2-2-2-3g**-3-4 | M | N. America | ? |
| Cervenakova[57] | R1-2-2-**2c-2-2-2-2-2**-3-4 | M | Britain | ? |
| Gelpi[120] | R1-2-2-**2-2-2-2-2-2**-3-4 | M | Austria | 1 |
| Kovacs[213] | R1-2-2-**3-2-3g-3-2-2**-3-4 | M | Hungary | 2 |
| Vital[368] | R1-2-2-**3-2-3g-2-2-2**-3-4 | V | France | 1 |

*Table 2.71 6-OPRI clinical phenotype (2)*

| Reference | Mean onset (years; range) | Mean Duration (range) | Codon 129 | FHx |
|---|---|---|---|---|
| Poulter[309] | 31.4 (SD 5.7) | 9 years * (SD 5) | MM | Positive |
| Poulter[309] | 41.7 (SD 5.3) | | MV | Positive |
| Nicholl[276] | 34 & 46 | 2 months & 4 years | Unknown | Positive |
| Oda[282] | 26 to 34 | 5 to 10 years | MM | Positive |
| Capellari[52] | 31 to 38 | 4 to 10 years | MV | Positive |
| Gelpi[120] | 65 | 4 months | MM | Negative |
| Kovacs[213] | 33 & 35 | 3 years | MM | Positive |
| Vital[368] | 65 | 9 months | MV | Negative |

* The disease duration was not significantly different in the MM and MV patients.

*Table 2.72 6-OPRI clinical phenotype (3)*

| Reference | Onset | Clinical features |
|---|---|---|
| Poulter[309] | Behavioural change; cognitive decline | Cortical dementia, cerebellar & pyramidal signs; myoclonus. Variable clinical phenotype |
| Nicholl[276] | Cognitive decline; unsteadiness & cortical visual loss in 1 case | Dementia, cortical blindness, pyramidal signs, myoclonus, paratonic rigidity, ataxia & akinetic mutism in 1 case. Cerebellar signs, chorea & dementia in 1 case |
| Oda[282] | Behavioural change, dysarthria | Dementia, cerebellar & extrapyramidal signs |
| Capellari[52] | Behavioural change, memory loss | Dementia, myoclonus, cerebellar, extrapyramidal & pyramidal signs; seizures in 2 cases |
| Gelpi[120] | Ataxia, dizziness, choreo-athetoid movements, rest tremor, apraxia | Saccadic speech, language problems, dementia, myoclonus |
| Kovacs[213] | Memory problems | Dementia, cerebellar signs, rigidity, behavioural change Anosmia in 1 case |
| Vital[368] | Rapidly progressive dementia | Dementia, cerebellar ataxia, extrapyramidal rigidity, myoclonus |

*Table 2.73 6-OPRI investigative findings*

| Reference | EEG | Neuroimaging | CSF 14-3-3 |
|---|---|---|---|
| Poulter[309] | Non-specifically abnormal | Normal or Atrophy | Positive (1 case) |
| Nicholl[276] | Periodic complexes in 1 case | Atrophy | Not reported |
| Oda[282] | Slow | Generalised atrophy | Not reported |
| Capellari[52] | Slow in 2 cases<br>Periodic complexes in 1 case | Atrophy | Not reported |
| Gelpi[120] | No periodic complexes | Diffuse atrophy | Positive |
| Kovacs[213] | Slow | Atrophy | Negative |
| Vital[368] | Slow waves & periodic paroxystic activity (sic) | Atrophy | Negative |

*Table 2.74 6-OPRI neuropathological findings*

| Reference | Neuropathological Findings |
|---|---|
| Poulter[309] | Atrophy in some cases; variable spongiosis with cerebellum usually spared<br>Dense $PrP^{Sc}$ staining in the cerebellar molecular layer;<br>perpendicular to pial surface<br>Plaque like deposits in parahippocampal gyrus in some cases. Type 2 $PrP^{Res}$ |
| Nicholl[276] | Widespread spongiform change with neuronal loss & gliosis in the cerebral cortex & basal ganglia; patchy changes in the cerebellum (molecular & granular layer)<br>Status spongiosis in the visual cortex in 1 case with cortical blindness<br>$PrP^{Sc}$ deposition in the cerebral cortex & cerebellum |
| Oda[282] | Diffuse atrophy; patchy neuronal loss<br>Spongiosis of the cerebral cortex (frontal area worst affected)<br>Astrocytosis of the frontal cortex, amygdala, hippocampus<br>$PrP^{Sc}$ positive plaques in the molecular layer of the cerebellum |
| Capellari[52] | Diffuse spongiform change, astrocytosis & neuronal loss in 1 case<br>No spongiform changes in 1 case<br>Elongated patches of PrP in the molecular layer of the cerebellum<br>Type 1 $PrP^{Res}$ present |
| Gelpi[120] | Moderate spongiform change in cerebral & cerebellar cortex; neuronal loss in basal ganglia & occipital cortex<br>Numerous eosinophilic globules in the molecular layer & parahippocampal gyrus |
| Kovacs[213] | Mild frontal atrophy; neuronal loss in all cortical areas<br>Mild spongiform change & astrocytosis of the cerebellar molecular layer<br>& marked $PrP^{Sc}$ deposition perpendicular to the leptomeningeal surface.<br>Strong $PrP^{Sc}$ deposition in the olfactory tract & bulb (1 case examined) |
| Vital[368] | Marked spongiosis of the cortex, caudate & putamen<br>Kuru plaques, especially in the molecular layer of the cerebellum<br>$PrP^{Sc}$ deposits perpendicular to the cerebellar parenchyma |

In the original reports of the Poulter[309] et al. family it was reported that premorbid personality disorders were common; however in a recent re-analysis of the family this finding was not upheld[250]. No premorbid personality disorders were reported in the Japanese family either[282] .

One case reported by Kovacs et al.[213] had $PrP^{Sc}$ deposition in the olfactory tract & bulb, associated with symptoms of anosmia. Interestingly the pathological appearances of eosinophilic globules found by Gelpi et al.[120] in their patient were also present in one individual from the Poulter et al.[309] family. Overall the clinical phenotype was variable, and to some degree influenced by the codon 129 status of the wild type allele, with MV individuals having an older age at onset, although the disease duration was unchanged[250] . This finding is borne out by the Basque family reported by Capellari et al.[52], where 2 MV individuals had disease onset at 38 years, with durations of 4 and 10 years. In the Poulter et al.[309] family an older age at onset seemed to be associated with a more aggressive disease course. This family is also of interest as multiple generations are known to have been affected, and genetic analysis has shown the mutation to be stable over time.

The only case to be reported with valine at codon 129 of the mutated allele is that reported by Vital et al.[368], which is notable for having neuropathological appearances consistent with GSSS, although the clinical picture resembled that of sCJDMM1. It is difficult to speculate further as to how codon 129 may have influenced the clinicopathological features when only 1 case with valine on the mutated allele has been described, but there does seem to be a trend for valine at the mutated allele to be associated with GSSS neuropathology.

## 2.XLVIII Seven-Octapeptide Repeat Insertion

Four families and 3 individuals have been reported to have prion disease due to the 7-OPRI mutation with the majority being neuropathologically confirmed. The clinical phenotype in those cases coupled to methionine on the mutated allele is one of a young age at onset and prolonged duration; the clinical features resemble those of sCJDMM1 with dementia being a dominant feature, accompanied by a pyramidal, cerebellar or extra-pyramidal signs and myoclonus. However the investigative findings do not resemble those of sCJDMM1, with neuroimaging typically showing atrophy, and EEG periodic complexes only being reported in 1 case. Pathologically spongiform encephalopathy is seen, and in 1 family[84] elongated $PrP^{Sc}$ deposits in the cerebellum. One member of this family also carried the E318G mutation of the *PSEN* gene, but the authors concluded that this had no effect on the phenotype seen.

Different repeat expansions have been reported in the different families. One case has been shown to have developed a de novo insertion, as genotyping of both parents was normal (with paternity being confirmed by haplotype analysis). Despite the different insertions seen, the clinical and pathological features are relatively consistent between the different families[246,146,373,48,84,41,134]. No asymptomatic mutation carriers have been reported, suggesting a high level of penetrance.

One Dutch family has been reported with a 7-OPRI coupled with valine at codon 129 of the mutated allele[184]. The age at onset was significantly younger compared to previously reported cases with 7-OPRI 129M, and the duration significantly shorter. As with many mutations associated with valine on the mutated allele, the neuropathology showed multicentric amyloid plaques. GSSS is a neuropathological diagnosis classically associated with an ataxic illness with late cognitive decline; however in many cases the clinical features and the pathology do not both match these criteria.

*Table 2.75 7-OPRI genotype and clinical phenotype (1)*

| Reference | Insertion | Mutated allele | Origin | No. patients reported |
|---|---|---|---|---|
| Goldfarb[134] | R1-2-**2c-3-2-3-2-3-2-3g**-3-4 | Unknown | N. America | 3 |
| Dermaut[84] | Not reported | M | Belgium | 3 |
| Lewis[231] | R1-2-2-**3-2-3-2-2-2-2**-3-4 | M | Australia | 3 |
| Wang[373] | R1-2-2-**2-2-2-3g-2-3g-2a**-3-4 | M | China | 3 |
| Canella[48] | R1-2-2-**3-2-2-3g-2-2-2**-3-4 | M | Italy | 1 |
| Guo[146] | R1-2-2-**2-2-2-3g-2-3g-2a**-3-4 | M | China | 1 |
| Jansen[184] | R1-2-2-**2-2-2-2-3g-2-2**-3-4 | V | Holland | 6 |

*Table 2.76 7-OPRI clinical phenotype (2)*

| Reference | Onset (years, mean) | Duration (years, mean) | Codon 129 | FHx |
|---|---|---|---|---|
| Goldfarb[134] | 27.3 (23 to 31) | 11.3 (10 to > 13) | Unknown | Positive |
| Dermaut[84] | 29 (24 to 32) | 9.7 (7 to 11) | MM | Positive |
| Lewis[231] | 28.3 (24 to 32) | 10 (7 to 16) | MV | Positive |
| Wang[373] | 53.3 (44 to 58) | 6 to 50 months | MM | Negative |
| Canella[48] | 18 | > 10 | MM | Negative |
| Guo[146] | 44 | 4 | MM | Positive |
| Jansen[184] | 52.2 | 2.4 | MV & VV reported | Positive |

*Table 2.77 7-OPRI clinical phenotype (3)*

| Reference | Onset | Clinical features |
|---|---|---|
| Goldfarb[134] | Behavarioural change, gait or cognitive problems | Dementia, behavioural change, cerebellar signs, myoclonus & rigidity |
| Dermaut[84] | Cognitive or cerebellar problems | Dementia, cerebellar, pyramidal, extrapyramidal signs, myoclonus |
| Lewis[231] | Behavioural change, forgetfulness | Dementia, involuntary movements, generalised tonic-clonic seizures |
| Wang[373] | Forgetfulness | Dementia, dysarthria, hypertonia, ataxia |
| Canella[48] | Behavioural change | Depression, psychosis, bipolar disorder, dementia, cerebellar ataxia |
| Guo[146] | Slowly progressive dementia | Dementia, myoclonus, pyramidal and cerebellar signs |
| Jansen[184] | Slowly progressive dementia | Dementia, depression, hypokinetic-rigid syndrome |

*Table 2.78 7-OPRI investigative findings*

| Reference | EEG | Neuroimaging |
|---|---|---|
| Goldfarb[134] | Slow | Unknown |
| Dermaut[84] | Slow | Atrophy |
| Lewis[231] | Slow | Atrophy |
| Wang[373] | Periodic complexes | Normal |
| Canella[48] | Unknown | Atrophy |
| Guo[146] | Periodic complexes & slow waves | Normal |
| Jansen[184] | Periodic complexes in 1 case; Non-specific slowing in 2 cases | Basal ganglia high signal in 1 case; atrophy in 1 case |

*Table 2.79 7-OPRI neuropathological findings*

| Reference | Neuropathological Findings |
|---|---|
| Goldfarb[134] | Spongiosis, gliosis & neuronal loss<br>Trace of $PrP^{Sc}$ present |
| Dermaut[84] | Atrophy, spongiosis, gliosis & neuronal loss<br>Elongated deposits of $PrP^{Sc}$ in the cerebellum, perpendicular to the leptomeningeal surface |
| Lewis[231] | Atrophy, marked spongiosis, gliosis & neuronal loss in the cerebral cortex & basal ganglia.<br>Atrophy & synaptic $PrP^{Sc}$ deposition in the cerebellum. Type 2 $PrP^{Res}$ |
| Wang[373] | Diffuse spongiform change, astrocytosis & neuronal loss<br>Type 2 $PrP^{Res}$ |
| Canella[48] | Unknown |
| Guo[146] | Spongiform change, diffuse neuronal loss, mild astrocytic gliosis<br>Predominantly monoglycosylated $PrP^{Res}$. Type 1 $PrP^{Res}$ |
| Jansen[184] | Numerous multicentric & unicentric amyloid plaques throughout the cerebrum & cerebellum & variable spongiform degeneration & astrocytosis<br>Type 1 $PrP^{Res}$ & a 8 kDa fragment |

The individual described by Cannella et al.[48] was shown to have a de novo mutation, as the insertion was absent from both parents (paternity was confirmed genetically). This is the first confirmed de novo case reported.

## 2.XLIX Eight-Octapeptide Repeat Insertion

Prion disease due an 8-OPRI has been identified in 3 families; all had an autosomal dominant pattern of inheritance[134,365,227]. Two families had an insertion on an allele carrying valine at codon 129, and 1 family on an allele with methionine. All families had different repeat insertions. In 2 individuals an EEG showing periodic triphasic waves was documented, and in 1 a MRI showing basal ganglia high signal. In other individuals the EEG was normal or non-specifically abnormal and neuroimaging was normal or showed atrophy. Overall the clinical and pathological features were consistent with GSSS[369].

*Table 2.80 8-OPRI genotype and clinical phenotype (1)*

| Reference | Repeat | Mutated allele | Origin | No. patients reported |
|---|---|---|---|---|
| Goldfarb[134] | R1-2-2-3-**2-2-2-2-2-2-2-2a**-4 | V | France | 4 |
| van Gool[365] | R1-2-2-**3g-3-2-2-2-2-2-2**-3-4 | V | Netherlands | 6 |
| Laplanche[227] | R1-2-2-3-**2-2-2-2a-2-2-2-3**-4 | M | France | 11 |

*Table 2.81 8-OPRI clinical phenotype (2)*

| Reference | Onset (years, mean) | Duration (years) | Codon 129 | FHx |
|---|---|---|---|---|
| Goldfarb[134] | 45 | 2.3 | Not reported | Positive |
| van Gool[365] | 43 | 3.3 | VV & MV | Positive |
| Laplanche[227] | 28 | 3.8* | MM | Positive |

* The mean duration for deceased patients is given; other members of the kindred were alive at up to 12 years after onset.

*Table 2.82 8-OPRI clinical phenotype (3)*

| Reference | Onset | Clinical features |
|---|---|---|
| Goldfarb[134] | Not documented | Behavioural change, cerebellar & pyramidal signs, myoclonus, mutism |
| van Gool[365] | Cognitive decline; personality & gait disturbance | Dementia, cerebellar signs, myoclonus, primitive reflexes, pyramidal signs, rigidity, |
| Laplanche[227] | Psychiatric or cerebellar features | Psychiatric symptoms, cerebellar signs, later dementia; terminal rigidity |

*Table 2.83 8-OPRI investigative and neuropathological findings*

| Reference | EEG | Neuroimaging | Neuropathological Findings |
|---|---|---|---|
| Goldfarb[134] | Triphasic complexes | NA | Spongiosis, gliosis, multicentric plaques |
| van Gool[365] | Triphasic waves | Atrophy; basal ganglia high signal | Mild spongiosis, gliosis & neuronal loss; multicentric plaques |
| Laplanche[227] | Non-specifically abnormal | NA | Atrophy & gliosis of the molecular layer of the cerebellar cortex; kuru plaques; multicentric plaques; $PrP^{Sc}$ positive; minimal spongiosis |

## 2.L Nine-Octapeptide Repeat Insertion

Two families with probable prion disease due a 9-OPRI have been described; in both cases only 1 family member has undergone *PRNP* genotyping[215,285]. The case descriptions are given in the tables.

*Table 2.84 9-OPRI genotype and clinical phenotype of the family reported by Owen[285]*

| Origin | Onset (years) | Duration (years) | Codon 129 |
|---|---|---|---|
| England | 53 | 2.5 | M* |
| **Onset** | **Clinical features** | | **FHx** |
| Behavioural change; gait problems | Dementia, apraxia, gait ataxia, myoclonus | | Positive |
| **Octapeptide repeat sequence** | | | |
| R1-2-2-**3-2-3g-2a-2-2-2-3g-2**-3-4 | | | |

* The insertion was combined with methionine at codon 129; the codon 129 status of the wild type allele was not documented.

*Table 2.85* *9-OPRI genotype and clinical phenotype of the patient reported by Krasemann*[215]

| Origin | Onset (years) | Duration (years) | Codon 129 |
|---|---|---|---|
| Germany | 32 | >2 (still alive) | MM |
| **Onset** | **Clinical features** | | **FHx** |
| Behavioural change; gait problems | Dementia, dysarthria, visual disturbance, gait ataxia, cerebellar signs, postural tremor. | | Positive |
| **EEG** | **Neuroimaging** | | **14-3-3** |
| Non-specific slowing | Cortical atrophy | | Not done |
| **Octapeptide repeat sequence** | | | |
| R1-2-2-**3-2-3-3g-2-2a-2-3-2**-3-4 | | | |

The English case had a FHx of early onset dementia; the German case had a family history of her mother, maternal grandmother and great-grandmother having died in their early 40s with dementia and spastic paraparesis.

No pathological details are known for either case.

## 2.LI Twelve-Octapeptide Repeat Insertion

One North American family with a 12-OPRI has recently been reported by Kumar et al.[221] 3 members with affected, all of whom had a clinical phenotype which resembled behavioural variant frontotemporal dementia, with slowly progressive personality and behavioural change, social disinhibition and increased appetite. Additional features not typically associated with frontotemporal dementia were present in the form of gait ataxia and seizures, leading to *PRNP* genotyping which demonstrated a 288 base pair insertion (presenilin 1 and microtubule protein associated tau were also analysed and no mutations found). In keeping with the clinical features were the MRI and PM findings of severe bilateral frontal atrophy. In the 2 individuals for whom neuropathology was performed a mixed picture of multi-centric $PrP^{Sc}$ positive plaques and numerous neurofibrillary tangles and neuropil threads was seen. This family further expands the possible phenotypes associated

with an OPRI of *PRNP,* which may be responsible for a frontotemporal type dementia.

*Table 2.86 12-OPRI genotype and clinicopathological phenotype*

| **Origin** | **Onset (years; mean)** | **Duration (years; mean)** | **Codon 129** |
|---|---|---|---|
| USA | 44 | 8 | Unknown |
| **Onset** | **Clinical features** | | **FHx** |
| Personality changes | Dementia, frontal type affect, seizures, ataxia | | Positive |
| **EEG*** | | **Neuroimaging*** | **14-3-3*** |
| Slowing, generalized spike and sharp wave discharges | | Generalised atrophy, particularly frontally | Negative |
| **Neuropathology** | | | |
| Bilateral frontal atrophy<br>Multi-centric $PrP^{Sc}$ positive plaques, particularly in the cerebellar cortex<br>Numerous neurofibrillary tangles and neuropil threads | | | |

* Investigation results were only known for the proband.

## 2.LII Animal Transmission Studies and Transgenic Animal Models

### *Introduction*

Animal transmission studies played a crucial role in elucidating the nature of prion diseases, or as they were previously called, 'slow virus' infections. Early studies used a variety of animals including non-human primates, cats and guinea pigs. More recently transgenic humanised mice have been developed which are more susceptible to human prion diseases, which facilitates research studies. Transgenic work has also led to the creation of animal models of gPD, which are useful to investigate whether or not *PRNP* mutations are truly pathogenic, and to study their clinico-pathological properties. Experimental transmission studies have involved surprisingly few *PRNP* mutations, most of which have been successful, although not always in wild type animals. Murine *PRNP* appears to exert an inhibitory effect on disease transmission, and the use of humanised mice with a transgenic form of *PRNP* encompassing human elements tends to be more successful[360]. In this section I will review those *PRNP* mutations which have been the subject of animal transmission studies or transgenic models.

### *P102L*

GSSS was one of the earliest gPD to be studied, due to the distinct clinicopathological features. The exact underlying mutation is not always identifiable in early studies, but patients from a family subsequently found to carry the P102L mutation were involved in the work of Masters et al[241]. They transmitted disease to various animals (primarily non-human primates) using brain tissue from a selection of GSSS patients. P102L was successfully transmitted from a male patient who had died at the age of 42 years after an illness consistent with GSSS, with spongiform change at post-mortem. Brain tissue was inoculated intracerebrally into spider and squirrel monkeys and chimpanzees. Both types of monkey developed spongiform encephalopathy after between 20 to 30 months incubation, but the chimpanzee was unaffected 7 years post-inoculation. Transmission to spider monkey, chimpanzee and guinea pig was attempted using brain tissue from another affected member of this

family but the results were negative after 3 years incubation. Intriguingly, no primates developed amyloid deposits.

P102L has also been transmitted via intracerebral inoculation to marmosets, who after between 25 to 32 months incubation initially became quiet and dishevelled, followed by balance problems which progressed to a broad based ataxic gait[19]. They later developed social withdrawal, drooling, daytime somnolence and disturbed nocturnal sleep. Neuropathologically the thalamus was the most severely affected brain region, with additional vacuolation of the molecular and granular layers of the cerebellar and fine vacuolation of the brain stem. Samples from the affected animals were subsequently passaged into second line marmosets, with further successful transmission and shorter incubation times.

Asante et al.[17] attempted to create a mouse model of P102L using animals carrying humanised *PRNP* homozygous for P102L and the murine *PRNP* gene knocked out. These animals did not develop spontaneous disease, living to a normal life expectancy with no clinical or pathological, or immunohistochemical abnormalities. However these animals were susceptible to P102L when inoculated with human brain homogenate, with a high attack rate and relatively short (compared to similar animals inoculated with sCJD) incubation time of 185 to 191 days. Neuropathologically there was generalized synaptic PrP deposition in the cerebral cortex, basal ganglia, hippocampus and thalamus, with the brain stem and cerebellum being less severely affected. There was widespread spongiform change but no multi-centric plaques. The only difference between transgenic animals inoculated with P102L and sCJD was somewhat less intense PrP staining seen in P102L. This model is difficult to interpret, as it is not truly a mouse model of P102L, given that none of the animals developed spontaneous prion disease. An earlier, probably more useful model was reported by Telling et al[358], who bred transgenic mice over-expressing murine *PRNP* with the murine equivalent of the P102L mutation. These animals developed spontaneous disease, but with a variable age at onset. This led the authors to ablate the wild-type murine *PRNP* in mice over-expressing P102L, which led to a very consistent pattern of disease onset at 145 days of age, with myopathy and peripheral neuropathy. Spongiform change and plaques were seen, and disease could be transmitted to healthy animals. These studies possibly provide more new

questions than answers, with the identification of complex interactions between wild-type and mutated PrP. The wild-type murine *PRNP* appears to be an inhibitory factor to the development of disease due to P102L.

The codon 129 status of the patients used in the early studies of Masters et al.[241] and Baker et al.[19] is not known. It is likely that they would be P102L-129M, this being the commonest haplotype. P120L-129V has been used to attempt to transmit disease to humanised mice, but no clinical or pathological changes resulted[361]. However when brain homogenate from the same individual was inoculated into humanised mice with the P102L mutation (which develop spontaneous prion disease), these animals developed disease significantly earlier than uninoculated animals. As before, interpreting this result is somewhat difficult – carriage of the P102L mutation appears to be permissive for disease transmission, but it is unclear why.

### *A117V*

Transmission studies of the A117V mutation have not been reported, but a mouse model with the murine equivalent (A116V) and over-expression of PrP has been published[402], with animals developing spontaneous disease. Progressive ataxia began at a mean age of 140 days, with death by 170 days. This ataxia was reported to be more prominent and prolonged than that seen in mouse models of sCJD. Neuropathologically there was mild scattered vacuolation, and prominent PrP positive plaques, mostly affecting the cerebellum. A weakly protease resistant PrP fragment was identified, similar to that seen in human A117V cases.

### *D178N*

As with other mutations, it is difficult to sub-divide transmission studies of the D178N mutation by the codon 129 status as this is often not known in early studies, and as previously discussed, 129 M or V cases cannot be distinguished definitively by their clinical phenotype. Brain homogenate from D178N cases of various nationalities was transmitted to non-human primates by Brown et al.[39], with

an approximately 46% transmission rate. The authors also used inocula from cases of 7-OPRI and E200K, and all 3 mutations had similar disease durations.

The first successful transmission of FFI to wild-type mice found most animals became symptomatic after a 400 to 600 day incubation period. Neuropathologically there was spongiform change, astrocytic proliferation, neuronal loss and abnormal PrP deposition, with the thalamus being most severely affected[356].

Although the data is limited, there is some evidence that the codon 129 status influences transmissibility of D178N-129M[359]. Of the cases with D178N-129M, 3 of 4 methionine homozygous patient samples transmitted disease, whilst only 1 of 3 heterozygous patient samples did so. A single D178N-129V case failed to transmit. In affected animals there was a distinctive $PrP^{Sc}$ deposition pattern, with intense staining of the thalamus (in particular the antero-ventral and medio-dorsal nuclei) and rostral corpus callosum. After digestion with protease K a 19kDa fragment of PrP was found, unlike that seen in the same line of animals following E200K inoculation, where a 21kDa fragment was seen.

There is a trend for FFI to be harder to transmit than other forms of gPD, and some authors feel that this relates to the levels of detectable $PrP^{Sc}$ present, which are low in FFI[39]. Whilst this is a plausible theory, the numbers of donor patients and recipient animals are relatively small, making it is difficult to draw definite conclusions regarding this.

Animal models have been created of both D178N-129M and D178N-129V. Knock-in mice homozygous for D178N-129M developed age-related behavioural changes, with sleep disturbance and temperature disregulation. MRI at 16 months of age showed atrophy of the cerebellum and flattening of the motor cortex, with enlarged ventricles and abnormalities of the thalamus. At neuropathology there were changes similar to those seen in human cases, with deep cerebellar white matter vacuolation, reactive gliosis and severe neuronal loss in the thalamus. Protease resistant PrP was rarely detectable, with only one animal having positive findings[176]. The clinical and neuropathological findings resembled those seen in human FFI cases.

Transgenic mice with a murine homolog of D178N-129V also develop spontaneous disease, with motor dysfunction, memory impairment and severe

abnormalities of the sleep-wake cycle on EEG. The phenotype was altered by varying the level of PrP expression, with over-expressing animals having shorter incubation times and those with a below normal level of endogenous PrP not developing disease. Whilst sleep disorders are not considered a typical feature of D178N-129V in humans, this has been infrequently reported[89].

## *E200K*

As could be expected with the commonest cause of gPD world-wide, the E200K mutation has been the subject of a number of transmission studies and transgenic mouse models. Brain homogenate from patients of various nationalities has been inoculated intracerebrally into non-human primates with resultant spongiform encephalopathy after incubation periods varying from 13 months[40] to 6 years[59]. The most detailed description of disease in non-human primates comes from Chapman et al[59], who reported a chimpanzee which developed disease 6 years after intra-cerebral inoculation with a 10% suspension of brain homogenate from a Jewish Libyan patient. The animal displayed frightened behaviour, trembling, gait and truncal ataxia and terminal indifference, decreased spontaneous movement and vocalisation. The illness lasted 3 weeks, and on neuropathological examination there was extensive spongiform change.

Further information on the neuropathological and immunohistochemical patterns seen in animal studies of E200K comes from humanised mice (with the murine *PRNP* gene knocked out) inoculated with E200K brain homogenate. The same strain of animal was inoculated with D178N-129M. Compare with the FFI animals, E200K transmission resulted in more hypothalamic vacuolation but less severe changes to the corpus callosum. The PrP deposition in E200K was widespread throughout the brain, unlike FFI where the thalamus and corpus callosum were most severely affected, and the brainstem spared. Following protease K digestion, a 21kDa fragment of PrP was found in the E200K animals, which is similar to that seen in human patients[359]. Another study using similar transgenic humanised animals found diffuse $PrP^{Sc}$ deposition in the cerebral cortex, with intense staining of the hypothalamus and locus coeruleus of the brainstem[242].

The E200K mutation has also been the subject of transgenic animal models. Friedman-Levi et al.[100] used a chimeric mouse/human *PRNP* construct with an E199K substitution, equivalent to the E200K mutation in humans. This was generated on a background of either null/null murine *PRNP*, or wild-type *PRNP*. Both lines of mice were affected, with an illness characterised by asymmetrical hind limb weakness progressing to paraplegia, leg clasping and lower body atrophy and occasional tremor. The manifestations were similar in knock-out and wild-type mice. Around 10% developed myoclonus. The mean age at onset was 5.4 months in wild-type animals with a mean duration 14.9 months. The onset was slightly later (mean 6.7 months) and duration shorter (mean 11.6 months) in murine *PRNP* knock-out animals. PrP immunohistochemistry demonstrated intraneuronal dot-like and granular immunostaining in a widespread distribution, with neurons of the spinal cord, basal ganglia, thalamus, frontal cortex and brainstem being particularly affected. Focal spongiform change was seen at the end-stage of disease, mostly of the frontal cortex and basal ganglia.

After establishing that this line of mice spontaneously develop disease, animals were assessed for abnormal PrP deposition at different stages. Protease K resistant PrP was absent at 1 month of age, and then progressively accumulated over time, in a fashion which correlated with age and clinical disease manifestations.

Passaging into further transgenic mice from the same lines was performed, with successful transmission to asymptomatic animals. These passaged animals displayed a greater degree of spongiform change and astrogliosis, and diffuse synaptic type PrP immunoreactivity.

### *V210I*

The V210I mutation has been successfully transmitted to transgenic mice with humanised *PRNP,* and to wild type bank voles and mice[242,3]. Tissue from 3 different patients was used to inoculate transgenic mice, all of which transmitted disease with incubation times varying from 182 to 233 days. Affected animals developed ataxia, a hunched back and behavioural change. Pathologically there was spongiosis, astrocytic gliosis localized to the entorhinal cortex and cingulate gyrus.

No variation was seen between animals inoculated with samples from different patients, despite there being significant phenotypic variation between the patients. Neuropathological findings were compared to animals inoculated with sCJD, E200K or D178N-129M samples. V210I most resembled sCJD neuropathologically.

Similar neuropathological conclusions were drawn from the transmission of V210I to bank voles, which survived for 157 days. The disease was highly transmissible, with $PrP^{Sc}$ type 1 being present in both the human and animal subjects. The vacuolar degeneration and lesion pattern seen was very similar to animals inoculated with V120I, sCJD MM1, sCJD MV1 or E200K.

### *Q217R-129V*

One study used brain homogenate from 2 unrelated patients with Q217R-129V (1 from Sweden, 1 belonging to the Indiana kindred) to attempt to transmit disease to rodents; this was unsuccessful[170].

### *M232R*

The rare M232R mutation has been successfully transmitted via intracerebral inoculation of brain homogenate to New Zealand white mice, which developed disease after around 700 days incubation. Neuropathologically the animals displayed spongiform change of variable severity, astroglial proliferation and neuronal loss, with a neuropil distribution of abnormal PrP. Overall the neuropathological findings were similar to those seen when sCJD was transmitted to this line of mice. The authors[166] also attempted transmission using lymph nodes and spleen from the same patients, but this was unsuccessful.

## *Animal Transmission Studies and Transgenic Animal Models: Conclusions*

Animal studies have obvious limitations. The use of humanised transgenic animals partly compensates for genetic differences between humans and animals. However studying clinical features in animals is difficult, and subtle changes in areas

such as behaviour and cognition will be hard to detect. The main features recorded in such studies are the attack rate and incubation period, which are fairly crude disease characteristics. Useful neuropathological data can be obtained (albeit limited by anatomical differences between the different hosts), in particular the serial examination of animals at different stages of disease incubation or progression. The most striking feature of the literature regarding animal transmission studies and models of gPD is how few of the many *PRNP* mutations have been studied. There is scope for a great deal of interesting further work in this area, in particular establishing if *PRNP* mutations reported in a single case only are definitely pathogenic and transmissible. The use of the same animal strains in different studies should be encouraged to allow direct comparisons to be made of different results.

## 2.LIII Literature Review: Conclusions

In this chapter I have summarised the disease characteristics of all pathogenic *PRNP* mutations reported to date. This is not always straightforward, due to the marked variability between individuals carrying the same mutation, and limitations of the available data. Often only a very small number of cases are reported, with a wide range in the level of clinical, genetic and pathological detail provided. If the codon 129 status (and in particular the status of the wild type and mutated alleles) is not available, then classifying and analysing cases according to haplotype becomes very difficult. Frequently only a handful of cases of a known *PRNP* haplotype were found in the literature. There are likely to be significant number of undiagnosed cases, the clinicopathological features of which could be very different to the currently published cases. Making comparisons between small numbers of cases is difficult and may lead to invalid conclusions being drawn; however some general trends do emerge.

The majority of point mutations and octapeptide repeat insertions are in cis with methionine at codon 129. Heterozygosity at codon 129 is frequently reported, but methionine homozygotes are in the majority, and valine homozygosity is relatively unusual. Methionine appears to somehow be permissive for either mutational events or the development of disease (or both). gPD thus shows similarities with other forms of prion disease such as vCJD, the development of which is strongly linked to methionine at codon 129.

It is difficult to be clear as to how the codon 129 status of the wild-type and mutated allele influences the clinico-pathological phenotype. The most widely studied phenomenon is with the D178N-129M, where dramatic differences in clinico-pathological phenotype are determined by the wild-type allele codon 129. Even here though, there are reported exceptions to the rule that D178N-129MM causes FFI and D178N-MV causes a sCJD like phenotype. Another trend is for GSSS type cases to be associated with valine on the mutated allele, but again this is not a hard and fast rule. This link and the higher prevalence of methionine at codon 129 may explain why GSSS features are less common than sCJD-like cases. Where comparisons can be made between the same mutation in cis with either methionine or valine, this often (but not always) affects age at onset and disease duration, with a

tendency for valine to give rise to an older age at onset or longer disease duration. Heterozygosity at codon 129 also seems to have the same effect with a number of different mutations. Interestingly, this is seen with E200K in Slovakian cases but not in cases from other regions of the world, which implies that other, as yet unidentified genetic factors are at work. It seems highly likely that genetic factors other than the *PRNP* mutation and codon 129 influence disease development and phenotype; these may well involve genes other than *PRNP*.

The dominance of a small number of mutations world-wide is very interesting; the majority of individuals affected by gPD will have either E200K, D178N, or P102L. This is probably due to a combination of factors, including the likelihood of mutational events occurring at a particular *PRNP* locus, disease penetrance, age at onset and socio-economic factors (e.g. family size and emigration patterns).

Interpreting the data must be done with caution due to the small numbers of cases involved. In order to systematically examine these tendencies, it is crucial that gPD cases (and unaffected carriers) undergo complete sequencing of the *PRNP* gene, and that the codon 129 status of the wild-type and mutated alleles is established.

# CHAPTER THREE

# METHODOLOGY

## 3.I Case Ascertainment

Potential cases of gPD were identified from the records of the NCJDRSU (1990 to 2009), and from two earlier surveillance projects which are described below (1970 to 1990)[388,75,389,385].

### *The NCJDRSU & earlier studies*

The NCJDRSU (previously known as the National CJD Surveillance Unit) was established in 1990 as a result of Southwood Committee report [346]. The NCJDRSU aims to identify all individuals with prion disease in the UK. Referrals of potential cases are made by clinicians via a national reporting system or by informal discussion of doubtful cases, by neuropathologists encountering cases at PM, and by a twice yearly review of death certificates encoding any form of prion disease as a cause of death. In addition cases may be reported by allied heath professionals such as public health officers, organisations such as The UK CJD Support Network and members of the public.

Surveillance data was also available for the period 1970 to 1990 from the following studies:

'A retrospective study of CJD in England and Wales'[412].

In the 1970 to 1979 period, cases were ascertained primarily by a review of death certification.

'A retrospective epidemiological study of CJD in the UK' [386, 412]

From 1980 to April 1990, cases were obtained retrospectively from death certification and referrals of suspected cases of prion disease by UK clinicians.

## 3.II Visiting Patients

After a suspected case is referred to the NCJDRSU permission is sought from the patient (if possible), their family and local consultant for a research clinician to visit the patient. During the visit, the patient is interviewed (if possible), and examined, and an epidemiological questionnaire completed by an interview with a close relative. This includes questions regarding residential and occupational history, a detailed family tree, and a dietary history. A history of the illness is obtained, including past medical and drug history and investigative findings. Copies of medical records, the EEG and neuroimaging are taken with written consent from the patient or their next of kin. If a patient is referred after their death then the family is contacted (with the permission of the local consultant or general practitioner) and visited to complete the questionnaire, to obtain a clinical history and consent for review of the medical records. The family may decline the offer of a visit in which case the case notes are reviewed if possible. The quantity and quality of data collected therefore varies, and the investigations performed reflect the technology available at the time of the patient's illness and their specific clinical features.

## 3.III Clinical Symptoms and Signs

Data about the timing of disease onset, the presenting symptoms and the symptoms and signs present over the entire disease course were extracted from the available information in the questionnaire, history and medical notes. The point of onset was taken to be the earliest date at which novel symptoms attributable to prion disease were noted by the patient, their family or doctor. Where the symptoms could not be exactly dated an approximation was made using the available data (for example the date of referral; if a month of onset was given the 15$^{th}$ of the month was used; if a year was given then the 15$^{th}$ of June was used). In the large majority the onset was defined within 1 to 2 months. A certain amount of error will therefore be present, especially with regard to the precise date of onset of insidious symptoms. The disease duration was calculated using the date of onset and date of death (inclusive), or if the patient was still alive at the end of data collection then

30/10/2009 was used as the latest possible date of death for the purposes of this study. The presenting symptoms were taken from the questionnaire.

The presence or absence of each individual symptom or sign was recorded (a list of those recorded is given in Table 3.2). Difficulties sometimes arose when interpreting the examination findings documented by different clinicians and the symptoms described by the patient and their family. Clinical judgement was used to decide if what was described fitted with a symptom or sign; for example if the family described 'jerking movements' and clearly imitated myoclonus then this was interpreted as being myoclonus. However if the patient was described in the medical notes as having 'involuntary movements' without further details being given then this was classified as 'involuntary movement'.

## 3.IV Investigations

Investigations were carried out as clinically indicated by the referring physicians, who would at times request advice regarding this from the NCJDRSU. Not all patients underwent EEG, neuroimaging and lumbar puncture, either due to limitations in the services available in the referring hospital, or if tests were not felt to be clinically justified. A significant number of individuals presented prior to the widespread availability of MRI (the early 1990s) and CSF 14-3-3 analysis (which began at the NCJDRSU in November 1996).

### *CSF analysis*

The results of CSF analysis for total protein, glucose and the number of red and white cells present were obtained from the medical notes. Where possible the paired serum glucose level was also recorded. Analysis of CSF 14-3-3 and S100b were carried out by Dr A. Green in the NCJDRSU, according to previously published methodology[142]. In some early cases (during 1996) CSF analysis was carried out in the USA by Dr P.Brown.

### *EEG analysis*

Representative pages from EEG recordings were reviewed independently in the NCJDRSU by Prof R. Knight and Prof R. Will. These were classified into those showing periodic triphasic complexes, or those which were non-specifically abnormal or were normal. In some cases only an EEG report from the referring hospital was available; details of which EEGs were not reviewed in the NCJDRSU are given in the results section.

### *Neuroimaging*

Computed tomography (CT) and / or MR images were obtained either as hard copies or recordings on CD. These were reviewed by a consultant neuroradiologist with an interest in prion diseases (Dr D.Summers or Dr D.Collie). Details of any abnormal findings were noted, with particular reference to the presence or absence of changes associated with prion disease (basal ganglia high signal and / or cortical high signal in three or more brain regions on Fluid Attenuated Inverse Recovery; FLAIR or Diffusion Weighted sequences). Note was made of any cases with changes suggestive of vCJD (high signal in the pulvinar region of the thalamus).

### *Genotyping*

*PRNP* sequencing and codon 129 analysis were carried out according to previously published methodology[31]. Either blood or frozen brain tissue was used, depending upon which was available. Full gene sequencing (genotyping) in order to search for any *PRNP* mutations was only performed with informed consent from the patient or their next of kin. Data were also obtained from genetic testing performed by the National Prion Clinic, London. Genotyping became available in the early 1990s and therefore cases prior to this only had genotyping performed if appropriate stored samples and consent were available.

Individuals in the 4-OPRI group were studied in collaboration with the National Prion Clinic. *PRNP* microsatellite haplotype analysis and relatedness testing was performed by Dr S.Mead[192].

## *Neuropathology*

Where possible neuropathological tissue was examined in the NCJDRSU; in some cases tissue was not available but a PM report issued by another centre was available. As case ascertainment spanned 1970 to 2009, a range of neuropathological methods were used, and modern immunohisochemistry (IHC) and Western blotting was not always performed. Details of which cases were reviewed at the NCJDRSU and which underwent IHC and Western blotting are given in the results section.

Ideally both tissues fixed in formalin and frozen samples were obtained. Extensive neuroanatomical regions were sampled to provide representative tissue from frontal, temporal, parietal and occipital cortex, the basal ganglia, thalamus, hippocampus, hypothalamus and brain stem.

Histopathological examination made note of spongiform change, plaques, gliosis, neuronal loss and any other changes such as Alzheimer-type pathology or amyloid deposits.

IHC staining was performed using two anti-PrP antibodies[410]. The pattern of $PrP^{Sc}$ deposition was recorded, including features such as plaques or unusual $PrP^{Sc}$ patterns. The main patterns seen can be defined as follows:

Patchy/ perivacuolar : immunolabelling mostly around vacuoles

Diffuse synaptic : multiple tiny immunolabelled dots in the neuropil generally, with occasional coarser, larger deposits

Neuronal : pericellular punctuate or granular immunolabelling around neuronal perikarya.

Plaque : usually small to medium sized 'kuru' amyloid plaques with a fringed outline. These are typically reactive to periodic acid-Schiff, Congo red and alcian blue stains; the Congo red staining disappears after formic acid treatment.

Plaque-like : areas of dense PrP-positivity only visible following IHC staining.

Prion protein typing was carried out using Western blot analysis to determine the size and relative abundance of the different $PrP^{Res}$ glycoforms. These are classified according to their molecular weight into Type 1 (nonglycosylated form with a molecular weight of 21kDa) or Type 2 (nonglycosylated form with a molecular weight of 19kDa). If the diglycosylated band predominates in Type 2 then it is termed Type 2B. If the diglycosylated band does not predominate then it is termed Type 2A[409].

Neuropathological examinations at the NCJDRSU were carried out by Prof J. Ironside and Prof J. Bell. Immunohistochemical analysis and PrP isotyping was carried out by the team of Dr M.Head.

## 3.V Data Extraction

As a research registrar at the NCJDRSU between July 2007 and January 2010 I visited suspected cases of prion disease (of any type). This included 5 cases of gPD. I reviewed the case notes for all cases of gPD known to the NCJDRSU (N= 147). Additional records were reviewed for a number of cases which were not included in the final data set: in 3 cases a diagnosis of gPD was suspected on neuropathological grounds but there was insufficient clinical data to substantiate this; records of a number of non-UK cases were reviewed but it was later decided that non-UK cases should not be included. Data extraction was performed by reviewing the existing NCJDRSU databases, copies of medical records (both hospital and GP notes), the NCJDRSU questionnaires, the death certificates and the neuropathological records. In cases where the records held were incomplete I requested copies from the local hospital or GP. Some data was also provided by the National Prion Clinic, London.

I created an SPSS database and recorded the following data:

*Table 3.1* *Data collection (1)*

| ID number | Classification | Visited by NCJDRSU? | Date of onset |
|---|---|---|---|
| Age at onset | Date of referral | Date of death | Disease duration |
| EEG done? | Date of EEG | EEG result | Time from symptom onset to EEG |
| CT done? | Date of CT | CT result | Time from symptom onset to CT |
| MRI done? | Date of MRI | MRI result | Time from symptom onset to MRI |
| *PRNP* mutation | Codon 129 status | gPD suspected during life? | Family history |
| Lumbar puncture done? | Date of lumbar puncture | CSF 14-3-3 result | CSF S100b result |
| Sex | Birthplace (city) | Country of origin | Ethnicity |
| PM performed? | PM location | PM result | |
| Death certificate available? | Death certificate data | | |

I also recorded the presence or absence of the following symptoms and signs:

*Table 3.2* *Data collection (2)*

| | | | |
|---|---|---|---|
| Aggression | Akinetic mutism | Anxiety | Apathy |
| Cerebellar signs | Delusions | Depression | Extrapyramidal signs |
| Gait disturbance | Gegenhalten | Hallucinations | Headache |
| Involuntary movements | Myoclonus | Muscle wasting | Oculomotor signs |
| Pain | Primitive reflexes | Pseudobulbar problems | Psychiatric disturbance |
| Pyramidal signs | Rapidly progressive dementia | Sensory symptoms | Seizures |
| Social withdrawal | Speech disturbance | Vertigo | Visual disturbance |

The following definitions were used :

*Table 3.3* *Clinical definitions*

| Clinical symptom or sign | Definition |
|---|---|
| **Cerebellar signs** | Cerebellar ataxia, pronator drift, dysdiadochokinesia, nystagmus, intention tremor, staccato speech. |
| **Extrapyramidal signs** | Cogwheel rigidity, pill-rolling tremor, bradykinesia, festinant gait. |
| **Gait disturbance** | Description by family of change to walking such as shuffling, decreased balance, unsteadiness or falls.<br>Ataxia, festinant gait, or non-specific gait disturbance. |
| **Gegenhalten** | Increased tone where the patient apparently resists examination |
| **Involuntary movements** | Tremor, myoclonus, dystonia, chorea, twitching. |
| **Oculomotor signs** | Abnormality of saccadic or pursuit movements or palsy of the third, fourth or sixth cranial nerves. |
| **Primitive reflexes** | Grasp, pout, snout or palmo-mental reflex. |
| **Pseudobulbar problems** | Upper motor neurone lesions of the $10^{th}$, $11^{th}$ and $12^{th}$ cranial nerves. |
| **Psychiatric disturbance** | Anxiety, depression, psychosis, mania. |
| **Pyramidal signs** | Pathologically brisk reflexes, Babinski sign, weakness in a pyramidal distribution, spastic hypertonicity. |
| **Speech disturbance** | Description by family of slurring, word finding difficulties or change in speech quality.<br>Dysarthria, dysphonia, dysphasia or staccato speech. |

## 3.VI Case Classification

Cases were classified according to internationally accepted diagnostic criteria[395] (Boxes 3.1 and 3.2). In order to capture as many cases as possible, those meeting criteria for probable or definite prion disease in whom *PRNP* genotyping was not performed but where a second degree relative was affected by gPD were also included. *PRNP* genotyping was not always performed, either due to cases being referred prior to this being available, or due to family wishes, or due to the lack of availability of suitable tissue or blood samples. Details of the classification of individual cases are given in the results section, as is the exact justification for including those who did not meet the diagnostic criteria for gPD. The diagnostic criteria have changed over time; the criteria given below (which were current at the time of data collection and analysis) were applied to all cases.

*Box 3.1. Classification of definite and probable genetic prion disease*

| Definite genetic prion disease |
| --- |
| Definite (neuropathologically confirmed) prion disease<br>AND definite or probable prion disease in a first degree relative.<br>OR<br>Definite prion disease and a pathogenic *PRNP* mutation. |
| **Probable genetic prion disease** |
| Progressive neuropsychiatric disorder AND<br>definite or probable prion disease in a first degree relative.<br>OR<br>Progressive neuropsychiatric disorder and a pathogenic *PRNP* mutation. |

*Box 3.2.* *Neuropathological definitions of CJD, GSSS and FFI*

| |
|---|
| Genetic CJD<br><br>A person with an affected first degree relative<br>or a disease associated *PRNP* mutation AND<br>Spongiform encephalopathy in cerebral and/or cerebellar cortex,<br>and/or sub-cortical grey matter and/or<br>Encephalopathy with $PrP^{Sc}$ immunoreactivity<br>(plaque and/or diffuse synaptic and/or patchy/peri-vacuolar types). |
| GSSS<br><br>A member of a family with dominantly inherited progressive ataxia<br>and/or dementia and one of a variety of *PRNP* gene mutations<br>and encephalomyelopathy with multi-centric $PrP^{Sc}$ plaques. |
| FFI<br><br>A member of a family with a D178N-129M *PRNP* mutation,<br>and thalamic degeneration, and variable spongiform changes in the cerebrum. |

*Box 3.3.* *Clinical criteria for probable and possible sporadic prion disease*

Probable (in the absence of an alternative diagnosis):

Progressive dementia AND at least two of the following:

❋ Myoclonus ❋ Visual or Cerebellar disturbance

❋ Pyramidal or Extra-pyramidal dysfunction ❋ Akinetic mutism

AND

An EEG showing triphasic complexes with a periodicity of one Hertz

AND/OR positive CSF 14-3-3 AND disease duration of less than two years

AND/OR high signal in the basal ganglia on MRI

Possible:

Progressive dementia AND EEG atypical or not known AND

disease duration less than two years AND at least two of the following:

❋ Myoclonus ❋ Visual or Cerebellar disturbance

❋ Pyramidal or Extra-pyramidal dysfunction ❋ Akinetic mutism

# CHAPTER 4

# RESULTS

## 4.I Prion Disease in the UK, 1970 to 2009

The number of cases of prion disease on the database of the NCJDRSU for the time period 1970 to 01/10/2009 are given below, subdivided according to the type of prion disease found or suspected. Details of case classification and the source of cases for each time period are given in the methodology section.

*Table 4.1 Prion disease cases in the UK, 1970 to 2009*

| Time period | 1970 – 1979 | 1980– 1985 | 1986 – 1990 | 1991– 2009 | 1970-2009 |
|---|---|---|---|---|---|
| **Probable gPD** | 1 | 2 | 2 | 110 | 115 |
| **Definite gPD** | 1 | 2 | 4 | 25 | 32 |
| **gPD (all)** | **2** | **4** | **6** | **147*** | **159*** |
| **Possible sCJD** | N/A | N/A | 23 | 106 | 129 |
| **Probable sCJD** | 29 | 26 | 60 | 303 | 418 |
| **Definite sCJD** | 118 | 97 | 76 | 805 | 1096 |
| **sCJD (all)** | **147** | **123** | **159** | **1214** | **1643** |
| **Possible iCJD** | N/A | 0 | 0 | 0 | 0 |
| **Probable iCJD** | 1 | 0 | 1 | 14 | 16 |
| **Definite iCJD** | 0 | 0 | 5 | 47 | 52 |
| **iCJD (all)** | **1** | **0** | **6** | **61** | **68** |
| **Possible vCJD** | 0 | 0 | 0 | 4 | 4 |
| **Probable vCJD** | 0 | 0 | 0 | 54 | 54 |
| **Definite vCJD** | 0 | 0 | 0 | 116 | 116 |
| **vCJD (all)** | **0** | **0** | **0** | **174** | **174** |
| **Total no. cases** | **150** | **127** | **171** | **1596** | **2044** |

* Not all cases of gPD identified could be classified according to the current diagnostic criteria; details are given in section 4.III. A further 12 unclassifiable cases are included in the totals marked *.

*Table 4.2 Proportion of prion disease cases with full PRNP genotyping*

| Time period | 1970 – 1979 | 1980 – 1985 | 1986 – 1990 | 1991 – 2009 |
|---|---|---|---|---|
| sCJD (all) | 147 | 123 | 159 | 1214 |
| sCJD & full *PRNP* genotyping | 0 | 0 | 0 | 561 (46.2%) |
| iCJD (all) | 1 | 0 | 6 | 61 |
| iCJD & full *PRNP* genotyping | 0 | 0 | 1 (16.7%) | 15 (24.6%) |
| vCJD (all) | 0 | 0 | 0 | 174 |
| vCJD & full *PRNP* genotyping | 0 | 0 | 0 | 143 (82.2%) |
| Total no. cases of prion disease (sporadic, variant or iatrogenic) | 148 | 123 | 165 | 1449 |
| Total no. cases of prion disease (sporadic, variant or iatrogenic) & full *PRNP* genotyping | 0 | 0 | 1 (0.6%) | 765 (52.8%) |

In total there were 2044 cases of possible, probable or definite prion disease identified in the UK from the NCJDRSU database between 1970 and October 2009, of which 159 (7.8%) were gPD.

In addition to the gPD disease cases listed above, a number of cases were identified with a potential diagnosis of gPD, but where this diagnosis could not be substantiated. Three cases had definite prion disease, with neuropathological findings of widespread microvacuolar spongiform change and linear deposition of $PrP^{Sc}$ in the cerebellar cortex. In 1 case both type 1 and type 2 $PrP^{Res}$ were present. The findings were felt to be strongly suggestive of gPD due to an insertional mutation of *PRNP*. However *PRNP* genotyping was not performed in any of these cases, and the FHx was either unknown or negative for prion disease. These cases are therefore not included in the following results.

Of those classified as having sCJD, iCJD or vCJD, just over half underwent complete sequencing of *PRNP*. The majority of those with vCJD had *PRNP* genotyping, which reflects the difficulty diagnosing vCJD, and the emphasis placed on excluding all possible alternative diagnoses. Only 46.2% of those with sCJD had full *PRNP* genotyping, which raises the possibility that there are undiagnosed

patients with gPD in this group. As described above, several individuals with neuropathological findings suspicious for gPD were seen, but without *PRNP* sequencing this cannot be confirmed.

## 4.II Pathogenic *PRNP* Mutations Identified in the UK, 1970 to 2009

The different pathogenic *PRNP* mutations found in the UK between 1970 and 2009 are given below. Where known the codon 129 status of the mutated allele is shown.

*Table 4.3 PRNP mutations found in the UK*

| ***PRNP* Mutation** | **Number of Cases** |
|---|---|
| P102L – 129 M | 32 |
| P105L – 129 V | 1 |
| A117V – 129 V | 14 |
| S132I – 129 M | 1 |
| Y163X | 2 |
| D167G – 129 M | 1 |
| D178N – 129 M | 5 |
| D178N – 129 V | 2 |
| E200K – 129 M | 34 |
| D202N – 129 V | 1 |
| V210I – 129 M | 2 |
| Q212P – 129 M | 1 |
| 2-OPRI – 129 M | 1 |
| 4-OPRI – 129 M | 10 |
| 5-OPRI – 129 M | 10 |
| 6-OPRI -129 M | 36 |
| 7-OPRI | 1 |

Details of the exact octapeptide repeat insertion were available for some of the 4-OPRI and 6-OPRI cases, but not any of the 2-OPRI or 5-OPRI cases.

### *4-OPRI Insertion Sequence*

The complete *PRNP* open reading frame was sequenced in 9 patients. DNA was unavailable for patient 9. All patients tested were shown to have an additional 4 R2 repeats, and to be homozygous for methionine at codon 129. No other sequence variations were detected. The OPRI sequence in all cases was thus:

R1-2-2-**2-2-2-2**-3-4

### *6-OPRI Insertion Sequence*

The exact insertion was known for 24 of the 6-OPRI patients, who either underwent full sequencing or were related to an individual who had undergone sequencing.

*Table 4.4* *Octapeptide repeat sequences seen in 6-OPRI cases*

| **OPR sequence (insertion in bold)** | **Number of Cases Affected** | **Codon 129 of mutated allele** |
|---|---|---|
| R1-2-2-**3-2-3g-2-3g-2**-3-4 | 2* | Unknown |
| R1-2-2-**2-3-2-3g-2-2**-3-4 | 21** | Methionine |
| R1-2-2-**2c-2-2-2-2-2**-3-4 | 1*** | Methionine |

* Cases 1 and 12

** Cases 2 to 9, 14, 17, 20, 21, 24, 25, 27, 29 to 31, 33, 34 and 36

*** Case 13

## 4.III Case Classification (1)

Each case was classified according to internationally agreed diagnostic criteria (as detailed in the methodology) as being either 'definite' or 'probable' gPD. Relatives were considered to be affected if they had definite or probable prion disease, or were described by relatives or in the medical notes as having prion disease. Relatives with neurological or psychiatric problems of uncertain aetiology were not included. Certain cases were identified which were not classifiable using the diagnostic criteria; details of which are given below:

### *5-OPRI Group*

It was not known if Case 10 was symptomatic from gPD.

### *P102I Group*

It was not known if Case 30 was symptomatic from gPD.

### *A117V Group*

Case 3 in the A117V group had neuropathologically confirmed prion disease and is a second degree relative of 2 other A117V cases. He did not undergo *PRNP* genotyping and did not have any first degree relatives with probable or definite prion disease.

Case 7 of the A117V group was described as having GSSS on the death certificate. No further details are known; she is included in the A117V group because she has the same surname as other A117V cases and is from the same geographical area.

### *Y163X Group*

It was not known if Case 2 of the Y163X group was symptomatic from gPD.

### *E200K Group*

Case 18 had neuropathologically confirmed prion disease, but did not undergo *PRNP* genotyping. There were no known first degree relatives with prion disease, but 2 first cousins (Cases 26 and 32) were found to have the E200K mutation. Case 18 is therefore currently classed as having sCJD within the NCJDRSU surveillance system.

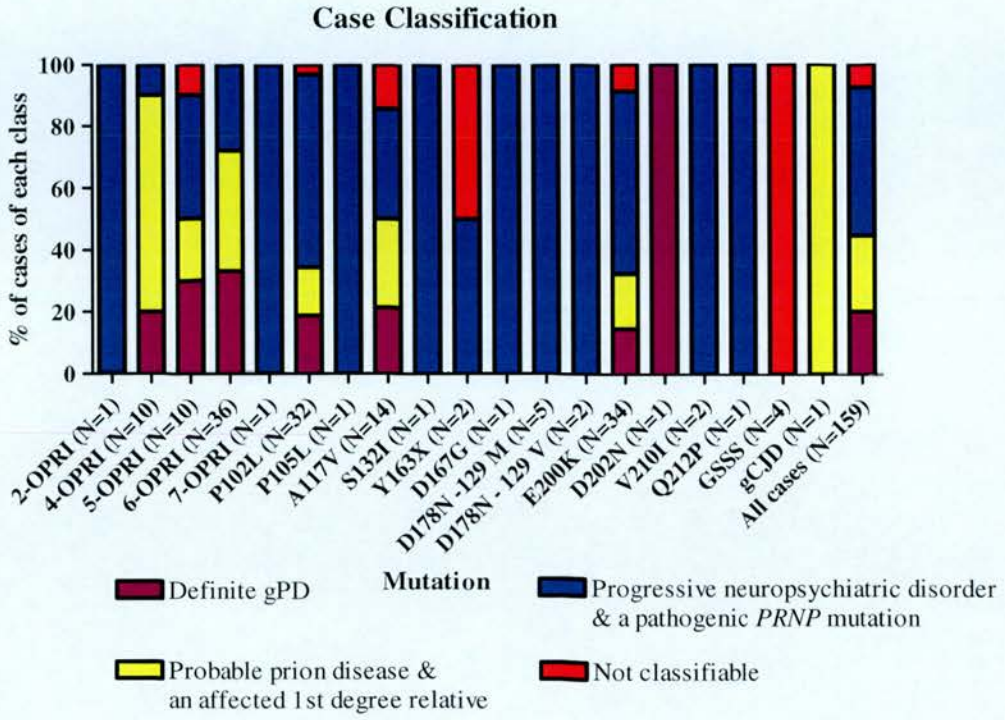
Case 19 had probable prion disease, and not undergo a PM or *PRNP* genotyping. There are no known first degree relatives with prion disease; she is a first cousin of Case 14.

Case 28 had probable prion disease, but did not undergo a PM or *PRNP* genotyping. There are no known first degree relatives with prion disease, but she is the niece of Case 27. Case 28 is currently classified by the NCJDRSU as having sCJD.

### *GSSS (PRNP mutation unknown)*

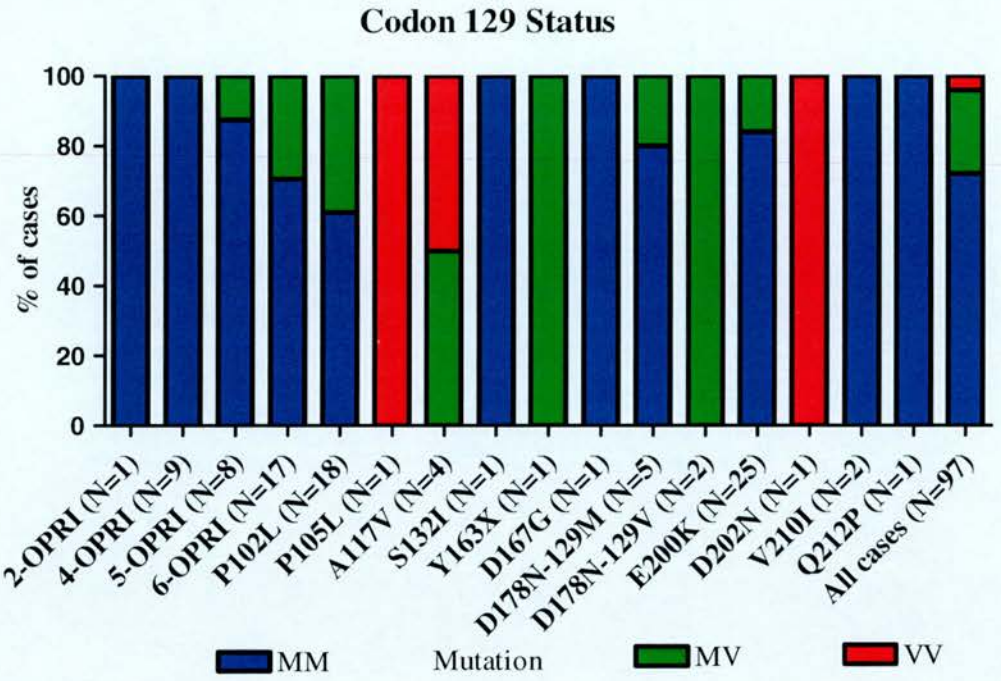
All 4 cases in this group had neuropathological evidence of GSSS with multi-centric amyloid plaques. None of the cases underwent *PRNP* genotyping and none had a FHx of GSSS. They therefore cannot be classified as having gPD according the NCJDRSU diagnostic criteria.

*Graph 4.1* *The percentage of each mutation group of each diagnostic classification*



*Graph 4.2* *The codon 129 status of each mutation group*

*The results are expressed as a percentage of those for whom this information is available (the number of cases in each group for whom this information was available is given in brackets).*



## 4.IV *PRNP* Genotyping

Not all patients underwent full *PRNP* sequencing. Some were classified as having a particular mutation based on the results of a relative(s)'s PRNP genotyping. A small number of cases were classified as having GSSS on neuropathological grounds without any genotyping data, and 1 individual was classed as having gCJD on the grounds of herself and her sister both having CJD.

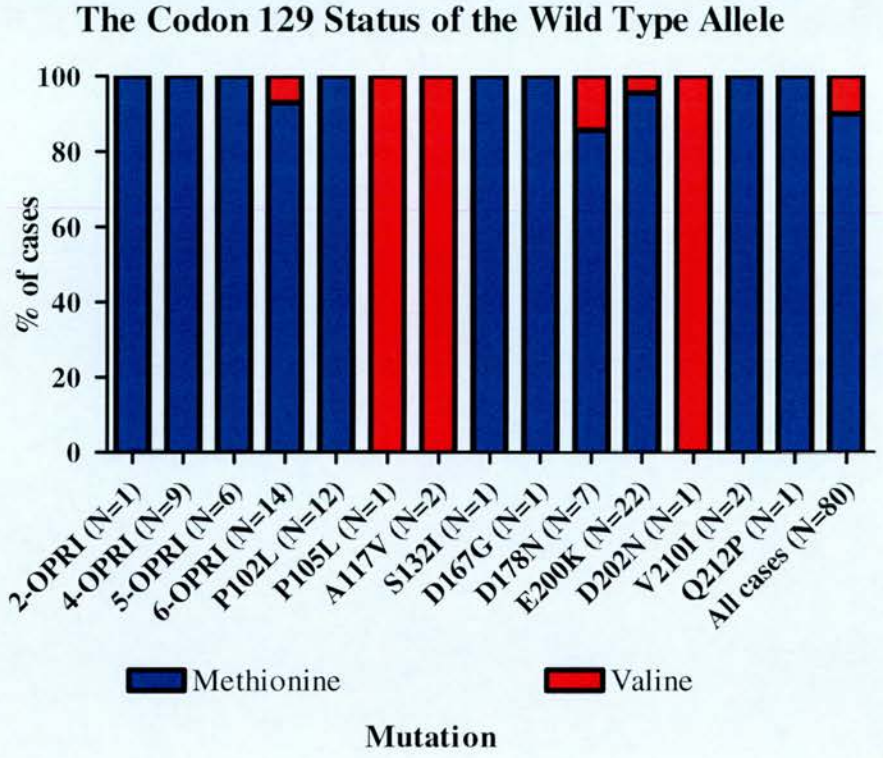
*Table 4.5 The percentage of each group for whom full PRNP genotyping data was available*

| *PRNP* Mutation | Total No. cases | % of cases with *PRNP* genotyping |
|---|---|---|
| **2-OPRI** | 1 | 100.0 |
| **4-OPRI** | 10 | 90.0 |
| **5-OPRI** | 10 | 80.0 |
| **6-OPRI** | 36 | 94.4 |
| **7-OPRI** | 1 | 100.0 |
| **P102L** | 32 | 90.6 |
| **P105L & V209M** | 1 | 100.0 |
| **A117V** | 14 | 78.6 |
| **S132I** | 1 | 100.0 |
| **Y163X** | 2 | 100.0 |
| **D167G** | 1 | 100.0 |
| **D178N – Codon 129 M** | 5 | 100.0 |
| **D178N – Codon 129 V** | 2 | 100.0 |
| **E200K** | 34 | 82.4 |
| **D202N** | 1 | 100.0 |
| **V210I** | 2 | 100.0 |
| **Q212P*** | 1 | 100.0 |
| **Genetic CJD (*PRNP* mutation unknown)** | 1 | 0.0 |
| **GSSS (*PRNP* mutation unknown)** | 4 | 0.0 |
| **All Cases** | 159 | **86.2** |

* The case with gPD due to the Q212P mutation was homozygous for this mutation.

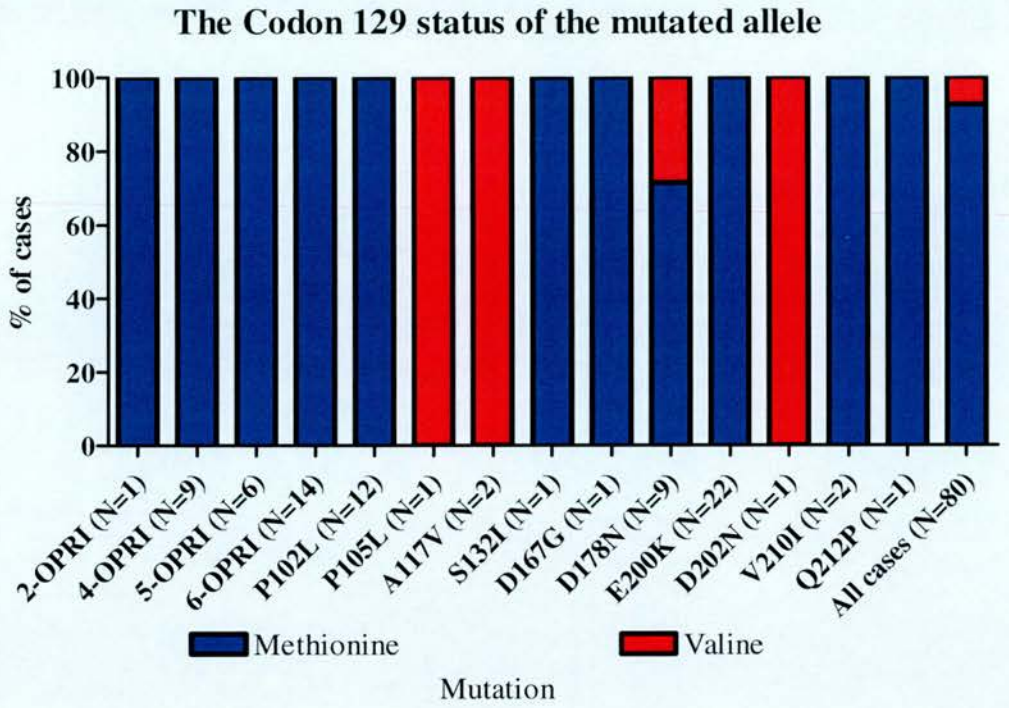
*Graph 4.3* *The PRNP codon 129 status of the wild-type allele*

*(this data was only available for a small number of cases; the total number is shown in brackets and the results expressed as a percentage of those for whom this information was known)*



*Graph 4.4* *The PRNP codon 129 status of the mutated allele*

*(this data was only available for a small number of cases; the total number is shown in brackets and the results expressed as a percentage of those for whom this information was known)*



## 4.V Other *PRNP* Polymorphisms (Other than Codon 129 & the Pathogenic Mutation)

A small number of individuals had other polymorphisms of the *PRNP* gene besides the pathogenic *PRNP* mutation and the polymorphic residue at codon 129. In all 12 such cases (8.8%) were found, details of whom are given below.

### *4-OPRI Haplotype Analysis*

In the 4-OPRI group additional analyses were performed in collaboration with D.Kaski and S.Mead of the National Prion Clinic, London.

The insertion sequence in all patients was the same; this is shown in Figure 4.1. Relatedness testing at unlinked microsatellites confirmed a mother-son relationship between patients 3 and 4 ($p = 6 \times 10^{-6}$, maximum likelihood of parent offspring 0.57). Other relatedness in this patient series, closer than second degree, were excluded by this analysis. For linked microsatellites, patients 2, 7, and 10 shared a haplotype of 2.5Mb, differing from the mother/son, and each other, at a single 3' marker (D20S194). Two further patients, 1 and 6 shared with the above 5 patients a central core haplotype spanning approximately 1.2Mb (D20S889 to D20S895). Finally, patients 5 and 8 demonstrated a shared 1Mb haplotype with those described above between D20S97 and D20S895 only, although these 2 patients shared an extended haplotype of 2Mb (D20S181 to D20S849). Overall the data was consistent with multiple (at least 2) ancestral occurrences of 4-OPRI in the UK.

rs1029273C (upstream of *PRNP*) and *PRNP* codon 129 (rs1799990) have been identified as risk factors for sCJD[253,370,288,254]. Genotyping revealed homozygosity for codon 129MM (rs1799990AA) and rs1029273CC in all 4-OPRI patients. At least 1 rs1029273C-129M haplotype would be expected in each patient if the 4-OPRI mutations had all occurred on this haplotype background. This assumption is conservative as the microsatellite genotyping suggests multiple separate events, however, the haplotype of the wild-type chromosome is independent of the presence of the mutation, and the haplotypes seen on the unaffected chromosome were not expected to be different from the control population. The

rs1029273C-129M haplotype occurs at a frequency of 0.339 in the control UK population (384/1132 UK control haplotypes). The probability that all wild-type haplotypes would be rs1029273C-129M is $P=5.9 \times 10^{-5}$ (binomial probability). rs1029273C is usually (92%) found on a 129M chromosome, but the significance of the association of rs1029273C and 4-OPRI could not be accounted for by an association driven by 129M alone (P=0.003, binomial probability, of finding that all 9 wild-type alleles were rs1029293C, with the assumption that all patients were genotype 129MM).

The clinical phenotype of 4-OPRI was compared with slightly larger insertional mutations. OPRI length had a profound effect on mean age of onset with 5- and 6-OPRI having earlier ages of onset. Mean age at onset in UK 5-OPRI patients was 42.3 in those with codon 129MM (range 26-63, SD = 12.4, $n = 6^{256}$). In UK 6-OPRI patients with 129MM mean age of onset was 31.4 years (range 20-49 years, SD = 5.7 years, $n= 30^{250}$); P=0.04 for comparison between 4-OPRI and 5-OPRI, and P<0.001 for 4-OPRI compared to 6-OPRI. Duration of disease was also highly significant (P<0.001) compared to 6-OPRI patients. However, there no significant differences were found between 4- and 6-OPRI for a number of clinical parameters, including myoclonus, cerebellar, extrapyramidal or pyramidal signs.

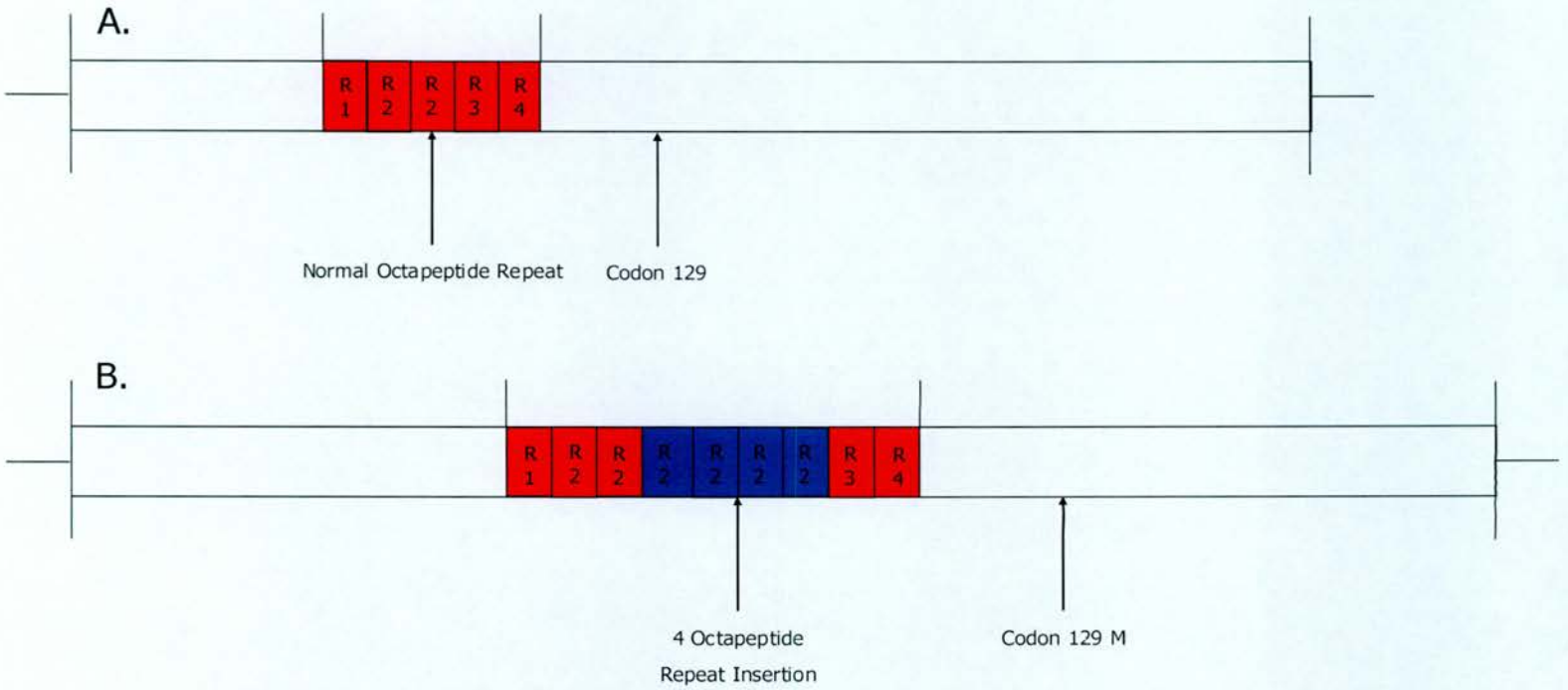



Figure 4.1 The 4 Octapeptide Repeat Insertion

A. The normal structure of the PRNP gene, with an octapeptide repeat region consisting of a nonapeptide followed by 4 copies of an octapeptide.

B. All of the cases described here had an insertion of 4 copies of the R2 octapeptide sequence and methionine homozygosity. They were also homozygous for rs1029273C, which is located outwith *PRNP*, 24,466 base pairs upstream of codon 129 (not shown)

### *P105L*

The single case with P105L mutation was also heterozygous for the V209M polymorphism.

### *A117V*

Cases 10 and 14 of the A117V group had the A117A silent polymorphism (GCA-GCG) on the wild-type allele and were G/A heterozygotes at -21nt from start of 5'UTR.

### *E200K*

Case 21 had a deletion of R34, a polymorphism found in healthy Caucasian populations, and which is not thought to influence prion disease susceptibility or clinico-pathological phenotype[31].

## 4.VI Case Demographics

The proportion of each group which was male or female is given below.

*Table 4.6 The sex distribution within each mutation group*

| *PRNP* Mutation | Total No. cases | Male (%) | Female (%) |
|---|---|---|---|
| **2-OPRI** | 1 | 1 (100.0) | 0 (0.0) |
| **4-OPRI** | 10 | 5 (50.0) | 5 (50.0) |
| **5-OPRI** | 10 | 5 (50.0) | 5 (50.0) |
| **6-OPRI** | 36 | 21 (58.3) | 15 (41.7) |
| **7-OPRI** | 1 | 0 (0.0) | 1 (100.0) |
| **P102L** | 32 | 9 (28.1) | 23 (71.9) |
| **P105L & V209M** | 1 | 0 (0.0) | 1 (100.0) |
| **A117V** | 14 | 5 (35.7) | 9 (64.3) |
| **S132I** | 1 | 0 (0.0) | 1 (100.0) |
| **Y163X** | 2 | 0 (0.0) | 2 (100.0) |
| **D167G** | 1 | 0 (0.0) | 1 (100.0) |
| **D178N – Codon 129 M** | 5 | 2 (40.0) | 3 (60.0) |
| **D178N – Codon 129 V** | 2 | 2 (100.0) | 0 (0.0) |
| **E200K** | 34 | 16 (47.1) | 18 (52.9) |
| **D202N** | 1 | 0 (0.0) | 1 (100.0) |
| **V210I** | 2 | 0 (0.0) | 2 (100.0) |
| **Q212P** | 1 | 0 (0.0) | 1 (100.0) |
| **Genetic CJD (*PRNP* mutation unknown)** | 1 | 0 (0.0) | 1 (100.0) |
| **GSSS (*PRNP* mutation unknown)** | 4 | 2 (50.0) | 2 (50.0) |
| **All Cases** | 159 | 68 (42.77) | 91 (57.23) |

Where possible the place of birth was identified from the death certificate. The distribution of cases according to geographical and ethnic origin is given below. Cases were classified as being of UK origin if they were born in the UK, or if their

origin was unknown and they were UK resident and not otherwise documented as being of non-UK origin.

Nine cases of were identified of non-UK origin based on their FHx and birthplace. Details are given below. In all this represented 5.7% of all cases.

Details of the non-UK origin cases are given on the next page. Details of the parental origin are given if they were known to be of non-UK origin; in most cases the parental origin was not known.

### *P102L Group*

Case 21 was born in Sicily and was of Italian ancestry.

### *E200K Group*

Case 4 was born in the UK but her mother was Syrian and her father a Libyan Sephardic Jew. Case 12 was a Caucasian individual born in the Republic of Ireland. Cases 21 and 30 were an Italian brother and sister. Case 23 was born in Spain and was of Spanish ancestry. Case 29 was a Caucasian individual born in Trinidad and Tobago.

### *V210I Group*

Case 1 was from the Campania region of Italian, and was of Italian ancestry. Case 2 was born in Libya (and was of Libyan ancestry).

## 4.VII Age at Onset

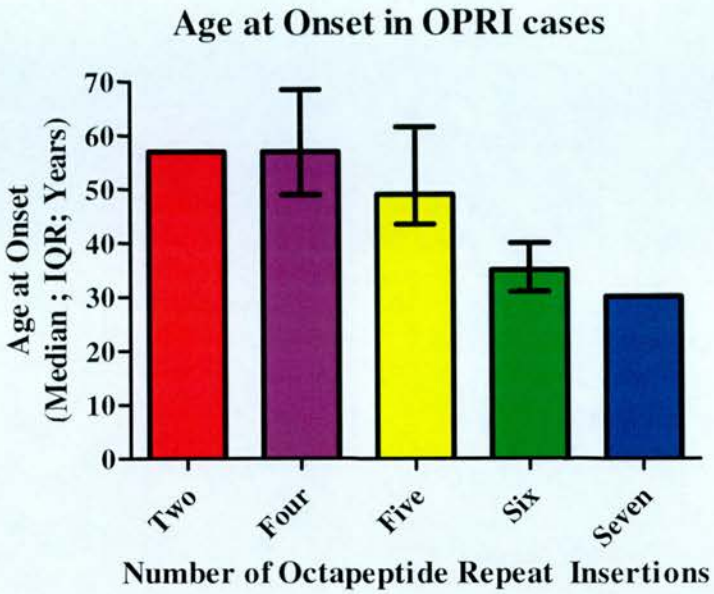
The age at onset of symptoms attributable to prion disease is given below. The median and inter-quartile range is given for groups of 5 or more individuals. Where the exact age at onset was not known an approximate value was calculated using the available information (details of how this was performed are given in the methodology section). If it was unknown if individuals were symptomatic or not they were not included. The E200K group was analysed as an entire group, and also subdivided into cases with methionine homozygosity or heterozygosity at codon 129. Therefore some E200K cases appear twice in the table. The age at onset was known or calculated for 148 cases.

*Table 4.7* *The age at onset*

*The median is given with IQR for groups of more than one individual.*

| *PRNP* Mutation | Total No. cases | No. cases with known / calculated age at onset | Median Age at onset (years; IQR) | Age at onset range (years) |
|---|---|---|---|---|
| **2-OPRI** | 1 | 1 | 57.0 | - |
| **4-OPRI** | 10 | 10 | 58.0 (52.0 ; 66.0) | 37.0 to 85.0 |
| **5-OPRI** | 10 | 9 | 49.0 (45.0; 61.0) | 41.0 to 68.0 |
| **6-OPRI** | 36 | 35 | 35.0 (31.0; 40.0) | 24.0 to 63.0 |
| **7-OPRI** | 1 | 1 | 30.0 | - |
| **P102L** | 32 | 32 | 50.0 (40.5; 57.8) | 27.0 to 65.0 |
| **P105L & V209M** | 1 | 1 | 34.0 | - |
| **A117V** | 14 | 10 | 41.0 (35.5; 42.0) | 24.0 to 45.0 |
| **S132I** | 1 | 1 | 61.0 | - |
| **Y163X** | 2 | 1 | 53.0 | - |
| **D167G** | 1 | 1 | 68.0 | - |
| **D178N Codon 129 M** | 5 | 5 | 59.0 (38.5; 62.0) | 38.0 to 62.0 |
| **D178N Codon 129 V** | 2 | 2 | 56.0 & 58.0 | 56.0 to 58.0 |
| **E200K (All cases)** | 34 | 34 | 58.5 (52.5; 65.5) | 37.0 to 77.0 |
| **E200K Codon 129 MM** | 21 | 21 | 56.0 (51.0; 60.0) | 42.0 to 70.0 |
| **E200K Codon 129 MV** | 4 | 4 | 63.0 (56.3; 66.0) | 55.0 to 66.0 |
| **D202N** | 1 | 1 | 72.0 | - |
| **V210I** | 2 | 2 | 64.0 & 61.0 | 61.0 to 64.0 |
| **Q212P** | 1 | 1 | 36.0 | - |
| **Genetic CJD (*PRNP* mutation unknown)** | 1 | 1 | 67.0 | - |
| **GSSS (*PRNP* mutation unknown)** | 4 | 4 | 55.0 (49.8 to 61.3) | 46.0 to 68.0 |
| **All Cases** | 159 | 152 | 49.0 (38.0 to 59.0) | 24.0 to 85.0 |

*Graph 4.5* *The age at onset in the OPRI cases*




The number of cases with known age at onset was as follows:

2-OPRI N = 1, 4-OPRI N = 10, 5-OPRI N = 9, 6-OPRI N = 35, 7-OPRI N = 1

## 4.VIII Disease Duration

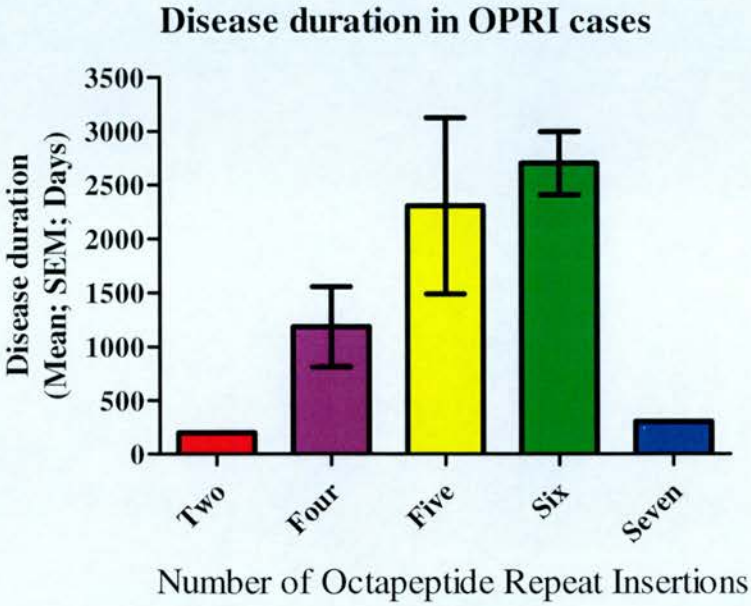
The disease duration (time from date of onset to date of death in days) is given below. The median and inter-quartile range is given for groups of 5 or more individuals. Where the disease duration was not known an approximate value was calculated using the available information (details of how this was performed are given in the methodology section). If it was unknown if individuals were symptomatic or not then they were not included. The E200K group was analysed as an entire group, and also subdivided into cases with methionine homozygosity or heterozygosity at codon 129. Therefore some E200K cases appear twice in the table.

When cases were still alive, 01/10/2009 was used as the earliest possible date of death in order to calculate the minimum disease duration. The disease duration was known or calculated for 158 cases.

*Table 4.8* *The disease duration*

| *PRNP* Mutation | Total no. cases | No. cases with known / calculated disease duration | Disease duration (days; median, IQR) | Disease duration range (days) |
|---|---|---|---|---|
| **2-OPRI** | 1 | 1 | 200.0 | - |
| **4-OPRI** | 10 | 10 | 441.0 (77.0; 2319.0) | 62.0 to 2904.0 |
| **5-OPRI** | 10 | 9 | 1038.0 (307.0; 4853.0) | 67.0 to 6258.0 |
| **6-OPRI** | 36 | 35 | 2832.0 (1200.5; 3877.0) | 43.0 to 7302.0 |
| **7-OPRI** | 1 | 1 | 305.0 | - |
| **P102L** | 32 | 32 | 1518.0 (1124.0; 2301.5 | 221.0 to 6515.0 |
| **P105L & V209M** | 1 | 1 | 3365.0 | - |
| **A117V** | 14 | 11 | 913.0 (572.0; 1197.0) | 284.0 to 1876.0 |
| **S132I** | 1 | 1 | 1382.0 | - |
| **Y163X** | 2 | 1 | 1359.0 | - |
| **D167G** | 1 | 1 | 514.0 | - |
| **D178N Codon 129 M** | 5 | 5 | 317.0 (270.5; 595.5) | 241.0 to 843.0 |
| **D178N Codon 129 V** | 2 | 2 | 717.0 & 438.0 | 438.0 to 717.0 |
| **E200K (All cases)** | 34 | 34 | 130.0 (72.0; 217.0) | 28.0 to 1952.0 |
| **E200K Codon 129 MM** | 21 | 21 | 103.0 (69.0; 150.5) | 28.0 to 589.0 |
| **E200K Codon 129 MV** | 4 | 4 | 267.5 (232.8; 408.8) | 223.0 to 454.0 |
| **D202N** | 1 | 1 | 2555.0 | - |
| **V210I** | 2 | 2 | 28.0 & 111.0 | 28.0 to 111.0 |
| **Q212P** | 1 | 1 | 2208.0 | - |
| **Genetic CJD (*PRNP* mutation unknown)** | 1 | 1 | 182.5 | - |
| **GSSS (*PRNP* mutation unknown)** | 4 | 4 | | |
| **All Cases** | 159 | 153 | 945.0 (219.5 to 2208.5) | 28.0 to 7302.0 |

*Graph 4.6* *The disease duration in the OPRI cases*




The number of cases with known disease duration was as follows:

2-OPRI N = 1, 4-OPRI N = 10, 5-OPRI N = 9, 6-OPRI N = 35, 7-OPRI N = 1.

## 4.IX The Earliest Noted Symptoms and Signs

### *P102L*

*Table 4.9 The earliest noted symptoms and signs in P102L cases*

| ***PRNP* mutation** | **Symptom or Sign** | **Number of cases affected (% of group)** |
|---|---|---|
| **P102L** | Cerebellar syndrome | 4 (12.5) |
| | Cognitive decline | 6 (18.8) |
| | Diplopia | 1 (3.2) |
| | Dizziness | 1 (3.2) |
| | Personality / behavioural change | 1 (3.2) |
| | Sensory symptoms | 6 (18.8) |
| | Seizures | 1 (3.2) |
| | Tinnitus | 1 (3.2) |
| | Tiredness | 2 (6.3) |
| | Unknown | 7 (21.9) |
| | Unsteadiness | 11 (34.4) |
| | Visual loss | 2 (6.3) |

Three of the cases with unsteadiness were described as being ataxic. 2 cases had both sensory symptoms and unsteadiness. In 1 case sensory symptoms were due to peripheral neuropathy. At onset, 22 (68.8%) of the P102L group had either cognitive decline, unsteadiness or sensory symptoms. Combinations of these features were not common.

### *P105L*

The single P105L case had unsteadiness at onset.

## *A117V*

*Table 4.10 The earliest noted symptoms and signs in A117V cases*

| ***PRNP* mutation** | **Symptom or Sign** | **Number of cases affected (% of group)** |
|---|---|---|
| **A117V** | Cognitive decline | 3 (21.4) |
| | Extrapyramidal features | 1 (7.1) |
| | Dizziness | 1 (7.1) |
| | Personality/ behavioural change | 3 (21.4) |
| | Speech problems | 2 (14.3) |
| | Tremor | 1 (7.1) |
| | Unknown | 5 (35.7) |
| | Unsteady | 1 (7.1) |

Of the cases with speech problems, 1 had slow speech (probably of an extra-pyramidal type) and 1 had dysarthria. The features at onset in the A117V were quite variable; cognitive decline and / or personality/ behavioural change were the commonest onset (5 cases, 35.7% of the group).

## *S132I*

The single S132I case had cognitive decline at onset.

## *Y163X*

In the Y163X group, the onset was unknown in 1 case and was with cognitive decline plus balance problems due to peripheral sensory neuropathy and autonomic neuropathy in the other case.

### *D167G*

The singe D167G case had cognitive decline at onset.

### *D178N-Codon 129 M*

*Table 4.11 The earliest noted symptoms and signs in D178N-129M cases*

| ***PRNP* mutation** | **Symptom or Sign** | **Number of cases affected (% of group)** |
|---|---|---|
| **D178N Codon 129 M** | Cognitive decline | 2 (40.0) |
| | Dizziness | 1 (20.0) |
| | Excessive sweating | 2 (40.0) |
| | Headache | 1 (20.0) |
| | Insomnia | 4 (80.0) |
| | Personality/ behavioural change | 1 (20.0) |
| | Tiredness | 3 (60.0) |
| | Visual hallucinations | 1 (20.0) |
| | Weight loss | 1 (20.0) |

All D178N-129M cases had either insomnia or excessive sweating at onset. All cases had more than 1 clinical feature at onset (e.g. insomnia plus tiredness plus weight loss; or excessive sweating plus tiredness plus visual hallucinations).

### *D178N-Codon 129 V*

Both of the cases with the D178N mutation and valine at codon 129 of the mutated allele had cognitive decline and low mood at onset.

*Table 4.12 The earliest noted symptoms and signs in E200K cases*

| Symptom or Sign | E200K All cases (%) | E200K Codon 129 MM (%) | E200K Codon 129 MV (%) |
|---|---|---|---|
| **Cognitive decline** | 10 (29.4) | 6 (28.6) | 2 (50.0) |
| **Diplopia** | 1 (2.9) | 1 (4.8) | 0 |
| **Dizziness** | 4 (11.8) | 4 (19.1) | 0 |
| **Gaze paresis** | 1 (2.9) | 1 (4.8) | 0 |
| **Head ache** | 1 (2.9) | 1 (4.8) | 0 |
| **Insomnia** | 4 (11.8) | 2 (9.5) | 1 (25.0) |
| **Involuntary movements (other than myoclonus) *** | 3 (8.8) | 3 (14.3) | 0 |
| **Limb weakness** | 1 (2.9) | 0 | 0 |
| **Low mood/ anxiety** | 6 (17.7) | 2 (9.5) | 1 (25.0) |
| **Myoclonus** | 2 (5.9) | 1 (4.8) | 0 |
| **Personality/behavioural change** | 9 (26.5) | 4 (11.8) | 1 (25.0) |
| **Sensory symptoms** | 3 (8.8) | 0 | 0 |
| **Speech problems** | 4 (11.8) | 2 (9.5) | 1 (25.0) |
| **Tiredness** | 2 (5.9) | 1 (4.8) | 1 (25.0) |
| **Visual disturbance / loss** | 2 (5.9) | 2 (9.5) | 0 |
| **Visual hallucinations** | 1 (2.9) | 0 | 1 (25.0) |
| **Weight loss** | 2 (5.9) | 1 (4.8) | 1 (25.0) |
| **Unknown** | 1 (2.9) | 1 (4.8) | 0 |
| **Unsteadiness** | 9 (26.5) | 6 (28.6) | 1 (25.0) |

* One case each had tremor, restless legs and chorea at onset.

The commonest features at onset were 1 or more of cognitive decline, personality or behavioural change or unsteadiness (present in 23 cases, 67.6% of the group). These were often combined with 1 or more of a variety of symptoms and signs.

### *D202N*

The single D202N case had cognitive decline at onset.

### *V210I*

Of the 2 cases of V210I, 1 had cognitive decline at onset, and 1 had tiredness and dizziness.

### *Q212P*

The single case of Q212P had unsteadiness due to gait ataxia at onset.

### *gCJD (PRNP mutation unknown)*

The single of gCJD (*PRNP* mutation unknown) had non-formed visual hallucinations at onset.

### *GSSS (PRNP mutation unknown)*

*Table 4.13* *The earliest noted symptoms and signs in GSSS (PRNP mutation unknown) cases*

| Group | Symptom or Sign | Number of cases affected (% of group) |
|---|---|---|
| **GSSS (*PRNP* mutation unknown)** | Cognitive decline | 2 (50.0) |
| | Gait & balance problems | 2 (50.0) |
| | Cerebellar problems | 1 (25.0) |
| | Low mood | 1 (25.0) |
| | Insomnia | 1 (25.0) |
| | Weight loss | 1 (25.0) |

*2-OPRI*

The single 2-OPRI case had sensory symptoms at onset.

*4-OPRI*

*Table 4.14 The earliest noted symptoms and signs in 4-OPRI cases*

| ***PRNP* mutation** | **Symptom or Sign** | **Number of cases affected (% of group)** |
|---|---|---|
| **4-OPRI** | Anxiety | 1 (10.0) |
| | Cognitive decline | 9 (90.0) |
| | Dizziness | 1 (10.0) |
| | Hypersomnolence | 1 (10.0) |
| | Myoclonus | 1 (10.0) |
| | Personality / behavioural change | 1 (10.0) |
| | Unsteadiness | 1 (10.0) |

The commonest onset in the 4-OPRI group was isolated cognitive decline.

*Table 4.15 The earliest noted symptoms and signs in 5-OPRI cases*

| ***PRNP* mutation** | **Symptom or Sign** | **Number of cases affected (% of group)** |
|---|---|---|
| **5-OPRI** | Cognitive decline | 4 (40.0) |
| | Headache | 1 (10.0) |
| | Unknown | 2 (20.0) |
| | Unsteadiness | 4 (40.0) |
| | Visual disturbance | 1 (10.0) |

Two 5-OPRI cases (20.0% of the group) had both cognitive decline and unsteadiness. The commonest onset in the 5-OPRI group was cognitive decline and/or unsteadiness.

## *6-OPRI*

*Table 4.16 The earliest noted symptoms and signs in 6-OPRI cases*

| ***PRNP* mutation** | **Symptom or Sign** | **Number of cases affected (% of group)** |
|---|---|---|
| **6-OPRI** | Cognitive decline | 26 (72.2) |
| | Cortical visual loss | 1 (2.8) |
| | Headache | 1 (2.8) |
| | Myoclonus | 1 (2.8) |
| | Paranoid delusions | 1 (2.8) |
| | Personality / behavioural change | 8 (22.2) |
| | Sensory symptoms | 1 (2.8) |
| | Seizures | 1 (2.8) |
| | Speech problems | 5 (13.9) |
| | Tremor | 2 (5.6) |
| | Unknown | 3 (8.3) |
| | Unsteadiness | 7 (19.4) |
| | Vertigo | 1 (2.8) |

Two of the cases with unsteadiness were documented as being ataxic. Two cases had both cognitive decline and unsteadiness; 5.6% of the group. One case with speech problems was documented as having dysarthria. Six cases (16.7%) had cognitive decline and personality/behavioural change. The commonest features at onset in the 6-OPRI group were 1 or more of cognitive decline, personality / behavioural change or unsteadiness.

## *7-OPRI*

The clinical features at onset were unknown for the single 7-OPRI case.

*Table 4.17 Summary of the earliest noted symptoms and signs in all cases*

| Symptom or Sign | Number of cases | % of all cases (N=159) |
|---|---|---|
| **Ataxia** | 6 | 3.8 |
| **Cerebellar syndrome** | 5 | 3.1 |
| **Cognitive decline** | 69 | 43.4 |
| **Diplopia** | 2 | 1.3 |
| **Dizziness/vertigo** | 10 | 6.3 |
| **Excessive sweating** | 2 | 1.3 |
| **Extrapyramidal features** | 1 | 0.6 |
| **Gaze paresis** | 1 | 0.6 |
| **Headache** | 4 | 2.5 |
| **Hypersomnolence** | 1 | 0.6 |
| **Insomnia** | 9 | 5.7 |
| **Involuntary movements (other than myoclonus*)** | 6 | 3.8 |
| **Limb weakness** | 1 | 0.6 |
| **Low mood or anxiety** | 10 | 6.3 |
| **Myoclonus** | 4 | 2.5 |
| **Neuropathy** | 1 | 0.6 |
| **Paranoid delusions** | 1 | 0.6 |
| **Personality/behavioural change** | 23 | 14.5 |
| **Seizures** | 2 | 1.3 |
| **Sensory symptoms** | 10 | 6.3 |
| **Speech problems** | 11 | 6.9 |
| **Tinnitus** | 1 | 0.6 |
| **Tiredness** | 8 | 5.0 |
| **Unknown** | 20 | 12.6 |
| **Unsteady** | 38 | 23.9 |
| **Visual hallucinations** | 3 | 1.9 |
| **Visual disturbance/loss*** | 6 | 3.8 |
| **Weight loss** | 4 | 2.5 |

* 1 was documented as having cortical visual loss

** 4 had tremor, 1 chorea and 1 restless legs syndrome.

Considering all cases with gPD, the findings at disease onset were variable. The commonest finding was cognitive decline, followed by unsteadiness, then by personality or behavioural change. In 20 cases (12.6%) the symptoms and signs at onset were not known.

## 4.X Symptoms and Signs Present over the Entire Illness

The following tables give details of the symptoms and signs present over the course of the illness, from onset to death. The percentages for each group were calculated using the group as a whole (i.e. including cases where the symptoms and signs present were unknown). In these tables the category 'involuntary movements' refers to involuntary movements other than myoclonus (i.e. chorea, dystonia, alien limb phenomenon etc.).

*Table 4.18* *Symptoms and signs present over the entire illness (1)*

| *PRNP* Mutation | Akinetic mutism N (%) | Autonomic problems N (%) | Cerebellar signs N (%) | Cognitive problems N (%) | Dysarthria N (%) | Dysphasia N (%) | Emotional lability N (%) | Extra–pyramidal signs N (%) | Hyper-reflexia N (%) | Hypo-reflexia N (%) | Hypo-tonia N (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **2-OPRI** | 1 (100.0) | 0 | 1 (100.0) | 1 (100.0) | 1 (100.0) | 0 | 0 | 0 | 1 (100.0) | 0 | 1 (100.0) |
| **4-OPRI** | 3 (30.0) | 0 | 3 (30.0) | 10 (100.0) | 3 (30.0) | 7 (70.0) | 2 (20.0) | 2 (20.0) | 1 (10.0) | 2 (20.0) | 0 |
| **5-OPRI** | 2 (20.0) | 0 | 3 (30.0) | 8 (80.0) | 1 (10.0) | 5 (50.0) | 3 (30.0) | 2 (20.0) | 5 (50.0) | 0 | 0 |
| **6-OPRI** | 1 (2.8) | 0 | 14 (39.0) | 34 (94.4) | 7 (19.4) | 7 (19.4) | 0 | 2 (5.6) | 11 (30.6) | 0 | 0 |
| **7-OPRI** | - | - | - | - | - | - | - | - | - | - | - |
| **P102L** | 0 | 0 | 18 (56.3) | 29 (90.6) | 16 (50.0) | 7 (21.9) | 8 (25.0) | 3 (9.4) | 10 (31.3) | 11 (34.4) | 0 |
| **P105L** | 0 | 0 | 1 (100.0) | 1 (100.0) | 1 (100.0) | 0 | 1 (100.0) | 1 (100.0) | 1 (100.0) | 0 | 0 |
| **A117V** | 0 | 0 | 7 (50.0) | 10 (71.4) | 4 (28.6) | 0 | 5 (35.7) | 5 (35.7) | 4 (28.6) | 0 | 0 |
| **S132I** | 0 | 0 | 0 | 1 (100.0) | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 0 |
| **Y163X** | 0 | 1 (50.0) | 1 (50.0) | 1 (50.0) | 0 | 1 (50.0) | 0 | 0 | 0 | 1 (50.0) | 0 |
| **D167G** | 0 | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 0 | 1 (100.0) | 0 | 0 |
| **D178N– Codon 129 M** | 1 (20.0) | 2 (40.0) | 3 (60.0) | 4 (80.0) | 2 (40.0) | 1 (20.0) | 0 | 0 | 3 (60.0) | 1 (20.0) | 0 |
| **D178N– Codon 129 V** | 0 | 0 | 2 (100.0) | 2 (100.0) | 1 (50.0) | 0 | 2 (100.0) | 0 | 1 (50.0) | 0 | 0 |
| **E200K– All cases** | 5 (14.7) | 0 | 29 (85.3) | 33 (97.1) | 17 (50.0) | 16 (47.1) | 7 (20.6) | 4 (11.8) | 17 (50.0) | 1 (2.9) | 1 (2.9) |
| **E200K– Codon 129 MM** | 4 (19.1) | 0 | 17 (81.0) | 20 (95.2) | 10 (47.6) | 11 (52.4) | 5 (23.8) | 3 (14.3) | 10 (47.6) | 0 | 1 (4.8) |
| **E200K– Codon 129 MV** | 1 (25.0) | 0 | 3 (75.0) | 4 (100.0) | 2 (50.0) | 3 (75.0) | 1 (25.0) | 0 | 2 (50.0) | 0 | 0 |
| **D202N** | 0 | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **V210I** | 2 (100.0) | 0 | 0 | 2 (100.0) | 0 | 2 (100.0) | 0 | 0 | 1 (50.0) | 0 | 0 |
| **Q212P** | 0 | 0 | 1 (100.0) | 1 (100.0) | 0 | 0 | 0 | 0 | 1 (100.0) | 0 | 0 |
| **Genetic CJD (*PRNP* mutation unknown)** | 0 | 0 | 0 | 1 (100.0) | 0 | 1 (100.0) | 0 | 0 | 0 | 0 | 0 |
| **GSSS (*PRNP* mutation unknown)** | 0 | 1 (25.0) | 2 (50.0) | 4 (100.0) | 0 | 1 (25.0) | 1 (25.0) | 2 (50.0) | 1 (25.0) | 0 | 0 |
| **All Cases** | 15 (9.4) | 4 (2.5) | 85 (53.5) | 144 (90.6) | 53 (33.3) | 48 (30.2) | 30 (18.9) | 21 (13.2) | 58 (36.5) | 16 (10.1) | 2 (1.3) |

*Table 4.19* *Symptoms and signs over the entire illness (2)*

| *PRNP* Mutation | Hyper-tonia N (%) | Involuntary movements** N (%) | Low mood/ anxiety N (%) | Muscle wasting N (%) | Myoclonus N (%) | Paratonia N (%) | Peripheral neuropathy N (%) | Personality/ behavioural change N (%) | Primitive reflexes N (%) | Pyramidal signs N (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **2-OPRI** | 0 | 1 (100.0) | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 1 (100.0) | 1 (100.0) |
| **4-OPRI** | 6 (60.0) | 7 (70.0) | 1 (10.0) | 0 | 8 (80.0) | 3 (30.0) | 0 | 3 (30.0) | 5 (50.0) | 2 (20.0) |
| **5-OPRI** | 5 (50.0) | 3 (30.0) | 2 (20.0) | 1 (10.0) | 5 (50.0) | 3 (30.0) | 0 | 3 (30.0) | 5 (50.0) | 4 (40.0) |
| **6-OPRI** | 8 (22.2) | 10 (27.8) | 5 (14.0) | 0 | 9 (25.0) | 4 (11.1) | 0 | 18 (50.0) | 7 (19.4) | 11 (30.6) |
| **7-OPRI** | - | - | - | - | - | - | - | - | - | - |
| **P102L** | 5 (15.6) | 11 (34.4) | 4 (12.5) | 1 (3.1) | 8 (25.0) | 2 (6.6) | 3 (9.9) | 10 (31.6) | 9 (28.1) | 9 (28.1) |
| **P105L** | 1 (100.0) | 1 (100.0) | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 0 | 1 (100.0) |
| **A117V** | 8 (57.1) | 6 (42.9) | 1 (7.1) | 0 | 6 (42.9) | 0 | 0 | 4 (28.6) | 3 (21.4) | 6 (42.9) |
| **S132I** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Y163X** | 0 | 0 | 0 | 1 (50.0) | 0 | 0 | 1 (50.0) | 0 | 0 | 0 |
| **D167G** | 1 (100.0) | 0 | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 0 | 1 (100.0) |
| **D178N – Codon 129 M** | 4 (80.0) | 2 (40.0) | 1 (20.0) | 0 | 3 (60.0) | 2 (40.0) | 0 | 1 (20.0) | 4 (80.0) | 3 (60.0) |
| **D178N – Codon 129 V** | 1 (50.0) | 2 (100.0) | 2 (100.0) | 0 | 2 (100.0) | 0 | 0 | 2 (100.0) | 1 (50.0) | 1 (50.0) |
| **E200K – All cases** | 28 (82.4) | 23 (67.7) | 9 (26.5) | 2 (5.9) | 29 (85.3) | 10 (29.4) | 0 | 20 (58.8) | 20 (58.8) | 21 (61.8) |
| **E200K – Codon 129 MM** | 17 (81.0) | 14 (66.7) | 1 (4.8) | 1 (4.8) | 18 (85.7) | 9 (42.9) | 0 | 12 (57.1) | 15 (71.4) | 12 (57.1) |
| **E200K – Codon 129 MV** | 3 (75.0) | 3 (75.0) | 1 (25.0) | 0 | 3 (75.0) | 2 (50.0) | 0 | 3 (75.0) | 2 (50.0) | 2 (50.0) |
| **D202N** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **V210I** | 2 (100.0) | 0 | 1 (50.0) | 0 | 2 (100.0) | 2 (100.0) | 0 | 0 | 2 (100.0) | 1 (50.0) |
| **Q212P** | 0 | 0 | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 0 | 1 (100.0) |
| **Genetic CJD (*PRNP* mutation unknown)** | 0 | 1 (100.0) | 0 | 0 | 1 (100.0) | 0 | 1 (100.0) | 0 | 1 (100.0) | 0 |
| **GSSS (*PRNP* mutation unknown)** | 2 (50.0) | 3 (75.0) | 1 (25.0) | 1 (25.0) | 1 (25.0) | 0 | 0 | 2 (50.0) | 1 (25.0) | 1 (25.0) |
| **All Cases** | 71 (44.7) | 70 (44.0) | 27 (17.0) | 6 (3.8) | 78 (49.1) | 26 (16.6) | 5 (3.1) | 63 (39.6) | 59 (37.1) | 63 (39.6) |

*Table 4.20* *Symptoms and signs over the entire illness (3)*

| *PRNP* Mutation | Seizures N (%) | Sensory symptoms /signs N (%) | Sleep disturbance N (%) | Speech disturbance N (%) | Swallowing problems N (%) | Unsteadiness N (%) | Visual disturbance /loss N (%) | Visual hallucinations N (%) | Visual or occulomotor signs N (%) | Unknown N (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **2-OPRI** | 0 | 1 (100.0) | 0 | 1 (100.0) | 1 (100.0) | 1 (100.0) | 1 (100.0) | 0 | 1 (100.0) | 0 |
| **4-OPRI** | 0 | 2 (20.0) | 1 (10.0) | 8 (80.0) | 3 (30.0) | 9 (90.0) | 1 (10.0) | 0 | 1 (10.0) | 0 |
| **5-OPRI** | 0 | 0 | 1 (10.0) | 7 (70.0) | 4 (40.0) | 8 (80.0) | 3 (30.0) | 1 (10.0) | 0 | 2 (20.0) |
| **6-OPRI** | 4 (11.1) | 2 (5.6) | 2 (5.6) | 11 (30.6) | 3 (8.3) | 27 (75.0) | 1 (2.8) | 1 (2.8) | 8 (22.2) | 2 (5.6) |
| **7-OPRI** | - | - | - | - | - | - | - | - | - | - |
| **P102L** | 1 (3.1) | 11 (34.4) | 5 (15.6) | 14 (43.8) | 7 (21.9) | 23 (71.9) | 9 (28.1) | 7 (21.9) | 14 (43.8) | 3 (9.4) |
| **P105L** | 0 | 0 | 0 | 1 (100.0) | 0 | 1 (100.0) | 0 | 0 | 1 (100.0) | 0 |
| **A117V** | 0 | 1 (7.1) | 3 (21.4) | 5 (35.7) | 0 | 8 (57.1) | 1 (7.1) | 0 | 5 (35.7) | 3 (21.4) |
| **S132I** | 0 | 0 | 0 | 0 | 0 | 0 | 1 (100.0) | 0 | 0 | 0 |
| **Y163X** | 0 | 1 (50.0) | 0 | 1 (50.0) | 0 | 1 (50.0) | 0 | 0 | 0 | 1 (50.0) |
| **D167G** | 0 | 0 | 0 | 0 | 0 | 0 | 1 (100.0) | 1 (100.0) | 0 | 0 |
| **D178N – Codon 129 M** | 0 | 2 (40.0) | 4 (80.0) | 3 (60.0) | 0 | 5 (100.0) | 1 (20.0) | 3 (60.0) | 1 (20.0) | 0 |
| **D178N – Codon 129 V** | 0 | 0 | 1 (50.0) | 2 (100.0) | 2 (100.0) | 2 (100.0) | 0 | 1 (50.0) | 1 (50.0) | 0 |
| **E200K – All cases** | 8 (23.5) | 9 (26.5) | 11 (32.4) | 24 (70.6) | 11 (32.4) | 30 (88.2) | 19 (55.9) | 13 (38.2) | 22 (64.7) | 0 |
| **E200K – Codon 129 MM** | 5 (23.8) | 6 (28.6) | 7 (33.3) | 16 (76.2) | 8 (38.1) | 18 (85.7) | 14 (66.7) | 7 (33.3) | 12 (57.1) | 0 |
| **E200K – Codon 129 MV** | 1 (25.0) | 0 | 3 (75.0) | 3 (75.0) | 1 (25.0) | 3 (75.0) | 2 (50.0) | 2 (50.0) | 2 (50.0) | 0 |
| **D202N** | 0 | 0 | 0 | 0 | 0 | 1 (100.0) | 0 | 0 | 0 | 0 |
| **V210I** | 0 | 1 (50.0) | 0 | 2 (100.0) | 1 (50.0) | 2 (100.0) | 2 (100.0) | 1 (50.0) | 1 (50.0) | 0 |
| **Q212P** | 0 | 1 (100.0) | 0 | 1 (100.0) | 0 | 1 (100.0) | 0 | 0 | 1 (100.0) | 0 |
| **Genetic CJD (*PRNP* mutation unknown)** | 0 | 1 (100.0) | 0 | 1 (100.0) | 0 | 1 (100.0) | 1 (100.0) | 1 (100.0) | 0 | 0 |
| **GSSS (*PRNP* mutation unknown)** | 1 (25.0) | 1 (25.0) | 1 (25.0) | 2 (50.0) | 0 | 3 (75.0) | 2 (50.0) | 0 | 1 (25.0) | 0 |
| **All Cases** | 14 (8.8) | 33 (20.8) | 29 (18.2) | 83 (52.2) | 32 (20.1) | 123 (77.4) | 43 (27.0) | 29 (18.2) | 57 (35.9) | 12 (7.6) |

## 4.XI Investigations: EEG analysis

All available EEG results are given below. The results are shown the number of EEGs of each type and as a percentage of the number of EEGs in each group. 61.2% (N=52) of the EEGs were reviewed in NCJDRSU. Three of the EEGs classified as showing periodic complexes were not reviewed in the NCJDRSU.

*Table 4.21 EEG findings*

| *PRNP* mutation group | Number of EEGs | Periodic complexes (%) | Non-specifically abnormal or slow (%) | Normal (%) |
|---|---|---|---|---|
| **2-OPRI** | 1 | 1 (100.0) | 0 | 0 |
| **4-OPRI** | 9 | 2 (22.2) | 6 (66.7) | 1 (11.1) |
| **5-OPRI** | 4 | 0 | 4 (100.0) | 0 |
| **6-OPRI** | 15 | 2 (13.3) | 11 (73.3) | 2 (13.3) |
| **7-OPRI** | 0 | - | - | - |
| **P102L** | 8 | 0 | 7 (87.5) | 1 (12.5) |
| **P105L** | 1 | 0 | 1 (100.0) | 0 |
| **A117V** | 4 | 0 | 4 (100.0) | 0 |
| **S132I** | 0 | - | - | - |
| **Y163X** | 0 | - | - | - |
| **D167G** | 1 | 0 | 1 (100.0) | 0 |
| **D178N – Codon 129 M** | 4 | 0 | 4 (100.0) | 0 |
| **D178N – Codon 129 V** | 2 | 0 | 1 (50.0) | 1 (50.0) |
| **E200K – All cases** | 28 | 8 (28.6) | 20 (71.4) | 0 |
| **E200K – Codon 129 MM** | 17 | 7 (41.2) | 10 (58.8) | 0 |
| **E200K – Codon 129 MV** | 3 | 0 | 3 (100.0) | 0 |
| **D202N** | 1 | 0 | 1 (100.0) | 0 |
| **V210I** | 2 | 1 (50.0) | 1 (50.0) | 0 |
| **Q212P** | 1 | 0 | 1 (100.0) | 0 |
| **Genetic CJD (*PRNP* mutation unknown)** | 1 | 1 (100.0) | 0 | 0 |
| **GSSS (*PRNP* mutation unknown)** | 1 | 0 | 1 (100.0) | 0 |
| **All Cases** | **83** | **15 (18.1)** | **63 (75.9)** | **5 (6.0)** |

## 4.XII Investigations: CSF analysis

The percentage of each group with an abnormal CSF result is given in table 4.22 (the number in each group tested is given in brackets). Not all cases underwent analysis of all CSF components, thus the numbers tested for each CSF component varied within each group. 14-3-3 was negative in 17 cases; of these 76.5% had an elevated S100b. The white cell count was known for 45 cases; it was less than 5 cells per $mm^3$ in all cases tested.

The normal values are as follows: CSF protein 0.15 to 0.45 g/l
Tau, pTau, NSE, and S100b were considered to be abnormal if they exceeded the following cut-offs: tau protein >1200pg/ml pTau-181 >120pg/ml S100b >0.41

*Table 4.22* *Results of CSF analysis for protein, 14-3-3, S100b, Tau & pTau*

| *PRNP* mutation | Protein raised (% ; N tested) | 14-3-3 positive (%; N tested) | S100b raised (%; N tested) | pTau raised (%; N tested) | Tau raised (%; N tested) |
|---|---|---|---|---|---|
| **4-OPRI** | 1 (20.0%; N=5) | 4 (80.0%; N=5) | 4 (100.0%; N=4) | 0 (0.0%; N=1) | 1 (50.0%; N=2) |
| **5-OPRI** | 2 (66.7%; N=3) | 2 (66.7%; N=3) | 3 (100.0%; N=3) | 0 (0.0%; N=3) | 2 (66.7%; N=3) |
| **6-OPRI** | 0 (0.0%; N=2) | 0 (0.0%; N=2) | 2 (100.0%: N=2) | 0 (0.0%; N=1) | 0 (0.0%; N=2) |
| **P102L** | 1 (16.7%; N=6) | 3 (60.0%; N=5) | 5 (100.0%; N=5) | 0 (0.0%; N=4) | 2 (50.0%; N=4) |
| **P105L** | 0 (0.0%; N=1) | 0 (0.0%; N=1) | 1 (100.0%; N=1) | 0 (0.0%; N=1) | 0 (0.0%; N=1) |
| **A117V** | 1 (33.33%; N=3) | 0 (0.0%; N=3) | 2 (66.67%: N=3) | 0 (0.0%; N=1) | 0 (0.0%; N=1) |
| **D167G** | 0 (0.0%; N=1) | 1 (100.0%; N=1) | 1 (100.0%; N=1) | - | 1 (100.0%; N=1) |
| **D178N – Codon 129 M** | 0 (0.0%; N=2) | 0 (0.0%; N=3) | 2 (66.7%; N=3) | 0 (0.0%; N=2) | 0 (0.0%; N=2) |
| **D178N – Codon 129 V** | 1 (50.0%; N=2) | 2 (100.0%; N=2) | - | - | - |
| **E200K–All cases** | 5 (25.0%; N=20) | 14 (82.4%; N=17) | 14 (87.5%; N=16) | 0 (0.0%; N=7) | 8 (80.0%: N=10) |
| **E200K– Codon 129 MM** | 2 (15.4%; N=13) | 8 (72.7%; N=11) | 9 (81.8; N=11) | 0 (0.0%; N=5) | 4 (80.0%; N=5) |
| **E200K– Codon 129 MV** | 1 (50.0%; N=2) | 2 (100.0; N=2) | 2 (100.0; N=2) | 0 (0.0%; N=1) | 2 (100.0%; N=2) |
| **V210I** | 0 (0.0%; N=2) | 1 (100.0; N=1) | 1 (100.0%; N=1) | - | - |
| **Q212P** | 0 (0.0%; N=1) | 0 (0.0%; N=1) | 1 (100.0%: N=1) | - | 0 (0.0%; N=1) |
| **Genetic CJD (*PRNP* mutation unknown)** | 0 (0.0%; N=1) | - | - | - | - |
| **All Cases** | **11 (22.5%; N=49)** | **27 (61.4%; N=44)** | **36 (90.0%; N=40)** | **0.0 (0.0%; N=19)** | **14 (51.85%; N=27)** |

## 4.XIII Investigations: Neuroimaging Findings

*Table 4.23 Results of MR brain imaging*

| *PRNP* mutation | N. with MRI | Positive N (%) | Normal N (%) | Atrophy N (%) | Other findings |
|---|---|---|---|---|---|
| **4-OPRI** | 5 | 0 | 1 (20.0) | 2 (40.0) | 1 (20.0%) with chronic subdural haematoma; 1 (20.0%) 'suspicious' of basal ganglia/cortical high signal |
| **5-OPRI** | 4 | 0 | 0 | 4 (100.0) | 1 (25.0%) also had spinal cord atrophy & on later imaging a chronic subdural haematoma |
| **6-OPRI** | 8 | 0 | 0 | 7 (87.5) | 1 (12.5%) foci of high signal (uncertain significance) |
| **P102L** | 8 | 0 | 3 (37.5) | 1 (12.5) | 3 (37.5%) 'suspicious' of basal ganglia /cortical high signal 1 (12.5%) non-specifically abnormal |
| **P105L** | 1 | 0 | 0 | 1 (100.0) | - |
| **A117V** | 6 | 0 | 1 (16.7) | 4 (66.7) | 1 (16.7%) atrophy & a cystic lesion in the right frontal lobe; 1 (16.7%) atrophy & foci of high signal in the basal ganglia & caudate nucleus |
| **D167G** | 1 | 0 | 0 | 0 | Poor quality images only available; non-diagnostic |
| **D178N – Codon 129 M** | 3 | 0 | 2 (66.70) | 1 (33.30) | - |
| **D178N – Codon 129 V** | 2 | 2 (100.0) | 0 | 0 | - |
| **E200K – All cases** | 25 | 12 (48.0) | 3 (12.00) | 4 (16.0) | 4 (16.0%) poor quality images which were non-diagnostic |
| **E200K – Codon 129 MM** | 16 | 8 (50.0) | 2 (12.50) | 2 (12.5) | 2 (12.50%) non-diagnostic |
| **E200K – Codon 129 MV** | 4 | 1 (25.0) | 0.00 | 2 (50.0) | 1 (25.0%) non-diagnostic |
| **V210I** | 1 | 1 (100.0) | 0.00 | 0 | - |
| **Q212P** | 1 | 0 | 0 | 1 (100.0) | - |
| **All Cases** | **65** | **15 (23.1)** | **10 (15.4)** | **25 (38.5)** | - |

23 (35.4) of all the MRIs were reviewed in the NCJDRSU. No MRI results were available for the following groups: 2-OPRI, 7-OPRI, S132I, Y163X and D202N. Definitions of 'positive' MRI findings are given in the methodology section.

### *CT Findings*

Of the 58 cases with CT brain results available, 23 (39.7 %) were normal and 23 (39.7 %) showed atrophy.

## 4.XIV Neuropathology

### *P102L Neuropathological Findings*

Neuropathological tissue was available for review in the NCJDRSU for 11 cases and the local PM report was available for a further 6 cases.

All cases were described as having either amyloid plaques, multi-centric plaques or both. Kuru-type plaques were seen in 6 cases (35.3%), neuritic plaques in 4 (23.5%), multi-centric plaques in 11 (64.7%) and amyloid plaques in 12 (70.6%). In 1 case (Case 9) there were an unusually low number of plaques seen (for a case of GSSS).

Spongiform change was seen in 16 cases (94.1%); in 7 cases (41.2%) the changes were severe, whilst in 9 cases (52.9%) it was mild to moderate, or varied between different brain regions. Neuronal loss was seen in 12 cases (70.6%); gliosis in 15 (88.2%) and atrophy in 7 (41.2%).

IHC was performed in 14 cases, all of which were positive for $PrP^{Sc}$. In all cases tested the plaques were positive for $PrP^{Sc}$. In 7 cases (50.0% of those which underwent IHC) there was additional $PrP^{Sc}$ staining seen outwith the plaques; in 3 cases this was synaptic in nature.

Additional findings were: cerebral sinus thrombosis in 1 case; occasional neurofibrillary tangles in 2 cases.

### *A117V Neuropathological Findings*

Of the A117V group, a full PM report was available for 3 cases (Cases 1, 4 and 6), and in 1 additional case (Case 4) tissue was available for review at the NCJDRSU. Brief PM summaries were available for Cases 2 and 3, which stated that the PM findings were consistent with GSSS, and similar to those of Case 1. All were concluded to have findings consistent with GSSS.

Of the 3 cases with detailed PM data available, 2 (66.7%) had spongiform change, 1 (33.3%) had neuronal loss, and 2 (66.7%) had gliosis. Atrophy was seen in 1 case (33.3%).

$PrP^{Sc}$ positive plaques were seen in all cases; in 2 cases (66.7%) these were described as being amyloid plaques, whilst in the remaining 1 case they were multicentric in nature. The distribution of the plaques was variable; in 1 case the cerebral cortex was primarily affected, whereas in the other 2 cases they were widely distributed in the cerebrum, basal ganglia, thalamus and cerebellum. In 2 cases the frontal and temporal lobes were particular affected by plaque deposition. In 1 case plaques were present in the cerebellar molecular layer. In all 3 cases the plaques were described as being confluent masses in some areas. The cerebellum was relatively spared in all cases. $PrP^{Sc}$ deposition was not seen outwith plaques.

### *S132I Neuropathological Findings*

Neuropathological tissue was reviewed at the NCJDRSU for the single S132I case.

There were multi-centric amyloid plaques, accompanied by neuronal loss and gliosis. There was severe cerebellar involvement and atrophy. There was mild spongiosis of the frontal lobes. $PrP^{Sc}$ IHC was positive. There was moderate tau positivity. The plaques were larger and more rounded than those typically seen in GSSS due to the P102L mutation. The findings were consistent with GSSS.

### *D167G Neuropathological Findings*

Neuropathological tissue was reviewed at the NCJDRSU for the single D167G case.

Widespread spongiform change, neuronal loss and gliosis were present. These changes were marked in the caudate nucleus and putamen and cerebellum (with the molecular layer of the cerebellum being the most severely affected area). No plaques were seen. $PrP^{Sc}$ deposition was present, mostly in a synaptic fashion. In the molecular layer of the cerebellum a coarse aggregated pattern of $PrP^{Sc}$ was seen. Type 1 $PrP^{Res}$ was seen. The findings were consistent with CJD.

### *D178N–129M Neuropathological Findings*

PM tissue from 3 cases was reviewed at the NCJDRSU, and a local report was available for 1 further case. The changes seen were variable. In Case 1 there was atrophy, neuronal loss and gliosis of the cerebral cortex, neuronal loss and astrocytosis in the basal ganglia, gliosis of the thalamus and hypothalamus, and in the cerebellum severe atrophy and neuronal loss, with Purkinje cell deposition in the granular layer of the cerebellum. There was neuronal loss in the dentate nucleus. In Case 2 there was marked gliosis on the thalamus, mamillary bodies, medial subiculum, caudate nucleus, putamen and inferior olives, with the thalami and mamillary bodies being worst affected. There was also mild cerebellar atrophy, and spongiform change in the anterior corpus striatum.

In Case 3 there was neuronal loss and gliosis in the cerebral cortex and basal ganglia, and a few areas of vacuolation in the temporal cortex. In Case 5 there was moderate patchy gliosis in the frontal and occipital cortex, with moderate gliosis in the cerebellum and severe gliosis in the white matter.

Overall, in 2 cases (50.0%) very mild spongiform change was seen, neuronal loss was present in 3 cases (75.0%), gliosis was present in all cases, and atrophy in 2 cases (50.0%). In only 2 cases (50.0%) was the thalamus particularly affected, and in 1 (25.0%) the olivary nuclei. The 2 cases with spongiform change were those with the longest disease duration (317 and 348 days vs. 241 and 300 days).

IHC was performed in 3 cases. In 1 case there was faint and patchy positive reaction in the thalamus; in another there were multiple, fluffy and diffuse plaques in the frontal and occipital cortex, heavy $PrP^{Sc}$ staining of numerous neurones in the cortex, and foci of synaptic and dendritic $PrP^{Sc}$ deposition in the cerebellum. In the remaining case there was no evidence of $PrP^{Sc}$ deposition.

Additionally, in 1 case there were multiple small infarctions in the occipital cortex and subcortical white matter. In 2 cases a diagnosis of prion disease was made, and in 2 cases (Cases 1 and 3) no definitive diagnosis was made.

### *D178N–129V Neuropathological Findings*

A local PM report was available for both D178N-129V cases.

Both of the D178N-129 V cases had severe spongiform change of the cerebral cortex, with neuronal loss and astrocytosis. The most severely affected areas were in Case 1 the frontal and occipital lobes, and in Case 2 the temporal lobes. In both cases the cerebellum was spared. Both had a synaptic pattern of $PrP^{Sc}$ deposition, and also $PrP^{Sc}$ plaques. In Case 1 there was a ribbon-like appearance to the $PrP^{Sc}$ deposition in the deeper cortical layers, and $PrP^{Sc}$ plaques in the cerebellum. In Case 2 there were numerous cortical amyloid plaques, heavy tau deposition and neurofibrillary tangles. Overall both cases had findings consistent with CJD, and in Case 2 co-existent Alzheimer's pathology (Braak & Braak stage VI).

## *E200K Neuropathological Findings*

Neuropathological tissue was reviewed in the NCJDRSU for 14 E200K cases; in another 6 cases a PM report was available. In 1 further case (Case 2) only a limited macroscopic report was available, with no overall diagnosis being made, and this case was therefore not included in the analysis. In all cases (except Case 2) the final neuropathological diagnosis was one of CJD.

Considering the group as a whole, all cases showed spongiform change; gliosis was seen in 15 (75.0%) and neuronal loss in 13 (65.0%). Plaques were not seen in any cases. In 16 cases 1 or more areas were described as being most severely affected. The most severely affected areas were: occipital cortex 11 cases (64.7%); frontal cortex 6 cases (35.3%); basal ganglia 5 cases (29.4%); temporal cortex 3 cases (17.7%); cerebellum 3 cases (17.6%); parietal cortex 2 cases (11.8%); hippocampus 1 case (5.9%).

IHC was performed in 15 cases; various different patterns of $PrP^{Sc}$ deposition were seen, with synaptic $PrP^{Sc}$ in 9 cases (60.0% of those tested); perivacuolar $PrP^{Sc}$ in 6 (40.0%); granular $PrP^{Sc}$ in 5 (55.6%); perineuronal $PrP^{Sc}$ in 4 (26.7%); and punctate $PrP^{Sc}$ in 1 case (6.7%). Plaque-like $PrP^{Sc}$ deposition was seen in 6 cases (40.0%). In addition 2 cases had linear $PrP^{Sc}$ deposition in the cerebral cortex; 1 of these cases also had an unusual $PrP^{Sc}$ linear staining pattern in cerebellum suggesting periaxonal accumulation of $PrP^{Sc}$. $PrP^{Sc}$ was predominantly found in the cerebral cortex (12 cases; 80.0%) or cerebellum in (10 cases; 66.7%).

Various cases had some unusual findings; Braak & Braak stage I or II tau deposition was seen in 1 case, and a few Lewy bodies were seen in the substantia nigra of 1 case. Mild cerebral arteriosclerosis was present in 1 case, and 6 had occasional plaque-like structures.

The group was then sub-divided according to the codon 129 status. PM data was available for 13 E200K Codon 129 MM cases; spongiform change tended to be widespread and to primarily affect the cerebral cortex. Some cases had severe involvement of the basal ganglia whereas in others there was none. Changes to the molecular layer of the cerebellum were seen in 7 (53.8%) cases.

Nine E200K Codon 129 MM cases underwent IHC; in all cases this was positive for $PrP^{Sc}$. There were plaque-like deposits in 3 (33.3%) of cases. Synaptic $PrP^{Sc}$ deposition was seen in 5 cases. Overall the pattern of $PrP^{Sc}$ deposition and the anatomical areas affected were variable. Linear $PrP^{Sc}$ deposition was seen in the cerebral cortex in 1 case. Molecular typing of the abnormal prion protein was performed in 3 MM cases, 2 of which were shown to have Type 1B $PrP^{Res}$. The remaining case also had type 1 $PrP^{Res}$, but with a glycoform ratio intermediate between A and B (i.e. under-representation of the non-glycosylated band).

PM data was available for 3 E200K Codon 129 MV cases. All had widespread spongiform change; in 2 cases this was predominantly micro-vacuolar. In all 3 cases the basal ganglia and granular layer of the cerebellum were severely affected by spongiform change. Two cases had a granular and synaptic pattern of $PrP^{Sc}$ deposition; 1 also had a liner pattern of $PrP^{Sc}$ deposition in the temporal, parietal, frontal and occipital cortex and granular layer of the cerebellum. Type 1 protease-resistance $PrP^{Res}$ was present in this case, with a glycoform ratio intermediate between A and B (i.e. under-representation of the non-glycosylated band).

### *D202N Neuropathological Findings*

PM tissue from the sole D202N case was reviewed at the NCJDRSU.

There were numerous amyloid plaques throughout the cerebral cortex, basal ganglia, thalamus and particularly in the cerebellum. These were associated with neurofibrillary tangles. There were vast accumulations of $PrP^{Sc}$ positive amyloid plaques in an almost linear distribution throughout the cerebral cortex and the cerebellum. There was little associated spongiform change and occasional Aβ plaques. The findings were consistent with a GSSS variant.

### *V210I Neuropathological Findings*

Neuropathological tissue from the sole V210I case was reviewed in the NCJDRSU.

There was spongiform change, in particular of the cerebral cortex and the molecular layer of the cerebellum. This was accompanied by neuronal loss and astrocytosis. IHC was positive for $PrP^{Sc}$ in a synaptic pattern in the cerebral cortex, and also in the granular layer of the cerebellum. There were no plaques.
The overall conclusion was that the changes were consistent with CJD.

### *Genetic CJD (PRNP mutation unknown) Neuropathological Findings*

A limited local PM report was available for Case 1, which stated that there was evidence of CJD and frontal atrophy.

### *GSSS (PRNP mutation unknown) Neuropathological Findings*

Neuropathological tissue from all 4 cases in this group was reviewed at the NCJDRSU; all met diagnostic criteria for GSSS, with multi-centric $PrP^{Sc}$ positive plaques being present.

Cortical spongiform change was present in 2 cases (50.0%), with gliosis and neuronal loss being present in 3 (75.0%) and 2 (50.0%) cases respectively. Multi-centric plaques were present in the cerebellum in all cases, with the molecular layer being particularly severely affected in 2 of them (50.0%); plaques were seen in the cerebral cortex in 3 cases, and in 1 case the deep grey matter was also affected. In addition to the $PrP^{Sc}$ deposition in the plaques, synaptic $PrP^{Sc}$ deposition was found in Case 6. More unusual findings were the presence of neurofibrillary tangles in Cases 6 and 7; these were in an unusual linear pattern in Case 5, and accompanied by Aβ amyloid angiopathy. In Case 7 the number of neurofibrillary tangles was small, and they were confined to the medial temporal lobe.

### *2-OPRI Neuropathological Findings*

Neuropathological tissue from the single 2-OPRI case was reviewed at the NCJDRSU.

There was severe neuronal loss, spongiform change and astrocytosis in the cerebral hemispheres. Astrocytosis was most marked in the occipital lobe, whilst spongiosis was most marked in the frontal lobe. The caudate nucleus showed severe gliosis. The cerebellum was severely affected with almost complete loss of granular cells. Type 1A $PrP^{Res}$ was present.

### *4-OPRI Neuropathological Findings*

Neuropathological results were available for 7 of the 4-OPRI cases. Tissue from Cases 1, 2, 3, 6 and 7 was reviewed at the NCJDRSU.

The overall conclusion in all cases was that the neuropathological appearances were consistent with sCJD. All cases had spongiform change and gliosis; neuronal loss was seen in 6 cases (85.7%). In 6 cases (85.7%) the cerebral cortex, cerebellum and basal ganglia were all affected. The brain stem and spinal cord were either unaffected or showed only minor involvement. In 5 cases a comment was made as to which region was most severely affected; this was variable, being the temporal lobes in 1 case, occipital and parietal lobes in 1 case, the frontal lobes in 1 case and the cerebral cortex generally in 2 cases. Three cases (42.9%) had atrophy. Additional findings were of: rarefaction of the outer portion of the dentate gyrus in Case 1; a recent focal haemorrhage in the left posterior putamen in Case 2; occasional neurofibrillary tangles in Case 3; and a chronic subdural haemorrhage in Case 8.

IHC demonstrated $PrP^{Sc}$ in all cases; the cerebellum was involved in all cases, and the cerebral cortex in 6 (85.7%); the midbrain was affected in 1 case (14.3%). The molecular layer of the cerebellum was affected in 6 cases (85.7%); in 4 cases (57.1%) this was in the form of linear $PrP^{Sc}$ deposition. The granular layer of the cerebellum was affected in 1 case (14.3%). The pattern of $PrP^{Sc}$ deposition was granular in 2 cases (28.6%), synaptic in 1 case (14.3%), plaque-like in 1 case

(14.3%), perineuronal in 2 cases (28.6%) and diffuse in 2 cases (28.6%). The $PrP^{Res}$ type was only known for 1 case; this was type 1.

### *5-OPRI Neuropathological Findings*

Tissue from case 3 was available for review in the NCJDRSU. The local PM reports for cases 1 and 5 were also available. All reports concluded that the neuropathological appearances were consistent with CJD.

All cases had spongiform change, neuronal loss, gliosis and atrophy; this involved the cerebral cortex in all cases, and the cerebellum in 2 cases (66.7%). There was patchy spongiform change and gliosis in the basal ganglia of Case 3, and minor gliosis of the thalamus of Case 1. Overall the spongiform change was mild in 1 case (33.3%) and severe in 2 cases (66.7%). The most severely affected areas were the occipital lobes in 2 cases (66.7%), and the left parietal lobe in 1 case (33.3%).

IHC was performed in Cases 3 and 5; both had $PrP^{Sc}$ deposition in the molecular layer of the cerebellum. In Case 5 the only other $PrP^{Sc}$ deposition was in the form of limited deposits in the parahippocampal gyrus; IHC for Aβ showed mild to moderate deposits, mainly in the form of plaques and linear subpial deposits. Silver impregnation showed little Alzheimer-type pathology but many axonal swellings (torpedoes) of the Purkinje cells in the cerebellum. In Case 3 there was also strong $PrP^{Sc}$ deposition around areas of confluent vacuolation in the cerebral cortex, and smaller areas of $PrP^{Sc}$ in the hippocampus, thalamus and basal ganglia.

## *6-OPRI Group Neuropathological Findings*

Tissue was available for review in the NCJDRSU for 6 cases, and local PM reports were available for an additional 10 cases; 1 of which simply stated 'definite CJD' with no further details being available. This case was therefore excluded from the analysis, giving an overall total of 15 cases with available PM data. Spongiform change was seen in 10 cases (66.7%), neuronal loss in 7 (46.7%), and gliosis in 11 (73.3%). Atrophy was seen in 3 cases (20.0%). The area which was worst affected was variable; in 4 cases (26.7%) this was the cerebellum, and in 5 (33.3%) the cerebral cortex; in the remaining cases this was not commented on. It is of note that not all cases had spongiform change of any type.

IHC was performed in 13 cases; all were positive for $PrP^{Sc}$, and all had $PrP^{Sc}$ deposition in the molecular layer of the cerebellum. Nine (69.2% of those who had IHC) had dense $PrP^{Sc}$ deposition in the molecular layer in a linear arrangement at a right angle to the pial surface. Where IHC was not performed (Cases 2 and 4) there were comments that the appearances of the cerebellum were unusual. $PrP^{Sc}$ deposition in other areas and in other patterns was seen, but this was variable. Plaque-like $PrP^{Sc}$ deposits were present in 2 cases (15.4% of those which underwent IHC).

## 4.XV The Family History

The percentage of each mutation group with a FHx of prion disease, dementia or neurodegenerative disease, or psychiatric illness is given below.
Information regarding the FHx was obtained from family members (as documented in the medical notes of the index case). In most cases it was not possible to independently verify the accuracy of this information. For the purposes of analysis, only first and second degree relatives were included. Relatives who were affected after the index case are included.

*Table 4.24* *Family history of prion disease, dementia, neurodegenerative or psychiatric illness in gPD cases*

| Mutation | Total number of cases | FHx of Prion disease N (% of group) | FHx of Dementia, neuro-degeneration or psychiatric illness N (% of group) | FHx Negative N (% of group) | FHx Unknown N (% of group) |
|---|---|---|---|---|---|
| **2-OPRI** | 1 | 0 | 0 | 1 (100.0) | 0 |
| **4-OPRI** | 10 | 4 (40.0) | 3 (30.0) | 3 (30.0) | 0 |
| **5-OPRI** | 10 | 8 (80.0) | 0 | 2 (20.0) | 0 |
| **6-OPRI** | 36 | 31 (86.1) | 3 (8.3) | 1 (2.78) | 1 (2.8) |
| **7-OPRI** | 1 | 0 | 0 | 0 | 1 (100.0) |
| **P102L** | 32 | 18 (48.4) | 9 (28.1) | 2 (6.25) | 3 (9.38) |
| **P105L** | 1 | 0 | 0 | 1 (100.0) | 0 |
| **A117V** | 14 | 11 (78.6) | 2 (14.3) | 0 | 1 (7.14) |
| **S132I** | 1 | 0 | 1 (100.0) | 0 | 0 |
| **Y163X** | 2 | 0 | 0 | 0 | 1 (100.0) |
| **D167G** | 1 | 0 | 1 (100.0) | 0 | 0 |
| **D178N – 129 M** | 5 | 0 | 4 (80.0) | 1 (20.0) | 0 |
| **D178N – 129 V** | 2 | 0 | 2 (100.0) | 0 | 0 |
| **E200K** | 34 | 19 (55.9) | 8 (23.5) | 7 (20.59) | 0 |
| **D202N** | 1 | 0 | 0 | 0 | 1 (100.0) |
| **V210I** | 2 | 0 | 0 | 2 (100.0) | 0 |
| **Q212P** | 1 | 0 | 0 | 1 (100.0) | 0 |
| **gCJD (*PRNP* mutation unknown)** | 1 | 1 (100.0) | 0 | 0 | 0 |
| **GSSS (*PRNP* mutation unknown)** | 4 | 0 | 1 (100.0) | 2 | 1 |
| **All cases** | 159 | 92 (57.9) | 34 (21.4) | 23 (14.5) | 9 (5.7) |

## 4.XVI Death Certification

127 cases were known to have died. The death certificates were available for 115 of these. The commonest causes of death are given below, followed by causes of death given in only 1 or 2 individual cases.

*Table 4.25 Causes of death documented at death certification (1)*

| Cause of death | No. Cases (% of those with available data) |
|---|---|
| **Genetic prion disease** | 41 (34.8) |
| **Prion disease (not specified as being genetic)** | 55 (46.6) |
| **Prion disease NOT listed as a cause of death** | 21 (17.8) |
| **Dementia** | 10 (8.5) |
| **Respiratory sepsis or failure** | 44 (37.3) |

*Table 4.26 Causes of death documented at death certification (2)*

| Causes of death listed for 2 cases only |
|---|
| Alzheimer's disease |
| Pulmonary embolism |
| Pulmonary oedema |
| **Causes of death listed for 1 case only** |
| Asperger's syndrome |
| Cardiac arrest due to vagal inhibition |
| Cerebellar ataxia of unknown cause |
| Familial Alzheimer's disease |
| Familial neurodegenerative disease |
| Hereditary progressive corticospinal degeneration |
| Huntington's disease |
| Immobilisation |
| Lewy Body Disease |
| Multiple cerebral accidents with cerebral degenerations |
| Neuro-degeneration |
| Steele-Richardson-Olszewski syndrome |
| Urinary tract infection |
| vCJD |

In addition in several cases had multiple types of prion disease listed as a cause of death, for example both CJD and GSSS, or both inherited prion disease and vCJD.

## 4.XVII *PRNP* Polymorphisms in vCJD

One hundred and forty seven cases of vCJD underwent *PRNP* genotyping, all of whom were methionine homozygous at codon 129. One hundred and eighteen had full *PRNP* sequencing; 5 of these had polymorphic loci other than codon 129. A synonymous (silent) polymorphism at codon 202 (GAC-aspartic acid to GAT-aspartic acid) was found in 2 cases, whilst a further 2 had a non-synonymous change at codon 219 (GAG-glutamic acid to AAG-lysine; E219K), and 1 had a 24 base pair deletion (DelR34). The cases with D202D and the individual with DelR34 had definite vCJD, whilst the E219K cases were classified as probable vCJD. The E219K polymorphism has previously been described in Asian and Pacific populations, but not in white Caucasians. The 2 patients with this genotype in this study were not of white Caucasian origin (further details about their ancestry are not known). The D202D and E219K polymorphisms were not seen in the control group.

*Table 4.27 PRNP sequence variation in vCJD cases*

| Codon | Number Tested | Genotype Data |
|---|---|---|
| 129 (Methionine/Valine) | 147 | All cases MM |
| 202 (Aspartic acid/Aspartic acid) | 118 | DD (N = 2) |
| 219 (Glutamine/Lysine) | 118 | EK (N = 2) |
| 24 base pair deletion | 118 | DelR34 (N = 1) |

Of the 307 sCJD cases screened, 59.5% were MM, 21.4% MV and 19.1% VV. Full *PRNP* sequencing found 13 cases with the silent A117A polymorphism (GCA-alanine to GCG-alanine), with 1 of these having another silent polymorphism at codon 68 (P68P). Four cases with a 24 base pair deletion in the octapeptide repeat

region were also identified. Of these 17 patients, 13 were classified as having definite and 4 probable sCJD.

In addition 1 case with a non-synonymous polymorphism at codon 167 (GAT-aspartic acid to GGT-glycine; D167G) was detected; details of this case have been given earlier in this chapter.

The frequencies of A117A and 24 base pair deletion were not statistically different from control data.

*Table 4.28 PRNP sequence variation in sCJD cases and control populations*

| ***PRNP* Variation** | **309 sCJD Patients** | **192 UK DNA Controls** | **778 Scottish Blood Donor Controls** |
|---|---|---|---|
| Codon 129 (Methionine/Valine) | MM (N=184; 59.5%)<br>MV (N=66; 21.4%)<br>VV (N=59; 19.1%) | MM (N=90; 46.9%)<br>MV (N=87; 45.3%)<br>VV (N=15; 7.8%) | MM (N=337; 43.3%)<br>MV (N=344; 44.2%)<br>VV (N=97; 12.5%) |
| Codon 117 (Alanine/Alanine) | N = 13; 4.2% | N=9; 4.7% | N=47; 6.0% |
| 24 base pair Deletion | N=4; 1.3%<br>DelR34 (N=3)<br>DelR3 (N=1) | N=1; 0.5%<br>DelR2 | N=12; 1.5%<br>All DelR34 |
| Codon 167 (Aspartic acid/Glycine) | DG (N=1; 0.3%) | No sequence data available | |
| Codon 68 (Proline/Proline) | PP (N=1; 0.3%) | | |

# CHAPTER FIVE

# DISCUSSION

## 5.I Structure of Discussion

Human gPDs are a heterogeneous group of conditions attributable to *PRNP* mutations, of which over 50 have been described. The incidence of individual mutations varies between different countries and ethnicities. To further complicate matters the clinicopathological phenotype associated with each mutation is potentially highly variable, with cases from within the same family sometimes displaying very divergent features. Furthermore, several apparently non-pathogenic polymorphisms of *PRNP* have been located, the significance of which is unclear.

The aim of this thesis was to review all cases of gPD referred to the NCJDRSU between 1990 and 2009, plus those identified in earlier epidemiological studies from 1970 to 1989. The *PRNP* mutations and polymorphisms found, the resultant clinico-pathological phenotype, investigative findings and FHx have been presented. In the discussion the incidence of gPD in the UK will be considered and compared to that seen elsewhere in the world, as will the clinicopathological phenotype seen within each different haplotype. The effect of codon 129 on gPD and the influence of other polymorphisms of *PRNP* on cases of sCJD and vCJD will also be discussed.

Finally, comments will be made on the ethical implications of *PRNP* genotyping and the challenges researchers in this field face, with a review of the current diagnostic criteria for gPD.

## 5.II The Incidence of Genetic Prion Diseases in the UK

Over the time period 1970 to 01/10/2009, a total of 2044 cases of possible, probable or definite prion disease were reported to the NCJDRSU, of which 159 could be attributed to gPD. If we consider the period 1990 to 2009 only (on the grounds that ascertainment is likely to have been incomplete in the earlier years, and the initial studies did not cover the entirety of the UK), the figures are 1596 cases of prion disease with 147 being genetic. Thus gPD was responsible for 9.2% of the cases of prion disease in the UK, with an average incidence of 7.35 cases per year, or 0.13 cases per million population per year. This is comparable to reports from countries such as Germany, France and Australia, but considerably lower than Slovakia, Hungary, Libya and Chile, where the E200K mutation is particularly common. Italy and Austria also have a higher rate of gPD; E200K-129M was responsible for most Austrian cases, whilst in Italy V210I-129M was found in the majority, with most of the remainder being due to E200K-129M[210]. The British figures reported here are therefore consistent with the general European trend in those countries not affected by clusters of the E200K mutation.

Table 1.7 in Chapter One summarised the incidence of gPD in different countries, and the proportion of prion disease cases which are reported to be genetic. These figures are taken from the available literature, and will be influenced by the degree of prion disease surveillance in different countries, and the rate at full *PRNP* sequencing of patients with suspected prion disease or undiagnosed unusual neurological conditions. Table 5.1 shows a comparison of the international data with the British figures from the current study. Tables 1.5 and 1.6 in Chapter One listed all the currently reported *PRNP* mutations (point mutations, insertions and deletions). Tables 5.2 and 5.3 highlight mutations identified in the current study.

*Table 5.1* *The incidence of genetic prion disease worldwide*

Adapted from Kovacs et al. 2005[210] with additional information from other sources. UK figures from the current study are shown in bold.

| Country | Genetic prion disease cases (% of all prion disease cases) | Incidence (per million population per year) |
|---|---|---|
| **Australia** [210] | 10.2 | 0.14 |
| **Austria** [210] | 14.4 | 0.28 |
| **Canada** [210] | 8.5 | 0.12 |
| **China**[118] | 7.8 | - |
| **France** [210] | 9.0 | 0.18 |
| **Germany** [210] | 7.6 | 0.13 |
| **Italy** [210] | 17.4 | 0.30 |
| **Netherlands** [210] | 2.1 | 0.02 |
| **Slovakia** [210] | 69.5 | 1.07 |
| **Spain** [210] | 10.3 | 0.23 |
| **Switzerland** [210] | 1.2 | 0.04 |
| **UK** | **7.35** | **0.13** |
| **Hungary**[210] | 25.60 | 0.27 |
| **Libyan Jews in Israel** [417] | - | 44.14 |
| **Japan** [270] | 15.90 | - |
| **Chile** [113] | 45.00 | - |
| **Belgium** [289] | 4.00 | - |
| **Sweden** [235] | 0.92 | - |
| **Finland** [214] | 27.27 | - |
| **Total** [210] | 10.2 | 0.17 |

*Table 5.2 PRNP point mutations identified in Britain and elsewhere*

*Those in bold were found during the current study.*

| Haplotype | Epidemiology |
|---|---|
| S97–129M | China; single case |
| **P102L-129M** | **Extensive range worldwide** |
| P102L–129M, 219K | Japan; single family |
| P102L-129V | USA/Italian; 2 cases |
| **P105L-129V** | **Britain, Japan; rare** |
| P105S-129V | N. America; single family |
| P105T-129M | E. India; 2 families |
| P105T-129V | 1 family |
| G114V-129M | Uruguay, China, S. India |
| **A117V–129V** | **Europe & N. America** |
| G131V–129M | America, Holland; 2 cases |
| **S132I-129M** | **Britain; single case** |
| Y145X–129M | Japan; single case |
| R148H–129M | Germany, N. America; 2 cases |
| Q160X–129M | Austria, N. America; 2 families |
| **Y163X (codon 129 status unknown)** | **Britain; 2 cases** |
| D167N-129M | Turkey; single case |
| N171S–129V | Brazil; single family |
| **D178N–129M** | **Europe, N. America, Australia** |
| **D178N–129V** | **Europe, N. America, Israel** |
| D178N-129V & N171S-129V | Single African-American family |
| V180I–129M | Japan, S. Korea, France, N. America |
| V180I–129 & M232R-129M | Japan; single case |
| T183A–129M | Germany, Brazil; 2 families |
| H187R–129V | N. America; single family |
| T188A–129M | Australia; single case |
| T188K–129M | Austria, Germany; 4 cases |
| T188R–129V | Germany, N. America; 2 cases |
| T193I–129M | Greece; single case |
| E196K–129M | Germany; 3 cases |
| E196K-129V | Germany; 1 case |
| F198S–129V | N. America; large kindred |
| F198V–129M | China; single case |
| **E200K–129M** | **Commonest cause of gPD worldwide** |
| E200K–129V | Europe; rare |

*Continued on next page*

*Table 5.2 (continued)*

*PRNP point mutations identified in Britain and elsewhere*

| Haplotype | Epidemiology |
|---|---|
| D202G-129V | Germany; single case |
| **D202N-129V** | **Canada, 1 family; England, 1 case** |
| V203I–129M | Italian; single case* |
| V203I-129V | Korea; single case |
| R208H–129M | China, Europe, N.America; 4 cases |
| R208H–129V | France; single case |
| R208C–129M | China; single case |
| **V210I–129M** | **China, Europe, Japan, N.America** |
| E211Q–129M | France, Italy |
| **Q212P–129M** | **Britain, Ireland, N. America; rare** |
| Q217R–129V | N. America |
| Y218N | Spain; single case |
| M232R–129M | China, Japan, Korea |
| M232T | Poland; single case |
| Y226X-129V | Holland; single case |
| Q227X-129V | Holland; single case |
| P238S | Germany; single case |

* The patient was resident in France but described as being Italian[302]

*Table 5.3* *PRNP octapeptide repeat insertion mutations identified in Britain and elsewhere*

*Those in bold were found during the current study.*

| Mutation | Epidemiology |
|---|---|
| del 24 base pair 129M | Europe; 2 cases |
| 24 nucleotide insertion | Canada; 1 case |
| 1-OPRI 129M | France, Italy; 3 cases |
| **2-OPRI (codon 129 status unknown)** | **Britain** |
| 2-OPRI 129V | N. America, Holland; 2 families |
| **4-OPRI 129M** | **Britain, Italy, Japan** |
| 4-OPRI 129V | France; single case |
| **5-OPRI 129M** | **Britain, N. America, Ukraine, Germany** |
| 5-OPRI 129V | N. America, Germany |
| **6-OPRI 129M** | **Europe, Japan, N. America** |
| 6-OPRI 129V | France; single case |
| **7-OPRI (codon 129 status unknown)** | **Britain** |
| 7-OPRI 129M | N. America (single family) & Japan (single case) |
| 7-OPRI 129V | Holland |
| 8-OPRI 129M | France; single family |
| 8-OPRI 129V | France, Holland |
| 9-OPRI 129M | England, Germany, 2 families |
| 12-OPRI | N. America; single family |

In all, 15 different *PRNP* mutations with 16 different haplotypes were found in the UK, with 3 mutations (6-OPRI, E200K and P102L) being responsible for 64.2% of all cases. It is noteworthy that while E200K is the commonest cause of gPD in most of the world, in the UK 6-OPRI has caused the highest number of cases. The majority of 6-OPRI cases belonged to a single family in the South of England, which has been extensively studied[250]. Given the presence of this large family, it is somewhat surprising that the overall rate of gPD in the UK is not higher, particularly in comparison with countries such as Finland, where 27.3% of all cases of prion disease have been reported to be genetic in nature, due to one large family with the D178N mutation. This could be explained by under-ascertainment of gPD in the UK, by excellent ascertainment of other, non-genetic forms of prion disease, by a

combination of the two factors, or by natural variation in the de novo *PRNP* mutation rate and penetrance of different mutations. Another possibility is that the background level of mutations other than 6-OPRI is lower in the UK than elsewhere.

Of all the cases classified as prion disease over the study period, 41.7% were subject to genotyping to seek *PRNP* mutations. It is therefore entirely possible that some cases of gPD were misclassified as sCJD. Information on the rate of genotyping performed in other countries is sparse; the 2005 EuroCJD study cited the Netherlands as having the lowest rate of *PRNP* genotyping at 31.7%[210]. It is likely that the UK is at least on a par with other European countries, with genotyping targeted at those felt to be at high risk (either based on FHx, ethnicity or clinicopathological phenotype) rather than applied in a blanket fashion.

All the commoner *PRNP* mutations (E200K, P102L, D178N, V210I) were represented in the UK, although the relative scarcity of D178N and V210I compared to elsewhere in Europe is notable, as is the fact that both cases of V210I were in fact of non-UK origin, with one being Italian and one Libyan. In the E200K group there were no cases in persons from Slovakia or nearby countries, but there was one affected individual of Libyan Sephardic Jewish descent, two affected (and related) Italians and one Spanish case. In the Italian and Spanish cases there was a strong FHx of gPD, unlike the majority of the E200K cases of British ancestry. Whether or not this reflects differences in the penetrance of the E200K mutation between different families, or merely the combined effects of larger family size and chance, is uncertain.

### *5.II.a Cases of gCJD and GSSS Where the PRNP Mutation was Unknown: gCJD*

The second case identified during retrospective case ascertainment of prion disease during the period from 1970 to 1979 was labelled as having gPD; the patient had a 6 month long illness characterised by dementia, cortical visual disturbance and myoclonus, triphasic periodic complexes on EEG and evidence of CJD on neuropathological examination. There was a history of her sister having died following a 'similar illness', but no further information was recorded. Genotyping had

not been developed at the time of this patient's death, and no frozen tissue was stored. The final diagnosis is therefore unclear; the clinicians involved at the time diagnosed a hereditary problem. It was felt that this case should be included in the current study for completeness, and to highlight the difficulties surrounding the analysis of older cases.

### *5.II.b Cases of gCJD and GSSS Where the PRNP mutation was unknown: GSSS*

Four cases were found with neuropathological features of GSSS, namely multi-centric amyloid plaques, but for whom *PRNP* genotyping was not performed. Three also had neurofibrillary tangles, which in 1 formed an unusual linear pattern. They all also displayed a clinical picture which would be consistent with this diagnosis. However as the FHx was negative or unknown, these individuals cannot be definitively diagnosed with GSSS. In addition to a simple lack of information, there are a number of reasons why the FHx may have been uninformative: de novo mutations in the proband; the deliberate concealment of previously affected relatives; or non-paternity. Clinicians are therefore left with a quandary; the neuropathological features strongly point towards a dominantly inherited, fatal neurodegenerative disease, but this diagnosis cannot be confirmed. What should the family, in particular blood relatives, be told and should they be offered pre-symptomatic testing? In such circumstances specialist genetic counselling will be particularly invaluable, but the risk analysis of the public health risk posed by relatives in such cases will remain very difficult, and a precautionary approach would be advisable. Restricting the provision of full information to the family because of the diagnostic uncertainties is probably not an acceptable approach.

## *5.II.c Death Certification*

A review of the death certificates of deceased individuals highlighted the variable terminology used by the medical profession generally, and the frequent lack of accurate documentation. Descriptions such 'Jakob-Creutzfeldt disease' were found, and 1 patient was labelled as having both CJD and GSSS. Only 34.8% of patients were certified as having gPD, and in 17.8% no form of prion disease was mentioned. A wide variety of diagnostic labels from Asperger's syndrome to vCJD were listed. This partly reflects the difficulties in making the diagnosis of prion disease during life, but also demonstrates the inaccuracy of death certification generally, and possibly a reluctance on the part of the physician to document the diagnosis for fear of social stigmatisation of the family. This may cause public health problems if the family are not fully informed of their own at-risk status and precautions needed regarding surgical procedures and blood donation.

## 5.III Codon 129 & other *PRNP* polymorphisms

### *5.III.a The Effect of Codon 129 on Genetic Prion Disease*

The polymorphic residue at codon 129 of *PRNP* gene is potentially an important determinant of disease susceptibility and phenotype in sporadic and iatrogenic human prion diseases. Methionine homozygotes are more susceptible to sCJD, vCJD and iCJD, and of the gPD cases tested here 72.2% possessed this genotype. Around a quarter were heterozygotes, with valine homozygosity being very rare (4.1% of those tested). This is in line with previous European reports[210]. In the vast majority of cases tested, the mutated allele carried methionine at codon 129 (although this data was only available for a small number of individuals). When considering all the pathogenic mutations reported worldwide, the majority occur in conjunction with methionine on the mutated allele (including the top 4 commonest mutations). It would seem logical to postulate that many more individuals with *PRNP* mutations and heterozygosity at codon 129 exist, but either do not manifest disease or do so at such an advanced age as to not present to the attention of prion disease surveillance teams. Testing this hypothesis is more difficult, as large scale screening of asymptomatic individuals for an autosomal dominant, untreatable, fatal condition is clearly an ethical minefield. It may well be that the presence of valine somehow stabilises the structure of $PrP^{C}$ and reduces or nullifies the spontaneous conformational change to $PrP^{Sc}$ which presumably occurs in cases of gPD. Valine at codon 129 is less likely to be associated with pathogenic *PRNP* mutations (although it is entirely possible that those with valine at codon 129 and *PRNP* mutations simply do not develop any prion disease and are therefore not captured by surveillance mechanisms).

## 5.III.b *PRNP Polymorphisms other than Codon 129 in Sporadic and Variant CJD*

Analysis of *PRNP* in sCJD, vCJD and controls has demonstrated genetic variation in vCJD that is not seen in sCJD. Full *PRNP* sequencing of 118 confirmed UK vCJD patients found 5 with polymorphisms: 2 with E219K, 2 with D202D, and 1 with a 24 base pair deletion (del R34).

D202D is a synonymous polymorphism, with no impact on the amino acid sequence of the prion protein, and no effect on protein folding kinetics. It has been seen in isolated cases of sCJD and iCJD, but its frequency in healthy populations is unknown. It may be that it has an as yet unknown effect on vCJD susceptibility, but further investigation will be required to test this hypothesis.

E219K is a non-synonymous, miss-sense polymorphism causing a change from glutamic acid (E) to lysine (K). This has not seen in Western European populations, but is relatively common in Japan and Korea[340,188], where it has been reported to be protective against sCJD, and a modifying factor when associated with the P102L mutation[104]. E219K did not cause any detectable alteration to the clinicopathological phenotype of the 2 vCJD cases in whom it was found. Both patients were of non-Caucasian origin, and may therefore come from populations where this polymorphism is common. However a transgenic mouse model does suggest that lysine at codon 219 renders $PrP^{C}$ more susceptible to conversion to $PrP^{Sc}$.

The 24 base pair deletion influences the metal binding domain of the $PrP^{C}$ N-terminus, but it is not thought to affect prion disease susceptibility or phenotype. This is based on their occurrence in healthy controls, sCJD and vCJD patients at similar frequencies.

## 5.IV Family Histories

The 159 patients with gPD could be segregated into 90 separate families. Twenty four families contained more than 1 individual with gPD; 35 families had only 1 affected individual with a diagnosis of gPD but a positive history for neurodegenerative, dementing or psychiatric illness; and 31cases had a negative or unknown FHx. Interestingly, whilst in the E200K group 25 separate families existed, in the 6-OPRI group there were only 5. This supports previous research demonstrating a common founder for the British 6-OPRI cases in the South-East of England in (at the latest) the 18$^{th}$ century, whilst the E200K mutation is likely to have arisen on multiple different occasions. The presence of multiple unrelated families in the UK with the E200K mutation supports the theory that this is probably a mutational hotspot.

Considering the cases as a whole, only 19.5% had no known FHx of any form of gPD, dementia, neurodegenerative or psychiatric illness. In over half there was a history of some form of prion disease, in most instances gPD. It was often difficult to obtain any level of detail regarding a relative's illness, and untangling ancestors with common forms of age-related dementia from those with occult prion disease was frequently not possible. It has previously been reported that a FHx of dementia is significantly commoner in those with sCJD than in healthy, age-matched controls[216]. Krasnianski et al. also found those with sCJD and a positive FHx to have a higher incidence of the Apo E4 allele than those with sCJD and a negative FHx[216]. As the presence of the Apo E4 allele is a significant risk factor for Alzheimer's disease, this could explain the increased rate of relatives with dementia seen. In the present study, it is likely that there is an element of ascertainment bias, as all the family trees were subjected to extensive scrutiny, resulting in an increased rate of positive histories. If a history of prion disease is removed from consideration, we are left with 23.3% of individuals having a FHx of some other form of dementia or neurodegenerative disease; still a doubling on that found by Krasnianski et al. in sCJD patients, but this could be explained by the more detailed history taken here, or by the inclusion of second degree relatives.

## 5.V Investigative Findings in Genetic Prion Disease

### *5.V.a Electroencephalogram Findings*

Only a very low incidence of EEG periodic triphasic complexes was seen, with 18.1% (15 out of 83) of those tested having this feature. Interestingly, all bar 2 of the positive findings were in those with E200K or an OPRI. None of the mutations associated with neuropathological appearances of GSSS were shown to have periodic complexes. This trend follows the results of previous studies[210,214], although the incidence of periodic complexes tended to be much lower in the current group. This may reflect more stringent EEG classification criteria, or possibly when the investigation was performed. EEGs were reported as being normal in only 5 individuals, 4 with an OPRI and 1 with D178N-129V. In 4 cases it was possible to calculate when the EEG was performed relative to the disease duration; 2 were performed less than half way through the illness and 2 were carried out late in the illness (91.7% and 83.4% of the total illness duration). Of these EEGs only 1 was reviewed as the NCJDRSU.

### *5.V.b Cerebrospinal Fluid Analysis*

Analysing the diagnostic utility of CSF proteins in gPD is difficult owing to the large number of different haplotypes, and the small amount of data available. In the present study 14-3-3 results were known for 30.0% of cases, S100b for 27.2%, pTau for 13.7% and Tau for 18.4%. The CSF total protein level was available for 33.3% of cases; whilst it was modestly elevated in 22.5% of those tested, only 2 cases had a level greater than 1.0g/l. The marker most sensitive for gPD was S100b, which was elevated in 90.0% of those tested, whilst 14-3-3 was positive in 61.4%. Just over half of those analysed had a raised Tau, but pTau was not positive in any case tested (N = 20). Of those who had a negative 14-3-3 the majority (76.5%) had an elevated S100b, whilst all of those with a normal S100b were also negative for 14-3-3.

Further analysis of these results according to mutation group is problematic owing to the small groups sizes; it is notable that positive 14-3-3 and elevated S100b were seen in most of the different haplotypes tested. Previous authors have attempted to compare the results seen in FFI, GSSS, OPRI and gCJD[210,224]. There are problems with this approach: firstly the classification of cases partly according to mutation (i.e. FFI or OPRI), partly according to neuropathological appearance (GSSS) and partly by default (gCJD encompassing all other haplotypes) which will lead to considerable group heterogeneity. One recent study[224] found CSF biomarkers to be elevated in gCJD but not in GSSS or FFI. I did not find positive 14-3-3 in the FFI cases tested, but 2 of the 3 tested did have a elevated S100b. Looking at the P102L and A117V groups together, only 37.5% had a positive 14-3-3 but 87.5% had an elevated S100b.

The only gPD groups for whom reasonably sized data sets are available are E200K, V210I and FFI[224,262]. A high level of 14-3-3 and S100b sensitivity is reported for E200K and V210I, but not for FFI (although the largest group tested for S100b only comprised 10 patients[224]). Very little data is available on how the codon 129 status of the wild type allele influences CSF biomarkers. There is also the possibility that the pathogenic *PRNP* mutation may interact with other polymorphic residues and thus give rise to different CSF results in different families or ethnic groups.

Overall, 14-3-3 and S100b are useful tests when investigating possible cases of gPD, but further large scale collaborative studies will be needed to unpick how their utility varies between different mutation haplotypes. Compared with sCJD, S100b is more sensitive in cases of gPD and 14-3-3 less sensitive. In particular the pattern of a negative 14-3-3 with an elevated S100b should raise the possibility of gPD.

### *5.V.c Neuroimaging Findings*

The typical MRI features of basal ganglia and cortical high signal were not frequently seen in gPD. In fact, only a handful of E200K, D178N-129V and V210I cases showed these changes, representing 23.1% of the 65 MRIs reviewed. A much higher rate of typical MRI features has previously been reported in E200K[101], and they have also been observed in a smaller percentage of OPRI, FFI, P102L and

A117V cases[210]. All neuroimaging was performed in the patient's local centre for diagnostic purposes, with a variety of MRI scanner types and sequences used. The quality and type of images was not always ideal, with a number being non-diagnostic due to movement artefact. It is likely that if the same group of patients were imaged in a research setting the 'hit rate' of typical MRI changes would be higher, but the data shown here will more accurately reflect the results available to clinicians in the real world. Atrophy was seen in just over a third of patients, whilst 15.4% had normal neuroimaging. These findings should not dissuade a physician from a diagnosis of gPD, whilst a typical MRI can be supportive. It is notable that the pulvinar high signal associated with vCJD was not seen in any case.

### *5.V.d Summary of Investigative Findings in Genetic Prion Disease*

Overall the pattern of investigative findings appears to vary somewhat according to the underlying mutation. EEG and MRI are less sensitive than in sCJD, although it was unusual to see a normal EEG. CSF biomarkers are helpful, with S100b being more sensitive than 14-3-3, the reverse of the pattern seen in sCJD. It would be difficult to clearly distinguish gPD from sCJD based on investigative finding. In cases of unusual neurodegenerative illness with non-specific investigative findings, *PRNP* genotyping is required to exclude a diagnosis of gPD.

## 5.VI Comparison of Each Mutation Group Seen in the UK with Findings from Elsewhere in the World

### *5.VI.a Octapeptide Repeat Insertions: Two-Octapeptide Repeat Insertion*

Two-OPRI is a rare *PRNP* mutation, the only previous reports of which are in 1 North American family and 1 Dutch family. In the present study 2-OPRI was found in a British male who developed symptoms in early middle age. Of note is the unusual mode of symptom onset, which was with progressive paraesthesia of the

right hand and arm. In other respects the clinico-pathological phenotype resembled that of sCJDMM1, with $PrP^{Res}$ type 1A apparent at PM. The British patient was methionine homozygous; the codon 129 status of the North American family was not reported, whilst the single affected Dutch patient was valine homozygous. It seems likely that this was a de novo mutation, given its overall rarity and the lack of a suspicious FHx in the British case. Whether or not the codon 129 status affects the phenotype seen is difficult to say given the paucity of genetic and clinical data available.

### *5.VI.b Four-Octapeptide Repeat Insertion*

All of the 4-OPRI patients were homozygous for methionine at codon 129 and for the previously described sCJD risk allele, rs1029273C[253]. This significant finding suggests that the presence of the 4-OPRI mutation alone may be insufficient for disease manifestation within a natural lifespan. Rather, this clinical disorder is more likely to manifest in the presence of a mutation and additional genetic risk factors. These additional factors being absent from relatives could account for the reduced penetrance of the mutation indicated by a relatively low frequency of familial concurrence of disease in these pedigrees. Whilst this possibility might have been confirmed by genetic testing of healthy elderly relatives of 4-OPRI probands, there would be major implications for the families and this was not possible.

The potential effect of *PRNP* codon 129 heterozygosity on susceptibility to and/or incubation time is well documented in sporadic, acquired[254,42] and some forms of inherited prion disease[377]. In 5-OPRI, 6-OPRI, P102L and A117V, *PRNP*-129 heterozygosity is associated with an older clinical onset compared with homozygosity[377]. One theory of prion disease susceptibility is that it is conferred at a molecular level by homotypic protein-protein interactions with an important role for the degree to which the wild-type and mutant prion proteins can adopt the same pathogenic conformations[67]. Given that 4-OPRI-129MM has an older clinical onset than many other gPD it is reasonable to predict that the age of onset of 4-OPRI-129MV might lie beyond the average human lifespan. This would explain the absence of 129MV patients in the present cohort. Whilst this explanation is plausible,

the kinetics of prion replication are not well understood, and it is also possible that the presence of the 129V on the wild-type allele prevents the stable generation of prions in 4-OPRI.

The analyses support the concept of variable penetrance of the 4-OPRI mutation that is dependent upon more than just homology between mutant and wild-type PrP at codon 129. Additional factors upstream of *PRNP* presumably act by altered expression of wild-type PrP. Increased PrP expression has been known for many years to be a potent susceptibility factor in transgenic mice[44]. It was assumed that the rs1029273C-129M haplotype on the mutant chromosome was shared between all patients because of common ancestry. This conservative assumption was only partially supported by the haplotype analysis, which was in fact most consistent with several different mutational events. In this latter scenario, the observation that all mutant alleles were also associated with the rs1029273C-129M haplotype lends further support to the increased susceptibility conferred by this haplotype.

4- through 5- to 6-OPRI shows a dramatically earlier clinical onset. Insertional mutations larger than 5-OPRI show similar ages of clinical onset[251]. Thus a correlation between insertion size and clinical phenotype is clear over this short range, and not for shorter or longer insertions. These clinical observations may form the basis for an informed correlation between molecular properties of mutant prion proteins and the consequences for the onset and clinical phenotype of the human disease.

Epistasis is a term used to describe an interaction between different loci, and has recently received a great deal of interest in complex disease genetics. There are no reported examples of neurological diseases caused by a mutation associated with a common genotype at additional susceptibility polymorphisms. Despite a huge effort in exploring genome-wide associations, common susceptibility polymorphisms have been hard to identify in neurodegeneration, leading to speculation that rare high risk alleles may be generally more significant. This 4-OPRI case series may thus provide a useful precedent for understanding how a rare high-risk mutation might typically manifest as a sporadic neurodegenerative disease.

The IHC in one case showed a distinct pattern from sCJD, with a very delicate staining of axons and dendrites, rather than synaptic or plaque-forming

deposits seen in sCJD. The cerebellum also showed prion protein deposits which seem to follow the dendrites of the Purkinje cells, in that they extend perpendicularly to the surface, giving the cerebellum a "tigroid" appearance. Remarkably, there was frequent deposition of hyperphosphorylated tau in several cortical areas, even with a very subtle involvement of the cerebellum. Although this tau pathology may have arisen independently from the prion disease, the role of the prion disease in triggering hyperphosphorylation and a secondary tauopathy remains likely, in particular, as no deposition of amyloid beta was seen in any of the brain regions examined.

In summary, this is the first demonstration of the requirement of inheritance of a susceptibility allele in association with an OPRI mutation for manifestation of a human prion disease. Whilst the molecular basis of the susceptibility allele is unknown, one possibility remains that it is linked to homology at codon 129 and higher levels of expression of *PRNP,* although this is yet to be demonstrated. This example of epistasis may provide a valuable model for the understanding of sporadic neurodegenerative disease in man.

### *5.VI.c Five-Octapeptide Repeat Insertion*

The majority of those affected by the 5-OPRI mutation belonged to a single family based in Wales, which contained at least 7 affected individuals (and a further 11 relatives with diagnoses such as death at 32 years from 'creeping paralysis'). In all 5 generations were involved, with multiple cases in each generation bar the first. Assuming the first generation to contain the founding mutation, this instance of the 5-OPRI mutation arose in the mid-19$^{th}$ century, and appears to have a high penetrance. Unfortunately the exact insertion sequence present is not known for this family. In addition 3 other apparently unrelated individuals were found, two of whom had a negative FHx. The remaining case[256] had a parent described as having died of Pick's disease.

Overall the phenotype was typified by cognitive decline and/or gait disturbance at onset, with these features appearing in the majority as the disease progressed, accompanied by myoclonus, pyramidal signs and dysphasia. These

features are consistent with previous reports, a surprising finding given that the actual repeat structure shows considerable heterogeneity between different families. It has previously been hypothesised that the codon 129 status influences disease susceptibility and age at onset, with MV cases having a later age at onset. There was only one such case found in the current series, the age at onset for whom (49 years) was rather lower than that of the MM cases (who had a median age at onset of 55 years). However it is not possible to draw firm conclusions based on such a small sample size, and the paucity of MV affected individuals is certainly supportive of the idea that valine at codon 129 of wild type allele prevents or delays disease onset.

The investigative findings reported here add significantly to the world literature on 5-OPRI; no individuals with periodic complexes on EEG were seen, nor were basal ganglia high signal or cortical ribboning seen on MRI in any case. However on CSF analysis positive findings were made, with the majority having positive 14-3-3, elevated S100b, tau and total protein. All of the cases which came to autopsy had clear spongiform change, gliosis and atrophy of the cerebral cortex, with the cerebellum also being involved in the majority. Of the 2 which underwent IHC, both showed abnormal PrP in the molecular layer of the cerebellum, with one having plaques and linear subpial deposits. Overall a consistent picture emerges of the effect of a 5-OPRI, which does not appear to be significantly modified by the exact repeat composition. However the small number of cases known does limit the strength of this conclusion; nevertheless it is in keeping with the findings of previous authors.

### *5.VI.d Six-Octapeptide Repeat Insertion*

The main source of the 6-OPRI is postulated to be a person living in the 18$^{th}$ century in the South-East of England, amongst whose descendents at least eighty six cases of 6-OPRI have been described in detail (the 'Brain kindred' [250]). It is not surprising therefore, that most of the cases reported to the NCJDRSU belonged to this family. Only 3 individuals could be said to definitely not be related to this kindred; namely 2 brothers with a different insertion (R3-R2-R3g-R2-R3g-R2 as opposed to the Brain kindred's R2-R3-R2-R3g-R2-R2) and one female with an R2c-R2-R2-R2-R2-R2 insertion. On comparing the 'Brain' and 'non-Brain' cases, no

marked differences in clinical phenotype stand out; the typical pattern of dementia, cerebellar, pyramidal and/or extra-pyramidal signs and myoclonus remain the cardinal features. Of note however is the fact that 2 of the 3 'non-Brain' individuals were heterozygous at codon 129 and both had EEGs with periodic complexes. They were in fact the only 6-OPRI cases who had periodic complexes; none of the 'Brain' cases described here or elsewhere had this finding. Whilst this observation only applies to a very small number of individuals, it does raise interesting questions about the effects of the specific insertional sequence; could the sequence itself (rather than just its size) have an affect on the disease manifestations?

Whilst Mead et al. found the codon 129 polymorphism to materially affect age at onset, they did not see any affect on disease duration[256]. However in the present series there was a significant affect of codon 129 status on both age at onset and duration, with MM cases clearly having an earlier age at onset (a median of 34 years vs. 43 years in the MV cases) and a much longer disease duration (3,396 vs. 627 days). This finding could possibly be explained by our use of days to measure duration, whereas earlier authors have used months or years; the use of a broader measure of time may have masked differences in disease duration.

The degree of spongiosis, gliosis and neuronal loss seen varied considerably between individuals and indeed within the brain regions of single cases. Typically abnormal PrP was found deposited in the molecular layer of the cerebellum, often in a linear fashion (a finding which is now considered a pointer towards gPD). It is noteworthy that in the two related 'non-Brain' cases these linear deposits were not seen, again raising the possibility of the exact insertional sequence may be influencing the clinicopathological phenotype.

### *5.VI.e Seven-Octapeptide Repeat Insertion*

Of the 1 case of the 7-OPRI mutation found in the UK very little is known. The patient was symptomatic at the age of 30 years, and died at the age of 31; the maximal age at onset was therefore 30 years and the minimal disease duration 305 days. The age at onset is comparable to that reported internationally (where most cases are in their late twenties to early thirties) although the disease duration is

probably shorter, with the majority of previously published cases surviving for a decade or more; however as the age at onset cannot be said with precision in the British case no conclusions can be drawn from this. Further clinico-pathological information was not known.

### *5.VI.f Octapeptide Repeat Insertion Mutations Considered as a Whole*

A wide range of insertional mutations have occurred in the UK, with a correspondingly varied roster of clinical and pathological manifestations. The differences between individuals with the same number of insertions can only be partly explained by the polymorphic residue at codon 129, leading to speculation about the effects of the exact insertional sequence itself, or indeed the presence of other modifying genetic factors outwith *PRNP*. Within the 4-OPRI group a new susceptibility allele without which the disease does not seem to manifest itself was identified, a remarkable finding which raises the possibility of the existence of unaffected mutation carriers. Many unanswered questions remain: why do insertions of 2 or 4 repeats cause disease when 3 do not? What factors influence the stability of a mutation across generations? How often do de-novo mutations occur? Are there a significant number of unaffected, unidentified mutation carriers in the general population, and could such individuals ever pose a public health risk?

## *5.VI.g P102L*

The third commonest *PRNP* mutation in this study was P102L, with 32 cases found. These belonged to multiple different kindreds, with most having a FHx of either prion disease (48.4%) or dementia or neurodegeneration (28.1%). In only 2 cases was the FHx negative (in the remaining 3 it was unknown). This led to a genetic condition often being suspected during the patient's lifetime, although the clinical phenotype was very variable. Some cases developed rapidly progressive dementia and bore a close resemblance to sCJD-MM1, whilst others manifested a cerebellar syndrome in isolation for some time before other neurological features appeared. A review of the clinical phenotype by codon 129 status was made, but as full genetic data was only available for 18 cases, only limited conclusions can be drawn. Eleven patients were MM, whilst 7 were MV and none VV. Most of the MM cases presented with gait and balance problems, with cognitive disturbance being a late feature. Of the MV cases, 4 had an early onset of rapidly progressive dementia, whilst 3 displayed gait and balance problems followed by cognitive decline. Whether or not codon 129 significantly affected the clinical picture is difficult to say when the numbers of cases are so small. A recent large-scale international analysis of P102L cases investigated this point and did not find a correlation between codon 129 type and clinical features, although again numbers were small[376]. They did however, note the age at onset to be significantly younger in methionine homozygotes. This tendency was seen in the current study (median age at onset in methionine homozygotes 42 years vs. 56 years in heterozygotes) and the disease duration was also longer in MM cases (median of 1825 days vs. 867 days in heterozygotes). However the case numbers are too small for definite conclusions to be drawn from this data.

A number of cases met the clinical criteria for a diagnosis of sCJD, but of those tested none had EEG periodic complexes or MRI changes to support this. However 60% (3 of 5) were positive for CSF 14-3-3, all of whom also had an elevated S100b. On reviewing the clinical details for these cases, only one fulfilled the diagnostic criteria for sCJD (the other 2 having a duration greater than 2 years).

Therefore overall although the symptoms and signs are heterogeneous, the combination of a careful FHx and a lack of typical EEG and characteristic MRI changes should a pointer towards a diagnosis of gPD. Although the P102L mutation is described as being a cause of GSSS, it does not necessarily cause a classical GSSS clinical picture.

The neuropathological findings were more consistent than the clinical ones, with all of those who underwent PM examination having amyloid plaques. However the number of plaques present and the degree of spongiform change seen were very variable. Whilst spongiform change was present in 94.1%, this was severe in only 7 of the 17 cases with available data (41.2%). All of those who underwent IHC showed positivity in the plaques themselves, with half having additional abnormal PrP deposition outwith the plaques.

The finding of multiple, apparently unrelated cases is consistent with previously published haplotype analysis[376], and suggests that the P102L mutation has arisen independently on a number of occasions. The codon 129 status possibly influences the age at onset and clinical features seen, but the small numbers of cases in each group limit the conclusions that can be drawn from this data. The P102L mutation should be considered as a possible diagnosis not only in patients with unusual progressive cerebellar syndromes, but also in those with a rapidly progressive dementia resembling sCJD but negative EEG and MRI results, and a significant FHx.

### *5.VI.h P105L*

The single British case with the P105L mutation carried this in conjunction with the V209M polymorphism, a previously unknown combination. Indeed all of the previously published cases of P105L were of Japanese origin. A comparison of the Japanese and British phenotypes is thus of particular interest; the age at onset in the British case was outwith the age range observed in Japan (albeit by a mere 4 years) whilst the duration is so far comparable (the British case being still alive 10 years after onset, whilst the disease duration observed in Japan ranged from 5 to 12 years). The clinical features were very similar, with gait apraxia, cerebellar and

pyramidal signs and dementia, atrophy on neuroimaging and non-specific EEG abnormalities. CSF findings were not reported for any Japanese cases, whilst in the British case the 14-3-3 was negative with a modestly elevated S100b. However unlike the Japanese cases (which had a strong FHx) there was no suspicious FHx in the British case, which may therefore have been a de novo instance of this rare mutation.

### *5.VI.i A117V*

Four separate kindreds carrying the A117V mutation were identified; the largest of these has already been published elsewhere[237]. Several different patterns of clinical onset and progression were seen, with the majority having a rapidly progressive dementia with later cerebellar problems, pyramidal signs and myoclonus. In some cases there was a near simultaneous onset of gait and cognitive problems, whilst in a small number there were prominent extra-pyramidal features early on. The age at onset ranged from the third to the sixth decade, with a disease duration from less than 1 year to over 3. These results are consistent with previous reports, which have also commented on the tendency for valine homozygotes to have a younger age at onset. Codon 129 data was only available for 4 individuals in the present study; the 2 heterozygotes had older ages at onset and shorter disease duration than the 2 VV cases but given the small numbers of cases it is not possible to analyse this further. Data on investigations were also limited: 6 underwent MRI, none of whom had basal ganglia or cortical high signal; and 4 had EEGs, none of whom displayed periodic complexes. CSF 14-3-3 was negative in the 3 cases analysed, 2 of whom had an elevated S100b. The neuropathological findings were similarly variable; although all were felt to represent GSSS, there was variability in the distribution of amyloid plaques, which in some cases were widespread whilst in another primarily restricted to the cerebral cortex. All of those for whom detailed neuropathological data was available showed relative sparing of the cerebellum. The clinicopathological phenotype of A117V remains variable, and although all of those who underwent neuropathological examination were diagnosed with GSSS, this was often not a diagnosis made during their lifetime on clinical grounds. Indeed, on

reviewing the family trees, there was in all cases a strong history of neurodegenerative disease, but in most cases relatives died with a diagnosis other than prion disease. It is likely that the phenotypic heterogeneity and the frequent absence of classical features of GSSS such as early, prominent ataxia have often muddied the diagnostic waters, further emphasising the role that *PRNP* genotyping should play in investigating hard to diagnosis hereditary neurodegenerative diseases.

### *5.VI.j S132I*

The single instance of the S132I mutation described here is the only known case worldwide; there was a strong FHx with the patient's brother having died of ataxia and dementia, and ancestors in the previous 3 generations dying with dementia in middle-age. It is reasonable to assume that these individuals were affected by the same mutation which was transmitted in an autosomal dominant fashion. Although dementia was the dominant feature of the illness, it is noteworthy that the proband's brother was described as being ataxic, whilst the proband herself had no cerebellar signs. The neuropathological findings in the proband were of multicentric amyloid plaques similar to but larger than those seen with the P102L mutation. This novel mutation highlights the difficulties of using the terms 'gCJD' and 'GSSS'; here the clinical features of the proband most closely resemble CJD, but the neuropathology fits with GSSS criteria.

### *5.VI.k Y163X*

Y163X is a rare mutation resulting in the truncation of PrP with loss of the glycophospholipid anchor. Only 2 individuals with this mutation have been referred to the NCJDRSU, regarding 1 of whom very little information is available, to the extent that it is unknown whether or not the she is actually symptomatic. Therefore only 1 case of gPD due to Y163X can be said to have been identified in the current study. This codon 129 heterozygous individual is thought to be still alive, at least 4 years after the onset of cognitive problems, followed by cerebellar signs. Of note is

the pre-existing autonomic and sensory neuropathy, which may well in fact be part of the disease phenotype in light of the recent publication of a family carrying this mutation, characterised by autonomic failure, sensory neuropathy and chronic diarrhoea, followed some years later by cognitive decline and seizures[252]. Although temporally disparate, the 2 constellations of symptoms seem to be linked, as evidenced by the presence of abnormal PrP not only in the CNS but also in the duodenum. Further neuropathological data of the effects of this mutation (possibly from the same family although this is not specified by the authors) describe vascular and parenchymal PrP deposition with extensive neurofibrillary tangles[319]. These intriguing reports further demonstrate the heterogeneity of prion disease, and the need for vigilance when planning surgical procedures (even non-neurosurgical ones) in those with or at risk of gPD; transmission studies of gastrointestinal tissue from those with the Y163X mutation and abnormal PrP in the duodenum would be invaluable in furthering our knowledge of the iatrogenic potential of this condition.

### *5.VI.I D167G*

The single case of the D167G mutation described here remains the only known instance of this mutation; although there was a FHx of cognitive impairment this had been attributed to dementia puglisitica and may therefore not be relevant. The clinical phenotype seen closely resembled that of sCJDMM1, with the onset of cognitive impairment in old age, followed by pyramidal signs, visuo-spatial deficits, visual hallucinations, myoclonus and death after less than 2 years. It is understandable therefore, that the patient was originally classified as having probable sCJD (based on the clinical features and a positive CSF 14-3-3). Neuropathologically there was little to go against this diagnosis, with widespread spongiform change, neuronal loss and astrocytosis. The only slightly unusual finding was that the most severely affected region was the molecular layer of the cerebellum, where a coarse aggregated pattern of type 1 $PrP^{Res}$ was found. Evidence for this novel mutation being causative of gPD disease (rather than being a silent polymorphism in a case of sCJD) comes from the likelihood that the substitution of a large hydrophilic amino acid for a small hydrophobic one may disrupt the physical properties of $PrP^{C}$, and

from animal models suggesting that codon 167 is involved in the binding of chaperone molecules[207]. However in the absence of further affected family members D167G cannot be definitively said to be a pathogenic mutation.

### *5.VI.m* *D178N-129M*

Only 5 cases of FFI were seen in the UK, none of whom were related or had a FHx of gPD (although 3 did have first degree relatives with neurodegenerative disease). The onset typically involved sleep disturbance and non-specific complaints such as tiredness and headache. This was true of both the 4 MM cases and the single MV case (although having valine on the wild-type allele has previously been associated with an ataxic onset and later sleep disturbance, this was not seen here)[312]. The overall clinical phenotype was one of frequent sleep disturbance, dementia, visual hallucinations, cerebellar and pyramidal signs, with non-specific EEG and MRI findings. Three subjects were tested for 14-3-3 and S100b; 14-3-3 was negative in all but S100b elevated in 2 cases.

The neuropathological findings in the 4 MM subjects examined were variable, and were not entirely in accord with findings reported in the literature on the subject. Methionine homozygosity is typically associated with severe degeneration of the thalamus, but this was only seen in 2 of the 4 MM cases examined here. The degree of abnormal PrP deposition was very variable, with none in 1 case, faint patchy thalamic deposits in another, whilst in the remaining case there was heavy $PrP^{Sc}$ staining of neurones in the cortex, foci of synaptic and dendritic deposition in the cerebellum, and multiple, fluffy diffuse plaques in the frontal and occipital cortex. These unusual neuropathological findings were not associated with any strikingly different clinical features. Several very large FFI families have been described in the international literature[323,214], and it is of interest that the British cases were all apparently unrelated and none had relatives affected by gPD. This again raises the issue of de novo mutational events, or indeed interactions between the pathogenic *PRNP* mutations and other genetic or environmental factors, with a net effect on disease susceptibility and manifestation.

### 5.VI.n D178N-129V

The D178N mutation with valine on the mutated allele was even less common than FFI, with only 2 cases found in the UK. Both carried methionine on the wild-type allele, and displayed a clinical phenotype resembling sCJDMM1, presenting with rapidly progressive dementia followed by cerebellar problems and myoclonus. One of the cases did have sleep disturbance, but this was not a prominent or early feature. Both had extensive cortical ribboning on MR imaging and positive CSF 14-3-3, with non-specific changes on EEG. They had similar neuropathological findings with severe spongiform change of the cerebral cortex, neuronal loss, astrocytosis, and abnormal PrP deposited in a synaptic pattern plaques. In Case 1 there was a ribbon-like appearance to the $PrP^{Sc}$ deposition in the deeper cortical layers, and $PrP^{Sc}$ plaques in the cerebellum. In Case 2 there were numerous cortical amyloid plaques, heavy tau deposition and neurofibrillary tangles, felt to represent co-existent Alzheimer's pathology (Braack and Braack stage VI). Whilst the severe spongiform change is not unusual, the $PrP^{Sc}$ plaques are, especially as the cerebellum was involved (it typically being spared in D178N-129V).

### 5.VI.o E200K

A large proportion of the cases of gPD identified in this study were due to the E200K mutation, although in contradistinction to other countries it was not the commonest mutation seen. The difficulties in distinguishing gPD due to E200K and sCJD are highlighted by the fact that 44.1% of the cases in this series were initially diagnosed with sCJD, and therefore it is possible that case ascertainment was not complete.

The presenting complaint was typically one of cognitive decline, personality or behavioural change, gait disturbance or a combination of these features. This was very similar to that of sCJD, although sleep disturbance was a much commoner presenting feature with CJD-E200K, especially in the MV sub-group.

A comparison of clinical features seen over the entire illness revealed a similar rate of cognitive problems, cerebellar and pyramidal signs and myoclonus in

sCJD and CJD-E200K. However extra-pyramidal signs such as bradykinesia and cog-wheel rigidity were less common in CJD-E200K whilst involuntary movements other than myoclonus (e.g. chorea, dystonia and tremor) were much more frequently observed in CJD-E200K.

The incidence of seizures in the present series (23.5%) was lower than has been previously reported[191,40]. There was also a lower incidence of extra-pyramidal signs, and a higher rate of visual disturbance or loss, and of involuntary movements other than myoclonus. The median age at onset seen here agreed with that reported by Mead (58 years), although the disease duration in the present series was noticeably briefer (130 vs. 213 days)[251].

In the current series there was a lower incidence (28.6%) of periodic complexes than previously reported in CJD-E200K[40] and in sCJDMM1[295]. However these figures are comparable to those previously reported in sCJD by the NCJDRSU (34%)[74]. The present findings are unlikely to be attributable to EEG timing, as on average the EEG was performed later in those cases which did not have typical periodic complexes.

The CSF 14-3-3 was positive in 82.4% of those tested (comparable to the 86% sensitivity reported in sCJD[63]), whilst S100b showed a tendency to be higher than is usually seen in sCJD, with over half of those tested having a result over 1.00ng/ml, and 12.5% have a results greater then 2.00ng/ml.

Around half of the cases which underwent MRI of the brain showed basal ganglia or extensive cortical high signal, and the true figure may in fact be higher, given that 16% of the available scans were non-diagnostic due to poor image quality. A previous study of 15 individuals from 1 family with CJD-E200K found 87% to have basal ganglia signal abnormalities on FLAIR, a much higher hit rate than is usually reported in sCJD cases[101].

The neuropathological findings in the current series were similar to those of sCJDMM1, with the exception of the $PrP^{Res}$ type seen. Of the 4 cases in which the $PrP^{Res}$ type was determined, Type 1B (21kDa nonglycosylated band and diglycosylated band predominating) was found in 2 MM E200K cases, and a type intermediate between 1A and 1B (i.e. under-representation of the non-glycosylated band) in 1 further MM cases and in a single MV case. These $PrP^{Res}$ types are unusual

and typically associated with gPD, and provide an important feature distinguishing sCJD from E200K.

Only 11.8% of the cases in the present series were heterozygous at codon 129; the codon 129 status of the wild type allele was only known for 1 of these cases, which was methionine. These findings agree with the picture seen worldwide, where the majority are MM, and only a very small number with valine on the mutated allele have been described[210].

Overall our MM and MV E200K cases had a similar age at onset, symptoms and signs and CSF findings. The disease duration was longer in the MV cases, and there was a tendency towards a lower incidence of periodic complexes and MRI positivity in the MV group. They may also have more severe basal ganglia and cerebellar involvement on neuropathology. However the small size of the MV group makes it difficult to draw firm conclusions.

Of the 34 cases identified, only 2 could be linked to known clusters of the E200K mutation; 1 case was of Sephardic Jewish ancestry (with no known FHx of prion disease) and 1 case was of Spanish ancestry and had an extensive FHx of gPD. In total 25 separate families were identified, 3 of which were not of UK Caucasian origin. Detailed genealogical research was not done, therefore it is possible that there were hidden links between these families. Only 44% of families had more than 1 affected individual. This data supports the view that the E200K mutation has arisen on multiple occasions. Over a fifth of cases had no FHx of prion disease, neurodegeneration, or psychiatric illness, raising the possibility that these cases represent de novo mutational events. It is of interest that the majority of cases appear to occur in isolation, in contrast to the findings in the Slovakian and Sephardic Jewish clusters. This raises the possibility that the penetrance of the mutation may be variable, possibly depending on other polymorphisms of the *PRNP* gene. In conclusion it remains a difficult task to separate out cases of CJD-E200K from those of sCJD, and clinicians should be particularly alive to the possibility of gPD when unusual features such as a young age at onset, early sleep disturbance, chorea, dystonia or tremor or a very high S100b are present.

D202N is a rare mutation, with only 2 cases reported worldwide, 1 of them British. This lady had an unusual illness, with a slowly progressive dementia lasting for 7 years; additional gait problems were only noted a mere 5 months prior to her death. Whilst prion disease was suspected by her physician, the initial neuropathological analysis did not in fact support this, with a diagnosis of Alzheimer's disease being made in the early 1980s. It was only upon a review some years later that numerous amyloid plaques with associated neurofibrillary tangles were identified throughout the cerebral cortex, basal ganglia, thalamus and particularly in the cerebellum. These were positive for abnormal PrP, and in places aligned in almost linear fashion. These appearances were felt to be consistent with a GSSS variant, which lead to analysis of the *PRNP* gene. It is noteworthy that in common with mutations such as P105L and A117V which give rise to neuropathological appearances classifiable as GSSS, the individual with D202N was valine homozygous at codon 129. This is at variance with the mutation which classically causes a GSSS-type picture, P102L, in which group the majority are MM at codon 129. As has previously been discussed, this raises interesting points about the interactions between these point mutations and codon 129. Is valine at codon 129 a permissive factor in the occurrence of these mutations? Or is D202N in conjunction with methionine a non-pathogenic polymorphism? As such it has certainly not been detected in population studies to date. Transgenic animal studies would be of great interest in order to further explore the interactions between these mutations and codon 129.

## 5.VI.q V210I

Scattered instances of the V210I mutation have appeared across Europe, N. Africa, S. America and Asia. Both the cases seen in the UK were immigrants, with one being born in Italy to a family resident for several generations in the Campania region, and the other Libyan. Both manifested a similar clinical phenotype, with onset of a rapidly progressive illness resembling sCJDMM1 in the seventh decade, and methionine homozygosity at codon 129. The first case had periodic complexes on their EEG, whilst the second had asymmetrical basal ganglia high signal on MRI and positive CSF 14-3-3 with an elevated S100b. This led to both being classified in their lifetimes as having sCJD, neither having a positive FHx. This mutation has already been observed in a Moroccan person of Berber ancestry; the exact racial extraction of the present North African case was not known, but there was no FHx of neurodegenerative disease.

No link could be made between the Italian case and any reported V210I family in Italy, but this individual was from the Campania region, an area in which this mutation is particularly prevalent[223] and 90% of those with the V210I mutation have a negative FHx[210]. The additional finding of elderly asymptomatic relatives being carriers of the mutation implies that the penetrance of this mutation is low. Whilst disease has successfully transmitted to transgenic mice using brain tissue from affected individuals[242], no similar attempts have been made with samples from unaffected carriers. Questions regarding penetrance create quandaries for clinicians and families; if it is low then is pre-symptomatic genetic counselling justified? What public health risks would an as-yet asymptomatic person with a low penetrance mutation pose?

## 5.VI.r Q212P

The single previously published case of the Q212P mutation occurred in a Caucasian N. American gentleman, who had an 8 year long illness characterized by ataxia, incoordination, dysarthria and dysphagia in the absence of dementia. Amyloid and abnormal PrP deposition were found at neuropathology, leading to the case being

labelled as one of GSSS. In the present study 1 British woman in her fourth decade was affected by this mutation, with a similar presentation comprising initial gait disturbance followed by cerebellar and pyramidal signs and myoclonus. However cognitive impairment did occur, appearing over a year later. At the end of the study period she was still alive, over 6 years after symptom onset. As with the earlier case, EEG and MRI have shown only non-specific abnormalities and atrophy. CSF analysis demonstrated a negative 14-3-3 with an elevated S100b. The possibility of this mutation not being the cause of her illness was considered, but extensive investigations have failed to demonstrate an alternative diagnosis. It is of interest that the patient is homozygous for the mutation, although the FHx is negative and there is no known history of consanguinity. This might suggest a low penetrance but significant dc novo mutation rate (or concealed consanguinity); the finding of an asymptomatic elderly carrier sibling of the N. American case supports this theory.

## 5.VII Diagnostic Criteria for Genetic Prion Disease

The current internationally agreed diagnostic criteria divide cases into those with 'probable' gPD or 'definite' gPD. A probable diagnosis can be made if a patient has a progressive neuropsychiatric disorder and a disease-specific mutation, or if they meet diagnostic criteria for probable prion disease and have a first-degree relative with definite or probable prion disease. In order to reach a definite diagnosis there must be neuropathological evidence of prion disease and a pathogenic *PRNP* mutation and a first-degree relative with definite or probable prion disease. These criteria have been carefully drawn up to try and classify cases as accurately as possible, but there are certain circumstances where applying them becomes difficult. For example, in the present study, individuals were identified with definite prion disease (but no *PRNP* genotyping) and a second-degree relative with definite gPD. In one case the individual had 2 second-degree relatives with gPD. In these circumstances it seems highly likely that the diagnosis is one of gPD, but the case is not classifiable as such. An individual approach is needed in such circumstances to judge the likelihood of such cases representing sporadic or genetic disease.

The criteria also create the situation where an individual with definite prion disease and a mutation that is well known to be pathogenic such as P102L or E200K but with an uninformative FHx can be described as having only probable gPD, implying a degree of diagnostic uncertainty that is probably not really present.

Difficulties also arise with novel mutations such as D167G; the single reported individual with this mutation had previously been diagnosed with definite sCJD, and without a FHx or other case reports it is impossible to know if this is a pathogenic mutation or not. From a pragmatic point of view it seems sensible to assume that this mutation is pathogenic until proven otherwise and to advise the family appropriately. Transmission studies or transgenic animal work may prove useful approaches to determining the nature of such novel mutations.

There is now a trend to classify cases according to *PRNP* haplotype rather than splitting them into gCJD or GSSS. Given the clinicopathological heterogeneity seen this is probably the correct approach. For example, whilst P102L-129M is said to be prototypic mutation causing GSSS, cases may actually closely resemble sCJD (to the point of meeting the criteria for a diagnosis of probable sCJD). The division of cases into gCJD and GSSS is somewhat artificial, and as more novel mutations with unusual presentations and neuropathological findings are described, it becomes less and less useful. *PRNP* genotyping is also the only way to exclude a diagnosis of gPD and should be offered to all patients and families affected by prion diseases to try and minimise the risk of misdiagnosis.

## 5.VIII Limitations of This Work & Suggested Topics for Further Research

This thesis has reviewed data on individuals of gPD referred to the NCJDRSU over a 20 year period, with additional cases from epidemiological studies covering the previous decade. Despite this, the number of cases remains small, especially when each *PRNP* mutation is considered separately. This is an issue inherent to the study of any rare disease, and one encountered by previous researchers. The large number of *PRNP* mutations and the need to further sub-classify them according to the polymorphic codon 129 residue makes accumulating sufficient cases for statistical analysis very difficult. Interesting conclusions can still

be drawn, but care must be taken to not read too much into trends within small groups. Previous authors have amalgamated cases into groups according to various criteria, for example comparing those meeting the diagnostic criteria for GSSS with those with insertional mutations. Whilst this is an attractive approach, it is difficult to know how meaningful it is to compare such groups. By far the commonest insertional mutation in the UK is 6-OPRI, whilst the commonest cause of GSSS is P102L. A comparison of patients with insertional mutations vs. GSSS may therefore effectively be a comparison of 6-OPRI and P102L. It is preferable to classify cases by *PRNP* haplotype, despite the constraints this puts upon sample size. International collaborations such as the EuroCJD study are one way of overcoming this difficulty.

Detailed haplotype analysis was performed for the 4-OPRI group, with the outcome that all were found to be homozygous for both methionine at codon 129, and the previously described risk allele for sCJD, rs1029273C. Future work seeking other such risk polymorphisms would be of great interest, especially looking for factors at work in families where elderly, unaffected mutation carriers are found. This would be of particular utility in seeking to explain why the E200K mutation in Israeli Sephardic Jews appears to have a very high penetrance, whereas in other ethnic groups including the UK population, it does not. International collaborations such as the EuroCJD project have already yielded a great deal of useful data because of their access to a large population of affected individuals, and further collaborative work screening for other genetic factors which influence disease susceptibility and clinicopathological phenotype could be very rewarding.

Performing genotyping for an autosomal dominant mutation which causes an untreatable, fatal condition is understandably controversial. Explaining complex genetic issues to families coming to terms with a terminal diagnosis in a loved one is challenging, and clinicians must endeavour to present unbiased advice in an understandable and timely fashion. There may also be disagreement between family members as to the best course of action, with older relatives sometimes being against genetic testing whilst younger relatives who are considering starting their own family may be keener to be fully informed. Balancing the best interests of the patient, their family, and the desire to gain data for research is difficult. The involvement of specialist genetic counselling services is desirable in such circumstances. Ethical

considerations limit the volume of genetic testing performed, particularly on pre-symptomatic, at-risk individuals. This may well change if significant developments are made in the field of prion disease treatment, in which case more widespread *PRNP* genotyping of all individuals with prion disease and their blood relatives could be justified on clinical grounds. Until then the current policy of making all families aware of the risk of gPD masquerading as sCJD, and targeting genetic testing specifically to those at higher risk based on the clinical picture and FHx is appropriate. However this will undoubtedly continue to miss a (hopefully small) number of cases of gPD.

## 5.VIX Conclusions

In summary, the clinico-pathological phenotype of gPD remains highly diverse, and their diagnosis is often difficult. Whilst CSF analysis, MRI and EEG are useful tools, the mainstay of diagnosis should be formal *PRNP* genotyping. Patients and families should receive specialist genetic counselling, and care must be taken to ensure that they are fully informed of the implications of a diagnosis of gPD before testing is performed, particularly in pre-symptomatic individuals. Whilst the current, internationally agreed diagnostic criteria are robust, they may require individual interpretation in some cases. Finally, the terminology is now evolving towards subdivision of cases by haplotype, as the terms gCJD and GSSS are not the exclusive entities they were once thought to be. There seems to be a continuum of clinical and pathological appearances on a spectrum between classical CJD and GSSS, which can lead to diagnostic confusion. Thus *PRNP* genotyping is essential both for diagnostic and research purposes.

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# APPENDIX:

# PUBLICATIONS

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BRAIN
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# Inherited prion disease with 4-octapeptide repeat insertion: disease requires the interaction of multiple genetic risk factors

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Genetic factors are implicated in the aetiology of sporadic late-onset neurodegenerative diseases. Whether these genetic variants are predominantly common or rare, and how multiple genetic factors interact with each other to cause disease is poorly understood. Inherited prion diseases are highly heterogeneous and may be clinically mistaken for sporadic Creutzfeldt–Jakob disease because of a negative family history. Here we report our investigation of patients from the UK with four extra octapeptide repeats, which suggest that the risk of clinical disease is increased by a combination of the mutation and a susceptibilty haplotype on the wild-type chromosome. The predominant clinical syndrome is a progressive cortical dementia with pyramidal signs, myoclonus and cerebellar abnormalities that closely resemble sporadic Creutzfeldt–Jakob disease. Autopsy shows perpendicular deposits of prion protein in the molecular layer of the cerebellum. Identity testing, *PRNP* microsatellite haplotyping and genealogical work confirm no cryptic close family relationships and suggests multiple progenitor disease haplotypes. All patients were homozygous for methionine at polymorphic codon 129. In addition, at a single nucleotide polymorphism upstream of *PRNP* thought to confer susceptibility to sporadic Creutzfeldt–Jakob disease (rs1029273), all patients were homozygous for the risk allele (combined $P = 5.9 \times 10^{-5}$). The haplotype identified may also be a risk factor in other partially penetrant inherited prion diseases although it does not modify age of onset. Blood expression of *PRNP* in healthy individuals was modestly higher in carriers of the risk haplotype. These findings may provide a precedent for understanding apparently sporadic neurodegenerative diseases caused by rare high-risk mutations.

Keywords: inherited prion disease; octapeptide repeat insertion; Creutzfeldt–Jakob disease; epistasis
Abbreviations: CJD = Creutzfeldt–Jakob disease; OPRI = octapeptide repeat insertion

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# Introduction

Prions are lethal infectious pathogens, largely composed of an abnormally folded host protein (Collinge, 2001). As one of the prototypic protein misfolding disorders, prion diseases share fundamental mechanisms with other neurodegenerative conditions. Human prion diseases may be divided into three categories: acquired, inherited or unexplained—termed sporadic Creutzfeldt–Jakob disease (CJD). Clinically, sporadic CJD and inherited prion disease show remarkable diversity, being associated with several subtypes and eponymous syndromes, and are often misdiagnosed as a result. The aetiology and clinical heterogeneity of sporadic neurodegenerative diseases are poorly understood.

Inherited prion disease accounts for ~15% of human prion diseases, and are always associated with coding mutations in the prion protein gene (*PRNP*) (Mead, 2006). Two types of pathogenic *PRNP* mutation exist: point mutations leading to an amino acid substitution in the prion protein or the production of premature stop codons; and alteration of the number of octapeptide repeats. The normal prion protein octapeptide repeat region is composed of a nonapeptide followed by a tandem repeat of four copies of an octapeptide and lies between codons 51 and 91 (Kretzschmar *et al.*, 1986). Insertions of up to nine extra, and deletion of two, octapeptide repeats have been described in patients.

Typically, octapeptide repeat insertion (OPRI)-associated prion disease presents as a syndrome of slowly progressive multifocal dementia accompanied by dyspraxia and cerebellar ataxia. However, the clinicopathological phenotype, both within and between OPRI families shows significant variability. Age of onset varies from adolescence to old age, the clinical course from several months to >15 years and the dominant clinical features may be psychiatric, cognitive, cerebellar or extrapyramidal (Collinge *et al.*, 1992; Laplanche *et al.*, 1999; Moore *et al.*, 2001; Mead *et al.*, 2006; Webb *et al.*, 2008). This is partly due to a coding polymorphism of *PRNP* codon 129 (Poulter *et al.*, 1992; Mead *et al.*, 2006, 2007). Much of the clinical heterogeneity remains unexplained however; candidate factors include variation of modifier loci (Lloyd *et al.*, 2001) and heterogeneity of the misfolded prion protein itself (Wadsworth *et al.*, 2006).

In the first patient series of this mutation we report the clinical phenotype, investigative findings, molecular genetic and neuropathological features of all known UK patients with 4-OPRI. We show that the clinical phenotype of 4-OPRI prion disease is distinct from that seen in patients with 5- or 6-OPRI. For a genetically determined disease, we found that a family history of early-onset dementia was remarkably uncommon. To explain this, we provide evidence that the mutation alone appears not to be sufficient to consistently cause the disease: all UK patients were homozygous at two common polymorphisms, in and upstream of *PRNP*. Our findings provide a precedent for the mechanisms of penetrance and the aetiology of apparently sporadic neurodegenerative diseases.

# Materials and methods

## Molecular genetics

Genomic DNA was extracted from peripheral blood using the Nucleon Bioscience BACC2 DNA extraction kit. The entire open reading frame of *PRNP* was assessed by direct sequencing from genomic DNA. The size of insertions was confirmed by fractionation of amplicons from polymerase chain reaction designed to amplify the octapeptide repeat region by agarose gel electrophoresis. The exact nature of additional repeat units was confirmed by a combination of subcloning and by direct sequencing. Sequence data were analysed using allele discrimination assay Seqscape software. rs1029273 upstream of the *PRNP* open reading frame (Mead *et al.*, 2001) was genotyped using the Taqman 5′ nuclease allele discrimination assay. For *PRNP* haplotype analysis, microsatellite markers D20S181, D20S193, D20S473, D20S867, D20S889, D20S116, D20S482, D20S97, D20S895, D20S849, D20S873, D20S95 and D20S194 were amplified. Data were analysed using ABI GeneMapper software v4.0.

For relatedness testing, a subset of the Powerplex16 microsatellite marker set (Promega) was utilized and processed in a similar manner to *PRNP* microsatellites. This subset included microsatellite markers VWA, D5S818, D13S317, D7S820, D16S539, THO1, TPOX, CSF1PO, PENTA E and PENTA D. Following allele discrimination assay GeneMapper software analysis to provide allele sizes (basepairs), these data were subsequently converted to equivalent Powerplex16 allele sizes (numerical scores) for use in the ML-Relate program (Kalinowski *et al.*, 2006).

## Samples

Samples were obtained for 10 patients (Table 1 and Supplementary Material) with informed consent by the National Prion Clinic, London, or the National CJD Surveillance Unit, Edinburgh.

The clinical, genetic, biochemical and neuropathological studies were approved by the National Hospital for Neurology and Neurosurgery and University College London Hospital research ethics committee. UK control samples (566) were obtained from healthy blood donors.

## Statistical analysis

Means, standard deviations (SDs), tests of normality, Mann–Whitney U-test, binomial probability tests, linear regression and $\chi^2$-tests were performed using the Statistical Package for the Social Sciences package (SPSS Inc.). Genetic association studies were performed with the use of PLINK (Purcell *et al.*, 2007).

## Genealogical investigations

Family lineages were traced back to identify cause of death of parents of affected individuals. Birth and death certificates were obtained from the General Register Office via their website. Where records were not available the original clinical notes, out-patient letters and medical records were used. A family history of neuropsychiatric illness was considered positive when there was early-onset dementia (age <65 years) or a later-onset progressive dementia or behavioural disturbance in the context of abnormal neurological signs leading to death, in keeping with the clinical picture observed in this case series.

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Table 1 **Clinical features**

| Patient | Sex | Age at onset (years) | Age at death (years) | Duration (days) | Codon 129 | Symptoms at onset | Pyramidal signs | Extrapyramidal signs | Myoclonus | Chorea | Cerebellar signs |
|---|---|---|---|---|---|---|---|---|---|---|---|
| I | M | 56 | 56 | 59 | MM | Dementia, gait disturbance, myoclonus, drowsiness | No | Yes | Yes | No | No |
| II | M | 67 | 67 | 77 | MM | Deteriorating handwriting | Yes | No | Yes | No | No |
| III | M | 47 | 53 | 2319 | MM | Impaired memory and concentration | Yes | Yes | Yes | No | Yes |
| IV | F | 85 | NA | Alive | MM | Impaired memory | No | No | No | No | No |
| V | F | 39 | 45 | 2160 | MM | Impaired memory and concentration | No | Yes | No | Yes | No |
| VI | M | 58 | 58 | 73 | MM | Deteriorating handwriting | No | No | Yes | No | Yes |
| VII | F | 65 | 66 | 414 | MM | Impaired memory and anxiety | No | Yes | Yes | No | Yes |
| VIII | M | 70 | 73 | 1191 | MM | Impaired memory and personality change | No | Yes | Yes | Yes | No |
| IX | F | 51 | 52 | 93 | U | Dizziness | Yes | No | Yes | Yes | Yes |
| X | F | 53 | 59 | 1943 | MM | Dementia | No | No | Yes | No | Yes |

M = male; F = female; U = unknown, although identical twin of Patient X, and therefore highly likely to be 129MM.

## Neuropathology

Tissue was fixed in 10% buffered formol saline followed by incubation in 98% formic acid for 1 h. Following post-fixation for 24 h in 10% buffered formol saline, the tissue samples were processed through graded alcohols and paraffin wax embedded.

Paraffin sections were cut at a nominal thickness of 7 μm, boiled in a low ionic strength Tris–ethylenediaminetetraacetic acid buffer, pH 7.8 for 20 min, followed by 15 min in 98% formic acid. Primary antibodies used were anti-glial fibrillary acidic protein rabbit polyclonal anti-serum (Dako), anti-prion protein monoclonal antibodies ICSM 35 (D-Gen Ltd) and KG9 (TSE Resource Centre, Neuropathogenesis Division, University of Edinburgh), anti-Tau AT8 (Dako) and anti-Beta-amyloid (Dako). Antibodies were detected with the Ventana Medical Systems Inc. immunostainer using a biotinylated universal IgG secondary antibody (iView Biotinylated Ig; Ventana Medical Systems, Inc.) an avidin–biotin horseradish peroxidase conjugate system (iView SA-HRP; Ventana Medical Systems, Inc.) before development with 3, 3′-diaminobenzedine tetrachloride as the chromogen (iView DAB; Ventana Medical Systems Inc.). Haematoxylin was used as the counter stain. Haematoxylin and eosin staining of serial sections was performed using conventional methods. Appropriate controls were used throughout (Wadsworth *et al.*, 2008). At least eight blocks were analysed in each case, including frontal, occipital and parietal cortices, temporal cortex with hippocampus, basal ganglia, thalamus, cerebellum and brainstem.

## Expression analysis

Whole blood was drawn into ethylenediaminetetraacetic acid and PAXgene™ tubes at a Donor Suite of the National Blood Service with informed consent. DNA was extracted from ethylenediaminetetraacetic acid whole blood using the Nucleon BACC3 kit (GE Healthcare). Samples were genotyped for *PRNP* codon 129 (rs1799990) and rs1029273 by allelic discrimination using the SDS7500 (Applied Biosystems) system. Primer and probe sequences are available on request. RNA was extracted from PAXgene tubes using the PAXgene™ 96 Blood RNA kit (PreAnalytix). RNA (1 μg) from each individual homozygous for the M129 allele was reverse transcribed using Omniscript (Qiagen) with random hexamer priming. *PRNP* transcript levels were measured using the SDS7500 by relative quantification using the delta-delta-Ct method with two different primer limited endogenous controls (β-actin and TATA box binding protein) each duplexed seperately with *PRNP* (assay details on request). The endogenous controls were selected for suitability using geNorm (http://genomebiology.com/2002/3/7/research/0034) to minimize candidate endogenous gene variability in human whole blood. Results of the relative quantities of the *PRNP* transcript represent the normalization of real-time quantitative polymerase chain reaction data by geometric averaging of relative quantities calculated using the two internal reference genes.

## Immunoblotting

Human brain (frontal cortex) was prepared as 10% w/v homogenates in Dulbecco's sterile phosphate buffered saline lacking $Ca^{2+}$ and $Mg^{2+}$ ions and analysed after proteinase K digestion (12.5 or 50 μg/ml final protease concentration, 1 h, 37°C) by immunoblotting with anti-prion protein monoclonal antibody 3F4 using high sensitivity enhanced chemiluminescence as described previously (Wadsworth *et al.*, 2001; Hill *et al.*, 2006).

# Results

## Clinical findings

The principle clinical features are shown in Table 1 and investigations in Table 2. In summary, the predominant clinical syndrome was a rapidly progressive cortical dementia with myoclonus, motor and cerebellar signs resembling sporadic CJD. A pure cognitive syndrome was apparent in one patient (Patient IV) in which there had been a slowly progressive decline in episodic memory over 7 years in the absence of neurological signs. This patient has a moderate cortical dementia with early defects in episodic memory

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Table 2 **Investigations**

| Patient | Early-onset dementia in parent | EEG | CT | MRI | Neuropathology |
|---|---|---|---|---|---|
| I | NA[a] | Typical | Gross generalized atrophy | Not done | CJD |
| II | No | Typical | Atrophy | Not done | CJD |
| III | No[b] | Non-specifically abnormal | Normal | High signal in right caudate head and temporal lobe | CJD |
| IV | No | Not done | Not done | Parietal and temporal lobe atrophy | NA (patient alive) |
| V | Yes | Non-specifically abnormal | Atrophy | Not done | Not done |
| VI | No | Non-specifically abnormal | Not done | Normal | CJD |
| VII | No | Suggestive | Atrophy | Not done | CJD |
| VIII | No | Non-specifically abnormal | Mild atrophy | Left chronic subdural haematoma | CJD |
| IX | No[c] | Non-specifically abnormal | Atrophy | Not done | Not done |
| X | No[c] | Normal | Unknown | Atrophy | CJD |

A family history was considered positive if there was a history of dementia in a parent with onset prior to the age of 65 years, or a late-onset neurodegenerative disease associated with dementia and neurological signs consistent with inherited prion disease. For EEG, typical refers to classical findings associated with CJD.
NA = Not available.
a Family history was censored.
b Son of Patient IV; however, we cannot be certain that Patient IV has inherited prion disease as her clinical phenotype is consistent with Alzheimer's disease.
c Monozygotic twins, family history refers to parents.

and executive functions, clinically resembling Alzheimer's disease. Memory deficits and more widespread cognitive decline were the predominant features in Patients I, III, IV, VII, VIII and X. A parental history typical of inherited prion disease was only rarely seen in our patient series.

Age at onset of disease was known for all patients and disease duration was known for nine patients. Both showed considerable variability, the youngest patient presenting in her late thirties and the oldest aged 85 years (or possibly 70 years; see description of living Patient IV in the Supplementary Material). The longest illness duration was 77 months (Patient III) although Patient IV is still alive aged 92 years, with a minimum disease duration of 7 years. The mean (SD) age of onset was 60 (12.97) years (range 39–85 years) with a median disease duration of 414 days (range 59–2319 days).

Biochemical (urea and electrolytes, liver function tests, serum calcium, serum copper, serum angiotensin-converting enzyme, and serum vitamin B12 and folate), haematological (full blood count and erythrocyte sedimentation rate), serological (antinuclear antibodies, rheumatoid factor, syphilis serology and anti-cardiolipin antibodies) and thyroid function tests, when performed, were within normal limits. CSF examination was performed in six patients (Table 3). Protein 14-3-3 was consistently positive in those tested ($n = 4$).

## Genetic analysis

The complete *PRNP* open reading frame was sequenced in nine patients. DNA was unavailable for Patient IX (the identical twin of Patient X, monozygosity not genetically confirmed). All patients tested were shown to have an additional four R2 repeats, and to be homozygous for methionine at codon 129. No other sequence variations were detected.

We genotyped *PRNP*-linked and unlinked microsatellites to test: (i) the number of separate ancestral occurrences of the 4-OPRI mutation; and (ii) cryptic close kinship within our series. Relatedness testing at unlinked microsatellites confirmed a mother–son relationship between Patients III and IV ($P = 6 \times 10^{-6}$, maximum likelihood of parent offspring 0.57). Other relatedness in this patient series, closer than second degree, was excluded by this analysis. For linked microsatellites, Patients II, VII and X shared a haplotype of 2.5 Mb, differing from the mother/son and each other, at a single 3′ marker (D20S194). Two further patients, Patients I and VI, shared with the above five patients a central core haplotype spanning ~1.2 Mb (D20S889–D20S895). Finally, Patients V and VIII demonstrated a shared 1 Mb haplotype with those described above between D20S97 and D20S895 only, although these two patients shared an extended haplotype of 2 Mb (D20S181–D20S849). Overall our data were consistent with multiple (at least two) ancestral occurrences of 4-OPRI in the UK; however, we cannot exclude a single ancestral occurrence several centuries in the past with time for recombination and mutation events to disrupt the disease haplotype.

*PRNP* codon 129 (rs1799990) and rs1029273C (upstream of *PRNP*) have been identified as risk factors for sporadic CJD (Palmer *et al.*, 1991; Mead *et al.*, 2001; Vollmert *et al.*, 2006; Mead *et al.*, 2009). Genotyping revealed homozygosity for codon 129MM (rs1799990AA) and rs1029273CC in all 4-OPRI patients. At least one rs1029273C-129M haplotype would be expected in each patient if the 4-OPRI mutations had all occurred on this haplotype background. This assumption is conservative as our microsatellite genotyping suggests that there may have been multiple mutational events. However, the haplotype of the wild-type chromosome is independent of the presence of the mutation, and the haplotypes seen on the unaffected chromosome were not expected to be different from the control population. The rs1029273C-129M haplotype occurs at a frequency of 0.339 in

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Table 3 **CSF analysis**

| Patient | Glucose (mmol/l) | Protein (mg/ml) | Cell counts | CSF OCB | Plasma OCB | 14-3-3 | S100b (ng/ml, <0.38) |
|---|---|---|---|---|---|---|---|
| II | 6.7 | 0.44 | Normal | Positive | Positive | Positive | Not done |
| III | 3.0 | 0.44 | Normal | Not done | Not done | Positive | 2.42 |
| VI | 3.9 | 0.31 | Normal | Not done | Not done | Not done | Not done |
| VII | 4.4 | 0.25 | Normal | Not done | Not done | Positive | 3.8 |
| VIII | Unknown | Unknown | Unknown | Positive | Not done | Positive | 0.44 |
| IX | 3.6 | 0.63 | Normal | Not done | Not done | Not done | Not done |

OCB = oligoclonal bands.

the control UK population (384/1132 UK control haplotypes). The probability that all wild-type haplotypes would be rs1029273C-129M is $P = 5.9 \times 10^{-5}$ (binomial probability). rs1029273C is usually found on a 129M chromosome, but the significance of the association of rs1029273C and 4-OPRI could not be accounted for by an association driven by 129M alone ($P = 0.003$, binomial probability, of finding that all nine wild-type alleles were rs1029293C, with the assumption that all patients were genotype 129MM). With the conservative assumption that Patient IV had Alzheimer's disease rather than prion disease, these associations remain significant (for the rs1029273C-129M haplotype $P = 1.7 \times 10^{-4}$, for rs1029273C independent of 129M $P = 0.005$).

We also compared the clinical phenotype of 4-OPRI with slightly larger insertional mutations. We did not compare 4-OPRI with smaller insertions as these have been identified in healthy control populations and are not clearly pathogenic (Beck *et al.*, 2010). OPRI length had a profound effect on mean age of onset with 5- and 6-OPRI having earlier ages of onset. Mean (SD) age at onset in UK patients with 5-OPRI in those with codon 129MM was 42.3 (12.4) years (range 26–63 years, $n = 6$) (Mead *et al.*, 2007). Mean (SD) age of onset in UK patients with 6-OPRI in those with codon 129MM was 31.4 (5.7) years (range 20–49 years, $n = 30$) (Mead *et al.*, 2006); $P = 0.04$ for comparison between 4-OPRI and 5-OPRI, and $P < 0.001$ for 4-OPRI compared with 6-OPRI. The relationship between increasing size of octapeptide repeat insertion, codon 129, and age of clinical onset is shown in Fig. 1. Duration of disease was also significantly shorter ($P < 0.001$) compared with patients with 6-OPRI. However, we found no significant difference between 4- and 6-OPRI for a number of clinical parameters, including myoclonus, cerebellar, extrapyramidal or pyramidal signs.

We went on to consider the frequency and phenotypic effects of rs1029273C-129M in other inherited prion diseases. Here we tested samples from 144 individuals with various inherited prion diseases (5-OPRI, $n = 11$; 6-OPRI, $n = 51$; P102L, $n = 36$; P105L, $n = 1$; A117V, $n = 12$; D178N, $n = 5$; E200K, $n = 26$; and Q212P, $n = 2$). None of the other inherited prion diseases had an absolute association with the upstream risk factor; however, in the entire series, rs1029273C was strongly associated with disease status compared with healthy controls (odds ratio = 1.62, $P = 2.5 \times 10^{-4}$, $\chi^2$-test, 1 degrees of freedom) with an increased frequency of the risk allele in inherited prion diseases. This overall association is potentially confounded by linkage between rs1029273C and certain mutations causing the rs1029273 allele linked to the

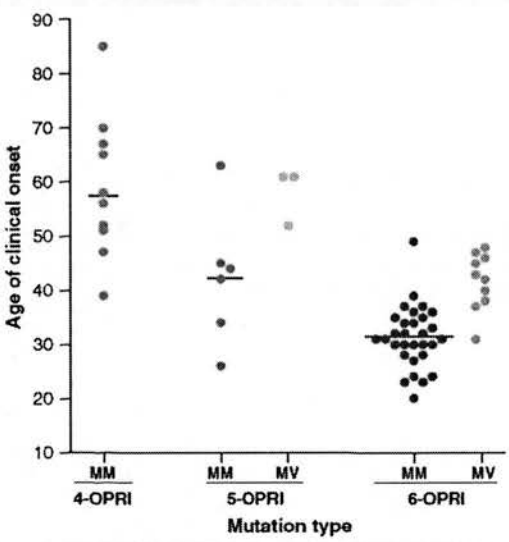


Figure 1 Correlation between mean age of onset and the number of OPRIs. Individual patient data shown (green, red and dark blue points) with means (horizontal black bars) for 4-, 5- and 6-OPRI, codon 129MM. Patients with a *PRNP* codon 129MV genotype are represented by yellow (5-OPRI) and light blue (6-OPRI) points, this genotype was not observed for 4-OPRI.

mutation to be more frequent because of hitch-hiking. It is known that the large UK kindred segregating a 6-OPRI mutation occurs on an rs1029273C-129M haplotype; however, large UK kindreds segregating P102L and A117V occur on rs1029273T-129M and rs1029273T-129V haplotypes. The haplotype background of other mutations is not known with certainty; microsatellite haplotype analysis in P102L, E200K, D178N and 5-OPRI is most consistent with multiple ancestral events. As a result, the mutation haplotype background has little overall impact on the frequency of rs1029273C and adjusting for the most probable background where known, had little effect on the statistical association reported above.

We went on to consider the strength of the association of rs1029273 in different inherited prion diseases, such as those most similar to 4-OPRI on the basis of reduced penetrance and a CJD-like clinical phenotype (e.g. 5-OPRI, E200K, D178N). In this

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analysis, we considered only 129MM individuals in cases and controls to exclude the possibility of an association being confounded by hitch-hiking with codon 129. In these three inherited prion diseases there were 47 rs1029273C alleles and 15 rs1029273T alleles, compared with 288/188 in controls, respectively ($P = 0.019$, $\chi^2$-test, 1 degrees of freedom). A similar analysis in the early-onset, highly penetrant inherited prion diseases (e.g. 6-OPRI, P102L, A117V) showed no significant associations independent of the codon 129 genotype. The rs1029273 genotype did not have any effect on the age of onset of inherited prion disease (for example, 6-OPRI, age of onset for rs1029273C = 33 years, rs1029273T = 31 years; $n = 27$), suggesting that in 4-OPRI, and possibly other similar inherited prion diseases, the wild-type chromosome determines the risk of a patient getting disease at any age rather than modifying the age of onset of disease itself.

## Electroencephalography

EEGs were performed in nine patients. Two showed characteristic periodic complexes associated with sporadic CJD. One was 'highly suggestive' of sporadic CJD and a further five were abnormal but not suggestive of CJD. One was normal. Brief summaries of individual reports are provided in the appropriate patient histories (Supplementary Material).

## Neuroimaging

CT scans were performed on seven patients (Patients I, II, III, V, VII, VIII, IX and X) and MRI on five (Patients III, IV, VI, VIII and X). Findings varied from normal appearances to generalized cortical atrophy with cerebellar involvement, with one case having right caudate head and temporal lobe high signal (appearances considered suspicious but not diagnostic of CJD; Case III). In one patient, a left-sided subdural haematoma was seen (Patient VIII). Detailed information about sequences performed for some patients was not available, and were often variable between patients as scans were performed at different stages of magnetic resonance technology.

## Neuropathology

Seven patients had post-mortem examinations. Patients II, III and VIII were examined in detail (refer to 'Materials and methods' section). A brief summary of salient neuropathological features for other patients with examined post-mortem is included in the patient histories (Patients I, VI, VII and X); however, the three described below are representative of the findings overall.

The macroscopic finding of a subdural haematoma was histologically confirmed in Patient VIII, showing dura mater as well as granulation tissue with intensive capillarization and haemosiderin deposition. In all three cases examined in detail, there was moderate to severe spongiform degeneration in all neocortical areas, often in a patchy distribution. This was associated with a moderate, focally severe neuronal loss with destruction of cortical grey matter (Figs 2A and Supplementary Fig. 1A). In these areas, there was also a severe proliferation of astrocytes, highlighted by glial fibrillary acidic protein immunostaining. Prion protein immunostaining showed a very delicate network of twisted prion protein-positive structures, which were most likely axons and dendrites (Fig. 2B). Typical synaptic prion protein deposits were not seen. Also, thin thread-like structures in the cortex and white matter were labelled, probably corresponding to intra-axonal abnormal prion protein (Fig. 2C and Supplementary Fig. 1B). There was no synaptic pattern and no deposition of plaques anywhere in the cortex. In contrast, the cerebellum showed features that are distinct, and very different from the pathology in the neocortex: there was no cerebellar spongiform degeneration in Case VIII, and only moderate gliosis that was pronounced in the molecular layer. Cases II and III showed moderate microvacuolar spongiform change in the molecular layer (Supplementary Fig. 1C). Prion protein immunostaining showed a very characteristic and unique pattern, where the staining appeared to extend along Purkinje cell processes or was contained within Purkinje cell processes in the molecular layer oriented perpendicularly to the surface. Small rounded deposits were also present in the granular layer in Cases II and III (Fig. 2F and G and Supplementary Fig. 1D). No amyloid plaques were present in the cerebellum. There was also deposition of hyperphosphorylated tau, which varied in shape and extent: while the occipital cortex showed the most intense deposition of hyperphosphorylated tau with formation of neuropil threads and occasional intraneuronal tangles (Fig. 2E), all other cortical areas showed much smaller, stub-like inclusions (Fig. 2D). The cerebellum was almost devoid of hyperphosphorylated tau structures, with only occasional granular inclusions (Fig. 2H). No beta-amyloid was seen in any of these areas.

## Expression analysis

We hypothesized that the susceptibility effect of the rs1029273C-129M haplotype was conferred by increased expression of wild-type prion protein. We tested this by measuring *PRNP* expression in whole blood against selected control genes. Healthy UK blood donors ($n = 145$) were sampled, 51 were homozygous for methionine at codon 129, 14/51 had rs1029273CC genotype, 24/51 were CT heterozygous and 13/51 were TT homozygous. Normalized *PRNP* expression levels varied from 0.76 to 1.44 with a mean of 1.14 (95% CI 1.02–1.26, $P = 0.03$, $t$-test) in CC homozygous individuals relative to TT homozygous individuals, with CT heterozygous individuals having intermediate levels of expression. When individuals were ranked by *PRNP* expression, the top four individuals with highest levels of relative *PRNP* expression were rs1029273C-129M homozygous.

## Prion protein immunoblotting

Frozen brain was available for analysis from Patients III, VI, VII and VIII. Frontal cortex (grey matter) was prepared as 10% w/v homogenate and analysed after limited digestion with proteinase K by immunoblotting using anti-prion protein monoclonal antibody 3F4. All four patient brain samples showed prominent protease-resistant $PrP^{Sc}$ fragments in the molecular mass range of ~19–30 kDa with no evidence for the generation of lower molecular mass prion protein fragments or for the co-existence of different $PrP^{Sc}$ types within the same brain sample (Fig. 3).

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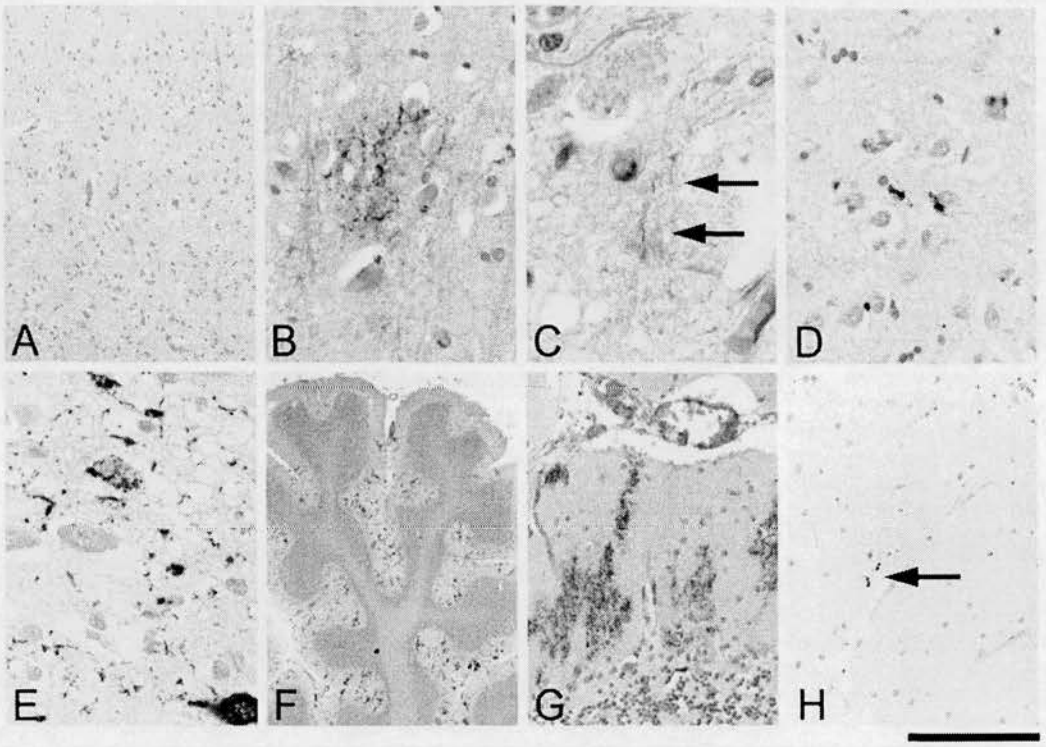


Figure 2 Pathology in Patient VIII with 4-OPRI. (**A**) Haematoxylin–eosin-stained section of the frontal cortex with severe spongiform degeneration and neuronal loss. (**B**, **C**) Prion protein deposition occasionally forms small 'woollen' structures in the deep cortical layers, and distinct, delicate labelling of axons is seen in the grey (**C**, arrows) and white matter. In the cortex, variable amounts of phosphorylated tau were seen, which were scattered and sparse in the frontal and temporal (**D**), and more abundant in the occipital cortex (**E**). Prion protein deposits form a striking, 'tigroid' pattern, which is oriented perpendicularly to the surface and is limited to the molecular layer (**F**, **G**). Very rarely, there are also deposits of phosphorylated tau (**H**, arrow).

Previous analysis of brain from 4-OPRI Patients VI and VII together with brain tissue from patients with 2- and 6-OPRI (Hill *et al.*, 2006) showed that the $PrP^{Sc}$ types associated with these OPRI mutations are apparently indistinguishable with respect to glycoform ratios and fragment sizes from those observed in classical CJD (Hill *et al.*, 2003; Wadsworth *et al.*, 2008). Consistent with these findings, 4-OPRI Patient III propagated $PrP^{Sc}$ with a type 2 fragment size (Fig. 3) that was indistinguishable from type 2 $PrP^{Sc}$ seen in 4-OPRI Patient VII (Fig. 3). 4-OPRI Patient VIII propagated $PrP^{Sc}$ with a type 3 fragment size (Fig. 3) that we have previously only observed in a single *PRNP* codon 129 methionine homozygous patient with sporadic CJD (Hill *et al.*, 2003). The clear demonstration of the propagation of different $PrP^{Sc}$ types among patients with 4-OPRI provides a molecular basis for phenotypic variability, although the small patient cohort examined here precludes significant analysis. However, in this context, it is interesting to note that Patient VI, who propagated type 1 $PrP^{Sc}$, had a short clinical duration and that this mirrors the association of type 1 $PrP^{Sc}$ and short clinical duration seen in sporadic CJD (Wadsworth *et al.*, 1999; Hill *et al.*, 2003).

# Discussion

We have presented the clinical, neuropathological and molecular genetic analysis of 10 patients with the 4-OPRI mutation of *PRNP*. All patients were homozygous for methionine at codon 129 and for the previously described sporadic CJD risk allele, rs1029273C. This statistically significant finding suggests that the risk of disease conferred by the 4-OPRI mutation alone may be enhanced by the wild-type chromosome. These additional factors being absent from relatives could account for the reduced penetrance of the mutation indicated by a relatively low frequency of familial concurrence of disease in these pedigrees. While this possibility might have been confirmed by genetic testing of healthy elderly relatives of 4-OPRI probands, there would be major implications for the families and this was not done. The sample of patients analysed was necessarily small and therefore the statistical significance of our genetic findings would not be robust to the addition of several further patients with 4-OPRI with different genotypes at codon 129 and rs1029273.

The effect of heterozygosity of the polymorphic codon 129 of *PRNP* on susceptibility to and/or incubation time is well

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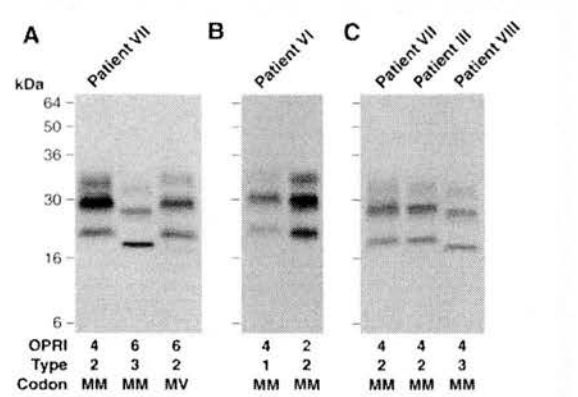


Figure 3 Prion protein immunoblots in 4-OPRI and other inherited prion diseases. $PrP^{Sc}$ types in Patients III, VI, VII and VIII. **(A–C)** Immunoblots of proteinase K digested brain homogenate from patients with different *PRNP* OPRI mutations. The size of OPRI mutation is designated below each immunoblot together with $PrP^{Sc}$ proteolytic fragment size [using the London classification of $PrP^{Sc}$ types (Hill *et al.*, 2003)] and *PRNP* codon 129 genotype, methionine (M) or valine (V). Immunoblots were developed with anti-prion protein monoclonal antibody 3F4 using a chemiluminescent substrate.

documented in sporadic, acquired (Collinge *et al.*, 1991; Palmer *et al.*, 1991) and some forms of inherited prion disease (Poulter *et al.*, 1992). In 5-OPRI, 6-OPRI, P102L, A117V and F198S, heterozygosity at codon 129 is associated with an older clinical onset compared with homozygosity (Dlouhy *et al.*, 1992; Poulter *et al.*, 1992; Mead *et al.*, 2007; Webb *et al.*, 2008, 2009). Prion disease susceptibility is thought to be conferred on a molecular level by homotypic protein–protein interactions (Palmer *et al.*, 1991) with an important role for the degree to which the wild-type and mutant prion proteins can adopt the same pathogenic conformations (Collinge, 1999; Hill and Collinge, 2003; Collinge and Clarke, 2007). Given that 4-OPRI with 129MM has an older clinical onset than many other inherited prion diseases, it is reasonable to predict that the age of onset of 4-OPRI with 129MV might lie beyond the average human lifespan. This would therefore explain the absence of patients with 129MV in our cohort. While this explanation is plausible, it would also be possible that the presence of the 129V on the wild-type allele prevents the stable generation of prions in 4-OPRI.

Our analyses support the concept of variable penetrance of the 4-OPRI mutation that is dependent upon more than just homology between mutant and wild-type prion protein at codon 129. Additional factors upstream of *PRNP* presumably act by altered expression of wild-type prion protein. Increased prion protein expression has been known for many years to be a potent susceptibility factor in transgenic mice (Bueler *et al.*, 1993), and we now report modestly increased expression of *PRNP* in blood conferred by the risk haplotype. We assumed that the rs1029273C-129M haplotype on the mutant chromosome was shared between all patients because of common ancestry. This conservative assumption was only partially supported by our haplotype analysis, which was in fact most consistent with several different mutational events. In this latter scenario, the observation that all mutant alleles were also associated with the rs1029273C-129M haplotype lends further support to the increased susceptibility conferred by this haplotype.

Four-OPRIs are the smallest insertional mutations of *PRNP* that are clearly pathogenic. Smaller (1-OPRI and 3-OPRI, but not 2-OPRI) insertions have been found incidentally and in healthy control populations (Beck *et al.*, 2010). Four- through 5- to 6-OPRI shows a dramatically earlier clinical onset. Insertional mutations larger than 5-OPRI show similar ages of clinical onset (Mead *et al.*, 2006). Thus, a correlation between insertion size and clinical phenotype is clear over this short range, and not for shorter or longer insertions. These clinical observations may form the basis for an informed correlation between molecular properties of mutant prion proteins and the consequences for the onset and clinical phenotype of the human disease.

Epistasis was initially used by Bateson in 1909 to describe the masking effect of an allele at one locus over a variant at another locus. The term is now more broadly used to describe an interaction between different loci, and has recently received a great deal of interest in complex disease genetics. Epistasis has been implicated in a number of human conditions, such as inflammatory bowel disease (Cummings *et al.*, 2007), colorectal cancer (Felix *et al.*, 2006) and among HLA class II alleles in human immune responses (Lincoln *et al.*, 2009). However, to our knowledge, there are no examples of neurological diseases caused by a mutation associated with a common susceptibility haplotype. Our 4-OPRI inherited prion disease case series may thus provide a useful precedent for understanding how a rare high-risk mutation might typically manifest as an apparently sporadic neurodegenerative disease.

The immunohistochemistry showed a distinct pattern from sporadic CJD. There was very delicate staining of axons and dendrites, rather than synaptic or uncommon plaque-forming deposits that are seen in the sporadic form. The cerebellum in these patients also showed prion protein deposits that seem to follow the dendrites of the Purkinje cells, in that they extend perpendicularly to the surface, giving the cerebellum a 'tigroid' appearance. This appearance is similar to previously reported cases with 4-OPRI, and the pattern of cerebellar prion protein deposition is also seen in cases with 5-OPRI (Mead *et al.*, 2007) or 6-OPRI (Capellari *et al.*, 1997; King *et al.*, 2003; Kovacs *et al.*, 2007) but not that of longer (8-OPRI) inserts, which show deposition of frequent amyloid plaques in the cerebellum and the forebrain (Laplanche *et al.*, 1999). In contrast to previous reports, we consistently observed fine, thread-like intra-axonal prion protein deposits in Case VIII and also in cases with 6-OPRI (Reiniger *et al.*, 2010), most likely due to a more sensitive detection method for abnormal prion protein. Remarkably, there was frequent deposition of hyperphosphorylated tau in several cortical areas, even with a very subtle involvement of the cerebellum as reported earlier (Reiniger *et al.*, 2010). Although this tau pathology may have arisen independently from the prion disease, the role of the prion disease in triggering hyperphosphorylation and a secondary tauopathy remains likely, in particular, as no deposition of beta-amyloid was seen in any of the brain regions examined.

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The demonstration that the $PrP^{Sc}$ types seen in patients with 4-OPRI are apparently indistinguishable from those seen in patients with sporadic CJD of the same codon 129 genotype appears difficult to reconcile with the distinct pattern of prion protein immunohistochemistry seen in 4-OPRI brain. However, in this context, it is important to note that we probe $PrP^{Sc}$ conformation by looking at accessibility to scissile bonds cleaved by proteinase K at the N-terminus of the protein. While the sensitivity of the N-terminal third of $PrP^{Sc}$ to cleavage by proteinase K does not appear to be altered by a 4-OPRI mutation, this mutation may confer distinct conformational preferences to the N-terminus, which could influence the kinetics of replication or the clearance of mutant $PrP^{Sc}$, thereby accounting for a distinct neuropathological phenotype. Methods other than protease digestion will now be required to reveal conformational differences between full-length $PrP^{Sc}$ in patients with sporadic CJD and 4-OPRI.

In summary, we report evidence for a susceptibility haplotype that may be an important determinant of penetrance in 4-OPRI and possibly other similar inherited prion diseases. While the molecular basis of the susceptibility allele is unknown, one possibility is that increased risk is conferred by a combination of both homology at codon 129 and higher levels of expression of *PRNP*. This example may provide a valuable model for understanding other sporadic neurodegenerative diseases.

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# Supplementary material

Supplementary material is available at *Brain* online.

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# Elevated phosphorylated tau pT-181 in a possible *PRNP* codon 129 MV vCJD case

AJE Green, MM Andrews, MT Bishop, et al.

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## LETTER

# Elevated phosphorylated tau pT-181 in a possible *PRNP* codon 129 MV vCJD case

### CASE REPORT

A 30-year-old man died of a progressive neuropsychiatric illness of approximately 17 months' duration with a clinical picture strongly suggestive of variant Creutzfeldt–Jakob disease (vCJD).[1] His initial symptoms, at the age of 28, were of personality change with anxiety and irritability. Lower abdominal and leg pain were troublesome early symptoms. At about 12 months of illness, he developed tremor and unsteadiness, leading to progressive walking problems. Increasing social withdrawal and behavioural problems were complicated by the development of progressive memory and general cognitive impairment. Visual hallucinations and paranoid delusions occurred in the later parts of his illness. During the course of his illness, he developed the following neurological signs: cognitive impairment, limb and gait cerebellar ataxia, mild dysarthria and mild pyramidal signs. Extensive neurological investigations revealed no cause other than prion disease, including consideration of a wide variety of inflammatory, neoplastic, immunological and neurodegenerative illnesses. The EEG showed diffuse slow activity without periodic complexes. The cerebral MRI showed changes suggestive of vCJD but did not show the characteristic pulvinar sign.[2] No tonsil biopsy or neuropathological examination was performed. Sequencing of the *PRNP* gene showed no pathogenic mutation and revealed the patient to be heterozygous (methionine (M)/valine (V)) at codon 129 and heterozygous for the common synonymous substitution at codon 117. The final formal case classification of this patient was of possible vCJD,[3] but the clinical opinion is that this is very likely to have been the first instance of vCJD in a *PRNP* codon 129 non-MM individual.

Cerebrospinal fluid (CSF) analysis was performed as part of the routine investigations, no abnormalities were found in white cell count, total protein or glucose concentrations, and no bacteria were grown on culture. In light of the normal white cell count and lack of bacterial growth, the CSF sample was considered to be sterile, and no further investigations were carried out. CSF was sent to the National CJD Surveillance Unit for the analysis of 14-3-3 and other brain-specific proteins. CSF 14-3-3 was detected using western blotting with chemiluminescent detection,[4 5] while CSF S-100b,[4] tau protein[5 6] and tau protein phosphorylated at threonine 181 (pT-181)[6] were measured using enzyme-linked immunosorbent assays (ELISAs). The results are shown in table 1.

**Table 1** Cerebrospinal fluid brain-specific protein results

| Analyte | Result |
|---|---|
| 14-3-3 | Positive |
| S-100b | 0.67 ng/ml (reference range: <0.41) |
| Tau protein | 2110 pg/ml (reference range: <500) |
| Phosphorylated Tau (pT-181) | 124 pg/ml (reference range: <120) |

### RESULTS AND DISCUSSION

Positive CSF 14-3-3 results and elevated S-100b and tau protein concentrations have been reported in both vCJD[4] and sporadic CJD (sCJD).[5] Thus, these results are consistent with either diagnosis. However, pT-181 concentrations have been reported to be raised only in vCJD and not in sCJD[6]; this study did not investigate the influence of *PRNP* codon 129 genotype on CSF pT-181 concentrations in sCJD. CSF pT-181 concentrations have been analysed by the National CJD Surveillance Unit (NCJDSU) in 64 neuropathologically proven and 43 clinically probable vCJD patients (57M:50F; age at onset disease 30.3±9.9 years, range 14–63 years) and 108 neuropathologically proven sCJD patients (58M:50F; age at onset of disease 64.5±12.5 years, range 27–87 years). The *PRNP* codon 129 genotype was available in 100 of the sCJD cases: 46 patients were MM, 37 were MV, and 17 were VV. Ninety-two of the vCJD cases had a *PRNP* codon 129 genotype analysis undertaken, and all were MM. The mean±SD CSF pT-181 concentrations for vCJD and sCJD were found to be 112.8±88.6 versus 49.3±28.4 pg/ml, $p<0.0001$ respectively. The mean±SD CSF pT-181 concentration for 65 disease control patients (38M:27F; age at onset of disease 61.3±15 years, range 14–89 years) who were initially suspected of having either vCJD or sCJD but who had neuropathological evidence of an alternative disease was found to be 40.9±31.0 pg/ml. Of these patients, 31 had neurodegenerative diseases such as Alzheimer's disease, Lewy body dementia, frontotemporal dementia, Parkinson's disease or Huntington's disease; 13 had malignant disease involving the central nervous system such as paraneoplastic syndrome or primary cerebral malignancies; six had cerebrovascular disease; six had no neuropathological evidence of CJD, but no alternative diagnosis could be made; two had hypoxic brain injury, and there was one case of each of the following: multiple sclerosis, subacute panencephalitis, encephalitis, multifocal demyelination, vasculitis,, progressive multifocal leucoencephalopathy and normal pressure hydrocephalus. A reference range for pT-181 of less than 120 pg/ml was calculated using the mean+2.5×SD of the pT-181 concentrations in these control patients. Elevated CSF pT-181 levels were found in 33 vCJD patients and in one patient with sCJD. The sCJD patient was heterozygous (MV) at *PRNP* codon 129. Thus, the CSF pT-181 concentration of 124 pg/ml found in our patient is more in keeping with a diagnosis of vCJD than of sCJD.

CSF brain-specific protein investigations are not part of the formal diagnostic criteria for vCJD, and while these results are not conclusive, they support the diagnosis of vCJD in this patient on the balance of probability. Further investigations are under way investigating the role phosphorylated tau plays in the pathogenesis of vCJD and whether phosphorylated tau can be exploited to develop more specific diagnostic CSF tests for vCJD.

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# The role of cerebrospinal fluid 14-3-3 and other proteins in the diagnosis of sporadic Creutzfeldt–Jakob disease in the UK: a 10-year review

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## ABSTRACT

**Background** It is 10 years since the detection of cerebrospinal fluid (CSF) 14-3-3 was included in the diagnostic criteria for sporadic Creutzfeldt–Jakob disease (sCJD) by the WHO. Since that time, other CSF proteins, such as S100b and tau protein, have been proposed as surrogate markers for sCJD. The authors aimed to investigate the diagnostic value of each of these three proteins.

**Methods** CSF samples collected from patients who were referred to the National CJD Surveillance Unit as suspected cases of sCJD during the period 1997–2007 were analysed for 14-3-3, S100b and tau protein. The sensitivity, specificity, positive predictive value and negative predictive value of each of these markers, either alone or in combination for the diagnosis of sCJD, were assessed. The impact of CSF 14-3-3 analysis on the case classification of sCJD was investigated.

**Results and discussion** CSF 14-3-3 had the greatest sensitivity (86%) when compared with tau protein (81%) and S100b (65%). The combination of a positive CSF 14-3-3 or an elevated tau protein with a raised S100b had the highest positive predictive power for sCJD. During the study period, 100 patients were classified as probable sCJD solely on the basis of the clinical features and a positive CSF 14-3-3. The most sensitive marker for sCJD was a positive CSF 14-3-3. The analysis of CSF 14-3-3 plays a crucial role in the case classification of sCJD.

## INTRODUCTION

Creutzfeldt–Jakob disease belongs to a family of fatal neurodegenerative diseases collectively known as human transmissible spongiform encephalopathies. Sporadic CJD (sCJD) remains the most prevalent form worldwide, with an annual incidence of 1.0–1.5/million/year.[1] Human spongiform encephalopathies are characterised by the accumulation of pathological prion protein (PrP) in the central nervous tissue. The pathological isoform is termed $PrP^{Sc}$, and this differs from the normal cellular isoform by its high content of β-sheet structure and partial resistance to protease digestion.[2] Additional histological changes identified include spongiosis, neuronal loss and gliosis. Neuropathological studies remain the only means of obtaining a definitive diagnosis; the initial diagnosis however is still dependent on the clinical phenotype, as defined by the WHO criteria.[3] Marked phenotypic heterogeneity is well documented in all human prion diseases, and in sCJD this observation is yet to be explained. Early experimental transmission studies on primates,[4] subsequent epidemiological studies[5 6] and isolated case reports have allowed diagnostic criteria to be refined over the years; however early diagnosis and in turn accurate surveillance remains a challenge. Atypical forms of sCJD are well recognised, it has been postulated that such variation is partially dependent on, or associated with, genetic and molecular factors, *PRNP* codon 129 genotype and PrP isotype respectively. The typical short duration disease phenotype is linked to methionine homozygosity and $PrP^{Sc}$ type 1.[2] The atypical and rarer variants (those defined as having a long duration of illness, young age of onset and unusual clinical or pathological features) are linked to valine homozygosity or heterozygosity at codon 129 and $PrP^{Sc}$ type 2.

The advent of novel diagnostic tests, specifically the cerebrospinal fluid (CSF) 14-3-3 protein, has also allowed improvements in classification over the last decade. The 14-3-3 has a better diagnostic utility than investigative tests such as the EEG.[7 8] However, the sensitivity of CSF 14-3-3 has been shown to vary, partially dependent on the genetic and molecular influences described above.[9] The detection of CSF 14-3-3 remains a supportive tool in the appropriate clinical context but as a solitary test, independent of clinical phenotype, has little value.

The major differential diagnoses of sCJD remain those of other irreversible neurodegenerative conditions; however a small proportion of patients may have a potentially treatable condition. Therefore, CSF analyses, such as cell count and total protein, are an important early investigation in these patients, and CSF 14-3-3 is often performed at this time. Many conditions associated with acute neuronal damage may result in a positive CSF 14-3-3 and thereby reduce the specificity of CSF 14-3-3 for sCJD.[8] Therefore, other brain-specific proteins in the CSF may be of value as diagnostic markers. These additional markers include the CSF astrocytic marker S100b and the neuronal marker tau, protein in isolation or in combination.

In this study, we aim to first review the sensitivity and specificity of each CSF protein in sCJD and also review the potential role of a combination of several markers to improve sensitivity and specificity in the clinical diagnosis of sCJD. Second, the impact of the CSF 14-3-3 on UK surveillance is


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of great importance and is therefore considered, specifically reviewing the number of cases of probable sCJD classified on the basis of CSF 14-3-3. In addition, those cases classified as a probable case of sCJD on the basis of a positive 14-3-3, but found to have an alternative pathological diagnosis, are investigated. The additional role of combining neuronal markers in order to potentially exclude sCJD is reviewed.

## MATERIALS AND METHODS

### Patients

The National CJD Surveillance Unit (NCJDSU) was established in May 1990 to prospectively identify and record all suspected cases of sCJD in the UK. The primary aim of the programme was to detect any change in the epidemiology of the disease that might be attributable to bovine spongiform encephalopathy, and a distinct clinical–pathological phenotype was described in 1996 (variant CJD). Global surveillance of CJD has continued within the UK, and active surveillance has improved due to strong collaborations with the neuroscience community. Patients are referred and, where possible, visited. A detailed history of the current illness and past medical history, including potential risk exposure, is undertaken. Each case is further investigated by clinical examination and review of clinical investigations. Investigative tests which are potentially supportive for a diagnosis of sCJD such as an EEG and MRI are reviewed by a member of the NCJDSU. The NCJDSU acts as a referral centre for CSF 14-3-3 analysis throughout the UK.

During the period 1997 and 2007 inclusive, 245 cases of neuropathologically confirmed sCJD[10] (117 female, 128 male aged 27–87 years (mean 65.8±9.7 years) at notification), 163 cases of clinically probable sCJD[3] (82 female, 81 male aged 41–92 years (mean 68.0±9.8 years) at notification) and 171 disease control cases (86 females, 85 males aged 28–89 years (mean 66.4±11.4 years) at notification) who had CSF 14-3-3 analysis were identified for this study. Cases classified as not suffering from CJD (disease control cases) included those with a pathologically proven alternative diagnosis or those provided with an alternative clinical diagnosis by either the clinical team or by a member of the NCJDSU (table 1).

**Table 1** Disease control cases (pathologically proven alternative diagnosis or alternative clinical diagnosis)

| Alternative pathological proven diagnosis | No of cases |
|---|---|
| Alzheimer's disease | 14 |
| Biopsy/post-mortem showed no evidence of CJD | 12* |
| Malignancy/paraneoplastic syndrome | 10† |
| Lewy body dementia | 4 |
| Cerebral lymphoma | 4 |
| Encephalitis | 4 |
| Parkinson's disease | 4 |
| Cerebrovascular disease | 3 |
| Alzheimer's disease and ischaemic change | 3 |
| Alzheimer's disease and Lewy body dementia | 3 |
| Progressive multifocal leucoencephalopathy | 1 |
| Normal pressure hydrocephalus | 1 |
| Fronto-temporal dementia | 1 |
| Corticostriatal degenerative disease | 1 |
| Demyelination | 1 |
| **Alternative clinical diagnosis** | **No of cases** |
| Dementia (unknown aetiology) or clinically not CJD | 22 |
| Clinically improved with or without steroids | 22 |
| Alzheimer's disease | 18 |
| Cerebrovascular disease/cerebral vasculitus | 9 |
| Fronto-temporal dementia | 7 |
| Lewy body dementia | 6 |
| Paraneoplastic syndrome | 6 |
| Psychiatric disorder | 4 |
| Huntington's disease | 3 |
| Hashimoto's encephalitis | 2 |
| Corticobasal degeneration | 2 |
| Multisystem atrophy | 1 |
| Granulomatous disease | 1 |
| Central pontine myelinolysis | 1 |
| Serotonin syndrome | 1 |

*One patient clinically not thought to have had Creutzfeldt–Jakob disease (CJD) and post-mortem conducted but brain tissue not examined.
†One patient had a western blot for $PrP^{Sc}$ which was negative; clinically this patient is thought to have a paraneoplastic process.

### CSF protein analysis

CSF samples are sent to the laboratory on dry ice and stored at −80°C prior to analysis. For this study, CSF 14-3-3, S100b and tau protein were analysed. Protein 14-3-3 in CSF was detected by western blotting after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) with chemiluminescent visualisation.[11–13] A positive, negative and two weak positive 14-3-3 controls were included on each run. These were controls from patients with neuropathologically confirmed sCJD (positive control) or from patients with either a clinical or pathological diagnosis of an alternative disease (negative and weak positive controls). The relative immunoreactivity of positive, negative and weak positive 14-3-3 CSF samples is given in figure 1. The blots were independently assessed by two people (AJEG, MA, GC or CP), and only positive CSF 14-3-3 results were used for case classification. CSF S100b was measured using a previously reported sandwich enzyme-linked immunosorbant assay.[11] A concentration of <0.5 ng/ml was considered to be normal, while a concentration of >1.0 ng/ml was considered to be diagnostic. CSF tau protein was measured using an enzyme immunoassay (Innotest hTAU-Ag, Innogenetics, Ghent, Belgium), according to the manufacturer's recommendations. A concentration of >1260 pg/ml was considered to be diagnostic.[14] This assay measures total tau protein concentrations and as such measures both normally phosphorylated tau protein and hyper-phosphorylated tau protein. Some CSF samples had insufficient volume for all three analytes to be measured.

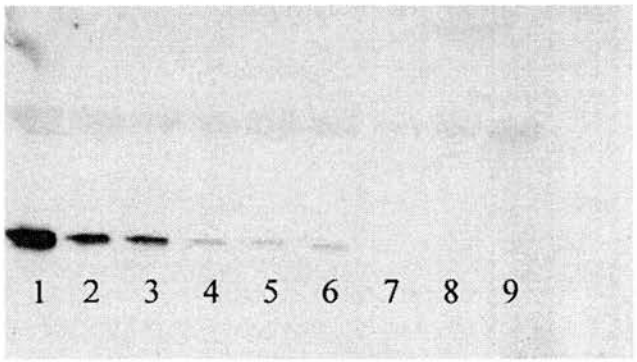


**Figure 1** Western blotting illustrating the presence of positive, weak positive and negative cerebrospinal fluid (CSF) 14-3-3. Lanes 1, 2 and 3 are positive for CSF 14-3-3; lanes 4, 5 and 6 are weakly positive for CSF 14-3-3; and lanes 7, 8 and 9 are negative for CSF 14-3-3. Lanes 1 and 2 are from two patients with sporadic Creutzfeldt–Jakob disease (sCJD), lanes 3 and 4 are from patients who have had a stroke, and the remaining lanes contain CSF samples from patients who do not have CJD.

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### *PRNP* codon 129 genotype and PrP isotyping

PrP isotyping was performed on all suspected cases of prion disease where fresh brain tissue was received by the NCJDSU. Small quantities of cerebral cortex were homogenised and treated with proteases, and the size and abundance of the three $PrP^{Sc}$ glycoforms was determined by western blot analysis.[15] Genotyping for polymorphism at codon 129 of the *PRNP* gene was carried out on all available blood specimens. DNA was extracted from blood using standard techniques and analysed using the Helsinki method.[16]

### Statistical analysis

A comparison of age by Mann–Whitney tests was carried out. Descriptive statistics were calculated for the sCJD and control patients. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency of each marker and for combinations of markers were obtained. To investigate the influence of the stage of disease on the sensitivity of CSF 14-3-3 and tau protein, we divided the disease duration for each patient into equal thirds. The stage in which the lumbar puncture (LP) was performed was noted, and the sensitivity of each marker for sCJD in each of the three stages was calculated.

## RESULTS

The sensitivity, specificity, PPV, NPV and efficiency of each neuronal marker for the diagnosis of neuropathologically confirmed sCJD cases are shown in table 2. CSF 14-3-3 is the most sensitive marker and has a higher sensitivity than CSF tau protein, 86% and 81% respectively. CSF S100b does not have adequate sensitivity (65%) to be used as an isolated marker of sCJD. The difference in sensitivity between CSF 14-3-3 and tau protein is not influenced by the different numbers of cases investigated for each analyte. The sensitivity of CSF 14-3-3 and *t* protein calculated using those samples where both analytes were measured is 85% and 81% respectively. The specificity of CSF 14-3-3 and tau protein in those samples where both analytes were measured is 74% and 85% respectively.

The combination of a positive CSF 14-3-3 with either an elevated S100b or an elevated tau protein increased the PPV of 14-3-3 from 83% to 94% or 91%, respectively (table 2, figure 2A, B). Likewise the combination of an elevated tau protein and an elevated S100b resulted in a higher PPV (95%) for sCJD when compared with using each marker alone (table 2, figure 2C). However, combining the tests either in pairs or taking all three markers together resulted in a reduction in sensitivity and a reduction in the efficiency of each marker. That is to say, the ability of each marker to distinguish between those patients with sCJD and those that present with symptoms similar to sCJD but turn out to have an alternative diagnosis is reduced if they are combined (table 2).

In the atypical subgroups, although the numbers are small, CSF 14-3-3 is more sensitive than an elevated tau protein (table 3). This finding is particularly noticeable in those patients who are 50 years old or younger at the onset of the disease. However, in those sCJD cases that were negative for either CSF 14-3-3 or tau protein, there did not seem to be any difference in the demographics of the patients or any obvious effect of codon 129 and $PrP^{Sc}$ isotype. In the 14-3-3 negative group, there were 35 sCJD cases (27–81 years (mean 62.0±10.9 years) at notification), and in the tau protein negative group there were 41 sCJD cases (44–81 years (mean 63.3±9.9 years) at notification). The disease duration was also comparable: 2–54 months (mean 15.1±11.7 months) and 2–54 months (mean 14.4±12.2 months) respectively. These disease durations are much longer than those in sCJD cases who are positive for CSF 14-3-3 (mean 6.6±6.8 months) or CSF *t*; protein (mean 6.5±6.6 months).

The effect of stage of disease on the sensitivity of CSF 14-3-3 and tau protein is shown in table 4. The time of the LP and the date of the onset of disease were only available in 209 patients. Only 7% of patients had an LP within the first stage of disease, while 42% and 51% of patients had CSF samples taken in the second and third stages of disease respectively. Both CSF 14-3-3 and tau protein show comparable sensitivity in the first two stages of disease, but in the final stage of the disease CSF 14-3-3 is more sensitive than CSF tau protein.

During the study period, 21 patients had a weak positive CSF 14-3-3. Of the 21 patients with weak positive CSF 14-3-3 results, nine patients had neuropathologically confirmed sCJD, while the remaining 12 patients had Alzheimer's' disease (three), Lewy body disease (two), no neuropathological evidence of CJD (two), frontal lobe degeneration (one), epilepsy (one), multifocal leucoencephalopathy (one) and angiotrophic lymphoma (one), and no further information could be obtained on the final diagnosis in the remaining patient.

The most specific individual marker was CSF S100b, but it also had the poorest sensitivity which limits its use as an isolated marker. CSF tau protein had a greater specificity than CSF 14-3-3 protein (85% vs 74%). Analysis of the control cases that had a positive CSF 14-3-3 and/or an elevated CSF tau protein showed that the most prevalent diagnoses included Alzheimer's disease, paraneoplastic syndrome and patients who clinically improved without an alternative diagnosis (table 5).

Out of a total of 242 cases of sCJD which had both CSF 14-3-3 and S100b measured, only 10 had a negative 14-3-3 and a normal S100b concentration of <0.5 ng/ml. Likewise, out of 216 sCJD cases that had both CSF tau protein and S100b measured, only 14 had concentrations of both markers within the normal range (figure 3A, B).

The impact of CSF 14-3-3 on UK surveillance over the last decade was assessed by examining the number of clinically

**Table 2** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency for each marker and combination of cerebrospinal fluid markers in neuropathologically confirmed sporadic Creutzfeldt–Jakob disease (sCJD)

| | 14-3-3 | Tau | S100b | 14-3-3 and S100b | Tau and S100b | 14-3-3 and tau | 14-3-3, tau and S100b |
|---|---|---|---|---|---|---|---|
| sCJD* | 210/245 | 175/216 | 158/243 | 151/242 | 127/216 | 162/216 | 123/216 |
| Not sCJD* | 44/171 | 20/135 | 17/169 | 9/169 | 7/135 | 16/135 | 6/135 |
| Sensitivity (%) | 86 (81 to 90) | 81 (75 to 86) | 65 (59 to 71) | 62 (57 to 69) | 59 (52 to 65) | 75 (69 to 81) | 57 (50 to 64) |
| Specificity (%) | 74 (67 to 81) | 84 (78 to 91) | 90 (84 to 94) | 95 (90 to 98) | 95 (90 to 98) | 88 (81 to 93) | 96 (91 to 98) |
| PPV (%) | 83 (77 to 87) | 90 (85 to 94) | 90 (85 to 94) | 94 (90 to 97) | 95 (90 to 98) | 91 (86 to 95) | 95 (90 to 98) |
| NPV (%) | 78 (71 to 84) | 74 (66 to 80) | 64 (58 to 70) | 64 (58 to 70) | 59 (52 to 66) | 69 (61 to 76) | 58 (51 to 65) |
| Efficiency (%) | 81 (77 to 85) | 83 (78 to 86) | 75 (71 to 79) | 76 (72 to 80) | 73 (68 to 77) | 80 (75 to 84) | 72 (67 to 76) |

Figures in parentheses are 95% confidence limits.

*The figures given are the number of positive or negative results divided by the total number of samples investigated. Efficiency was defined as the number of positive and true negative results divided by the total number of samples investigated.

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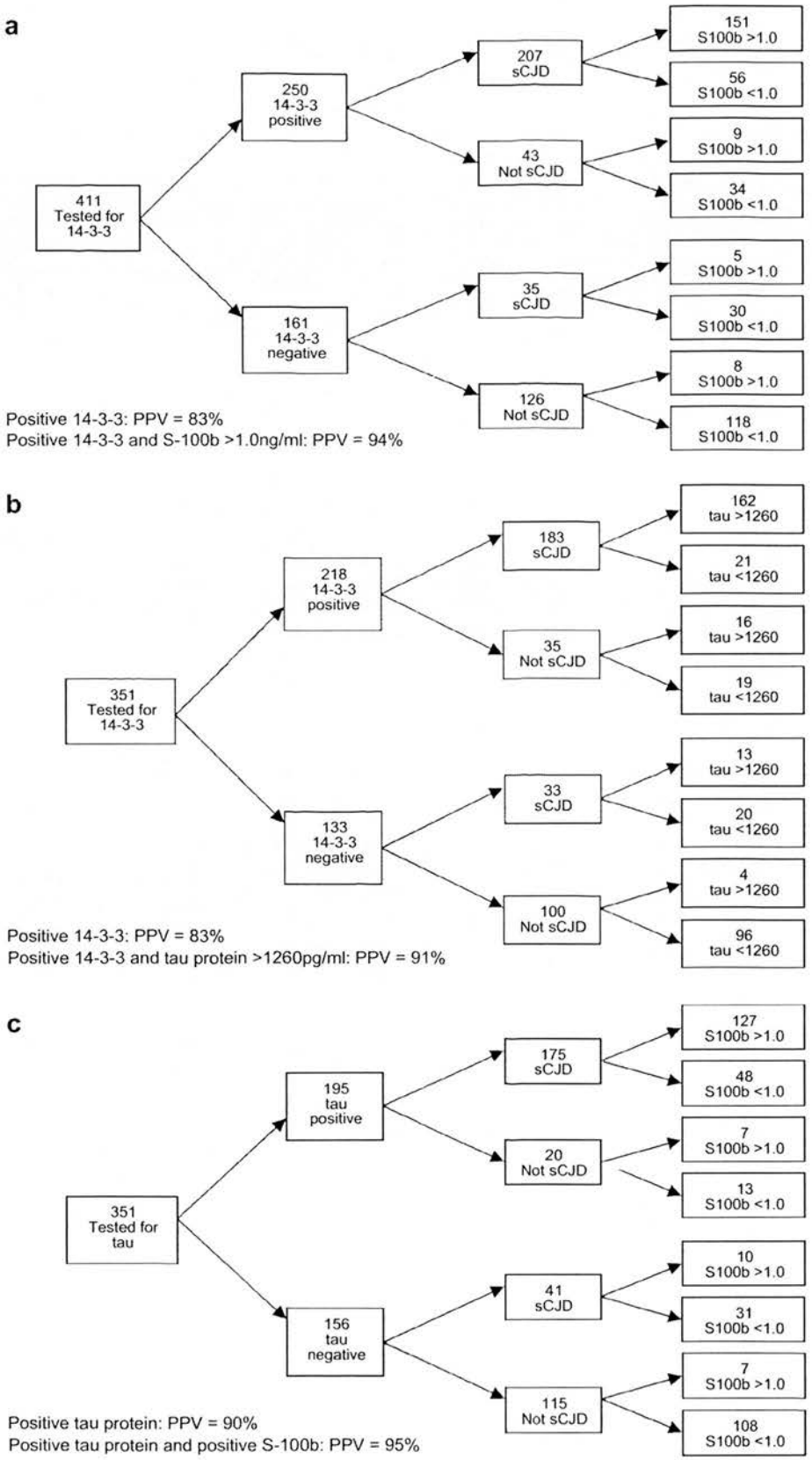


**Figure 2** (A) Role of a positive 14-3-3 and a diagnostic S100b in the diagnosis of sporadic Creutzfeldt–Jakob disease (sCJD). Positive 14-3-3: positive predictive value (PPV)=83%. Positive 14-3-3 and S-1000b>1.0 ng/ml=94. (B) Role of a positive 14-3-3 and an elevated τ protein in the diagnosis of sCJD. Positive 14-3-3: PPV=83%. Positive 14-3-3 and tau protein>1260 pg/ml: PPV=91%. (C) Role of a positive tau protein and an elevated S100b in the diagnosis of sCJD. Positive tau protein: PPV=90%. Positive tau protein and positive S-100b: PPV=95%.

probable cases classified as a result of a positive CSF alone. One hundred and sixty-three probable cases that died without post-mortem examination were identified. No information regarding whether an EEG had been performed was available in 25 cases. Of the remaining 138 cases, three had no EEG performed, and 135 had an EEG reviewed by a senior member of the NCJDSU.

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**Table 3** Effect of age at onset of disease, disease duration and *PRNP* codon 129 genotype on the sensitivity of CSF 14-3-3 and tau protein

| | 14-3-3 (%) | Tau protein (%) |
|---|---|---|
| <50 years at onset disease | 12/17 (71%) (44 to 90) | 10/16 (62%) (35 to 84) |
| ≥50 years at onset disease | 198/228 (87%) (82 to 91) | 165/200 (83%) (77 to 87) |
| >12 months' disease duration | 23/41 (56%) (40 to 72) | 21/39 (54%) (37 to 70) |
| ≤12 months' disease duration | 184/199 (92%) (88 to 96) | 151/175 (86%) (80 to 91) |
| *PRNP* codon 129-MM | 106/121 (88%) (80 to 93) | 92/108 (85%) (77 to 91) |
| *PRNP* codon 129-MV | 32/44 (73%) (57 to 85) | 26/39 (67%) (50 to 81) |
| *PRNP* codon 129-VV | 27/29 (93%) (77 to 99) | 24/27 (89%) (71 to 98) |

Results are expressed as number of positives divided by total number investigated. Figures in parentheses are the sensitivity of the individual marker in each subset of sCJD. The 95% confidence limits are given in parentheses after the percentages.

Of these 135 patients, 38 had an EEG classified as highly suggestive or typical and hence appropriate for use in classification. The remaining 97 were classified as probable with the aid of a positive CSF 14-3-3 (72%). Therefore, over the study period, at least 100 patients were classified as having probable sCJD on the basis of CSF 14-3-3 alone. Sixty-nine patients initially classified as probable sCJD on the basis of a positive 14-3-3 alone, with either an unsupportive or unobtainable EEG, had subsequent post-mortem examination. Neuropathological confirmation of sCJD was obtained in 66 of these cases. The remaining three cases were misclassified as probable sCJD but in fact had pathological confirmation of carcinomatosis of the meninges (14-3-3 positive, S100b 0.57 ng/ml, tau protein 3201 pg/ml) and Lewy body dementia (14-3-3 positive, S100b 0.57 ng/ml, tau protein 1170 pg/ml) and Alzheimer's disease (14-3-3 positive, S100b 0.94 ng/ml, tau protein 1294 pg/ml). This gives a misclassification rate of 4%.

## DISCUSSION

It is 10 years since a positive CSF 14-3-3 was added to the WHO diagnostic criteria for classifying probable sCJD. Since that time, many studies have reported a poorer sensitivity and specificity of CSF 14-3-3 than initially described, and have suggested that other markers of neuronal damage such as tau protein perform better. We have examined the diagnostic utility of CSF 14-3-3, tau protein and S100b analysis in the investigation of patients with suspected sCJD over a 10-year period. In addition, we have examined the overall impact that the inclusion of a positive CSF 14-3-3 has made on the diagnosis sCJD since it was introduced in 1997.

The sensitivity of a positive 14-3-3 in our study of sCJD is 86%, which is higher than that of tau protein (81%). The difference in sensitivity is more marked in the atypical forms of sCJD such as those who are younger than 50 years old at the onset of disease. In these cases, the sensitivity of CSF 14-3-3 is 71% compared with 62% for CSF tau protein. This supports the findings of a larger European-wide study that found that a positive CSF 14-3-3 was more sensitive than CSF tau protein in atypical sCJD cases.[9] CSF 14-3-3 is also more sensitive than CSF tau protein in the final stage of disease. This is important as this is the time at which the majority of CSF samples are taken.

**Table 4** Influence of time of cerebrospinal fluid sampling on cerebrospinal fluid 14-3-3 and *t* protein positive results

| Stage of disease | 14-3-3 (%) | Tau protein (%) |
|---|---|---|
| First stage (0–33% disease duration) | 9/14 (64%) (35 to 87) | 10/14 (71%) (42 to 92) |
| Second stage (34–66% disease duration) | 73/88 (83%) (73 to 90) | 72/88 (82%) (72 to 89) |
| Third stage (67–100% of disease duration) | 97/107 (91%) (83 to 95) | 89/107 (83%) (75 to 90) |

The time of the LP was calculated by expressing the time of LP from disease onset as a percentage of the total disease duration. The patients were classified into three groups depending on whether they had cerebrospinal fluid samples taken in the first, second or third stage of the disease. Results are expressed as the number of positives over total number investigated. The 95% confidence limits are given in parentheses after the percentages.

**Table 5** Diagnoses in disease controls with positive cerebrospinal fluid 14-3-3 and elevated tau protein cases

| Diagnosis (14-3-3 positive) | Pathological (26) | Clinical (18) |
|---|---|---|
| Alzheimer's disease | 7* | 2 |
| Malignancy/paraneoplastic | 5 | 1 |
| Improved/no evidence of Creutzfeldt–Jakob disease | 3† | 7 |
| Encephalitis/limbic encephalitis | 3 | 2 |
| Lewy body dementia | 1 | 0 |
| Parkinson's disease | 1 | 0 |
| Cerebrovascular disease | 0 | 2‡ |
| B cell lymphoma/cerebral lymphoma | 3 | 0 |
| Fronto-temporal dementia | 0 | 1 |
| Vasculitis | 0 | 1 |
| Central pontine myelinolysis | 0 | 1 |
| Multifocal demyelination | 1 | 0 |
| Anoxic brain injury | 1 | 0 |
| Normal pressure hydrocephalus | 1 | 0 |
| Corticobasal degeneration | 0 | 1 |
| **Diagnosis (*t* protein >1260 pg/ml)** | **Pathological (12)** | **Clinical (6)** |
| Alzheimer's disease | 3 | 1 |
| Malignancy/paraneoplastic | 3 | 0 |
| Improved or no evidence of Creutzfeldt–Jakob disease | 1 | 1 |
| Fronto-temporal dementia | 0 | 1 |
| Corticobasal degeneration | 0 | 1 |
| Encephalitis | 0 | 1 |
| Lymphoma | 1 | 0 |
| Cerebrovascular disease | 1‡ | 1 |
| Multifocal demyelination | 1 | 0 |
| Lewy body disease | 1 | 0 |
| Anoxic brain damage | 1 | 0 |

*Three had evidence of additional cerebrovascular disease, and two also had evidence of Lewy body dementia.
†One did not have their brain examined.
‡One had evidence of Alzheimer's disease.

A CSF S100b concentration of greater than 1.0 ng/ml increases the PPV of a positive CSF 14-3-3 from 83% to 94%, and an elevated CSF tau protein from 90% to 95%. CSF S100b is routinely analysed by the NCJDSU for this reason. CSF S100b is also useful in excluding disease, with a negative CSF 14-3-3 and CSF S100b concentration of less than 0.5 ng/ml having a NPV of 88%. Normal concentrations of CSF tau protein and CSF S100b concentration of less than 0.5 ng/ml have an NPV of 84%.

There are no differences in the age of onset of disease, disease duration or codon 129 status of sCJD patients who are negative for CSF 14-3-3 and those that are negative for CSF tau protein. Therefore, it is unlikely that the additional measurement of CSF tau protein will help improve the identification of CSF 14-3-3 negative sCJD cases.

Less than half the patients with weak positive CSF 14-3-3 results have sCJD, and this suggests that the diagnostic utility of weak positive 14-3-3 results is limited. However, a repeat CSF 14-3-3 analysis in this group of patients may be of value if the clinical circumstances warrant it.[9]

Elevated CSF tau protein has a better specificity for sCJD than CSF 14-3-3 (84% vs 74%). This is a similar finding to a recently published study where tau protein was felt to be the single best marker for sCJD with a specificity of 90%.[17] It is, however,

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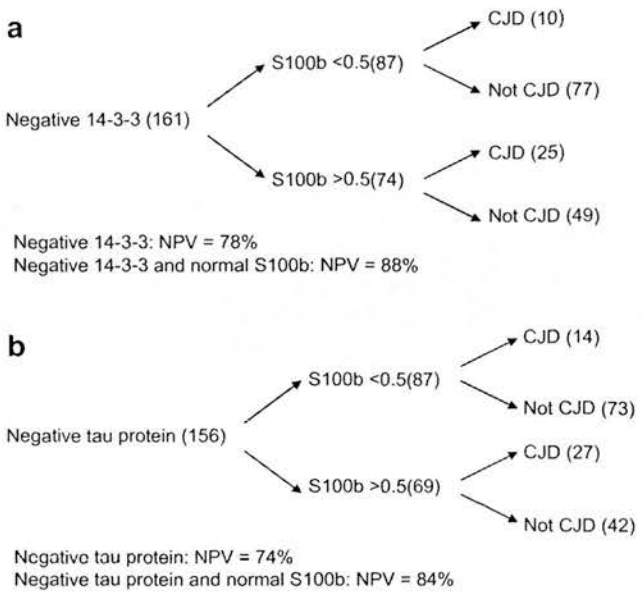


**Figure 3** (A) Role of a negative 14-3-3 and normal S100b in the diagnosis of sporadic Creutzfeldt–Jakob disease (CJD). (B) Role of a negative tau protein and a normal S100b in excluding sporadic CJD. NPV, negative predictive value.

important to note that the diagnostic test accuracy and the differences reported by these various studies are partially dependent on the cut-off concentration of tau protein used. A universally agreed level for tau protein is not currently available. The diagnoses in the non-CJD cases were similar for both tests. It is unclear why CSF tau protein should be of greater specificity than 14-3-3. The factors influencing the release of neuronal proteins in CJD and other conditions are not fully understood. Many studies have investigated the sensitivity of these two markers for the diagnosis of sCJD, but very few have compared their specificity. Indeed, specificity is highly dependent on the population investigated, and therefore it is very difficult to compare individual studies. However, the population investigated in this study is highly selected and consists of patients for whom the preceding pretest probability of sCJD is high.

### Box 1 Conclusions regarding the use of cerebrospinal fluid 14-3-3 and other markers in the diagnosis of sporadic Creutzfeldt–Jakob disease (sCJD)

- Cerebrospinal fluid (CSF) 14-3-3 as a sole marker has the highest sensitivity, particularly in the final stage of disease. As a sole marker CSF tau protein has the greatest specificity.
- A combination of CSF 14-3-3 and elevated S100b or elevated tau protein and S100b has a greater positive predictive value than CSF 14-3-3 alone
- The combination of CSF 14-3-3 and tau protein in CSF 14-3-3 negative sCJD cases has not been shown to be of any additional value, even in phenotypically atypical cases
- The combination of a negative CSF 14-3-3 and S100b is of value as a potential means of excluding sCJD
- The differential diagnosis of sCJD in the 14-3-3 positive non-CJD cases remains, as documented in previous studies

During the 10 years since its introduction, CSF 14-3-3 analysis has enabled 100 patients in the UK with suspected sCJD who died without a post-mortem and without supportive EEG data to be classified as probable sCJD. During this time, only three patients have been mis-classified as probable sCJD on the basis of a positive CSF 14-3-3.

We conclude that within the UK population referred with a clinical suspicion of sCJD to the NCJDSU over the last decade, brain-derived proteins such as 14-3-3, S100b and tau protein have immense diagnostic value (see box 1). In our experience, the combination of 14-3-3 and S100b remains the best predictor of supporting or excluding sCJD as a diagnosis when employed in an alogarithmic manner using 14-3-3 detection as the primary screening marker before utilising the S100b results. The importance of interpreting these results in the appropriate clinical context, however, is vital, especially as the phenotypic heterogeneity of sCJD remains wide.

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**Competing interests** None.

**Contributors** GC and CP visited the patients and their families, and collected clinical data; JMM collated the clinical data; DE provided statistical analysis; MA and AJEG analysed and interpreted the CSF proteins results; GC, AJEG, RGW and RSGK prepared the manuscript.

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# The role of cerebrospinal fluid proteins as early diagnostic markers for sporadic Creutzfeldt–Jakob disease

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ABSTRACT

The utility of cerebrospinal fluid (CSF) proteins such as 14-3-3, tau protein and S-100b as diagnostic markers in the early stages of sporadic Creutzfeldt–Jakob disease (sCJD) is unclear. We examined the diagnostic value of these CSF proteins in the early stages of sCJD (within 6 weeks of onset of symptoms). Four groups of patients were compared: patients with probable or neuropathologically confirmed sCJD with CSF taken within 6 weeks of onset ('sCJD < 6-week group', $n = 47$); patients with CSF taken within 6 weeks of disease onset but with a diagnosis other than CJD ('non-sCJD < 6-week group', $n = 21$); patients with neuropathologically proven sCJD where CSF was taken later than 6 weeks after onset ('sCJD > 6-week group', $n = 206$); patients with CSF taken later than 6 weeks after onset of symptoms but with a diagnosis other than CJD ('non-sCJD > 6-week group', $n = 166$). The sensitivity and specificity of different combinations of neuronal proteins were ascertained. The sensitivities of all three markers were similar and ranged from 96% to 98%. The sensitivity of these markers was greater in the 'sCJD < 6-week group' than in the 'sCJD > 6-week group'. This may be due to differences in the *PRNP* codon 129 and PrP isotype distribution between these groups. CSF tau protein had the greatest specificity (82%). We found all three CSF protein markers to be highly sensitive in the early stages of sCJD, with CSF tau protein having the greatest specificity and efficiency. Our findings indicate that CSF protein markers are effective tests in the early stages of sCJD.

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Sporadic Creutzfeldt–Jakob disease (sCJD) belongs to the family of transmissible spongiform encephalopathies, or prion diseases. It is a fatal neurodegenerative disease which is characterized by a rapidly progressive dementia, cerebellar ataxia and myoclonus. This progresses to akinetic mutism in the later stages [12]. Definitive diagnosis depends on neuropathological examination of brain tissue taken either at autopsy or by brain biopsy. Diagnostic criteria have been developed which allow for the classification of patients as probable cases during life. These criteria require the presence of typical clinical features and supportive investigations such as EEG or the presence of the protein 14-3-3 in the cerebrospinal fluid (CSF) [10]. The diagnosis of sCJD in the early stages can be difficult. While CSF 14-3-3 has proved to be a useful diagnostic test for sCJD since its inclusion into the WHO diagnostic criteria for probable sCJD [3,5,10,13], there have been a number of reports suggesting that CSF 14-3-3 may not be detectable in the early stages of disease [6–8]. A recent study reported that CSF tau protein was more sensitive than CSF 14-3-3 in early stages of sCJD [8]. In this study we compared the diagnostic value of CSF 14-3-3, tau protein and S-100b in the early stages of sCJD

'Early stage' sCJD was defined as the period within 6 weeks of symptom onset. This time point was chosen to enable a direct comparison with a previously reported study [8]. The NCJDSU database was reviewed and those patients for whom CSF samples were taken within 6 weeks (42 days) of disease onset were identified. Of these patients, 35 had neuropathologically confirmed sCJD and 12 had probable sCJD based on clinical features and a characteristic EEG pattern [11]. One patient had had two CSF samples sent within 6 weeks of disease onset; for the purposes of this study the first sample was used. This group of patients was designated the 'sCJD < 6-week group'. The male: female ratio was 25:22; the median age at disease onset was 67 years (interquartile range 61–75 years); the median total disease duration was 56 days (interquartile range 47–76. The median time from disease onset to lumbar puncture was 33 days (interquartile range 25–38 days).

Those patients who had a CSF sample taken within 6 weeks of symptom onset, who had a final diagnosis other than CJD were designated as the 'non-sCJD < 6-week group'. The male: female ratio was 10:11; the median age at disease onset was 70 years (interquar-

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**Table 1**
The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency for each marker and combination of CSF markers in the 'sCJD < 6-week group'. Efficiency was defined as: true positives + true negatives/total no. tested.

| CSF marker | 14-3-3 | Tau | S-100b | 14-3-3 and Tau | 14-3-3 and S-100b | Tau and S-100b | All three markers |
|---|---|---|---|---|---|---|---|
| sCJD < 6-week group | $N = 47$ | $N = 45$ | $N = 46$ | $N = 45$ | $N = 46$ | $N = 45$ | $N = 45$ |
| Non-sCJD < 6-week group | $N = 21$ | $N = 17$ | $N = 21$ | $N = 17$ | $N = 21$ | $N = 17$ | $N = 17$ |
| Sensitivity (%) (95% CI) | 96 (85, 99) | 98 (88, 99.9) | 98 (88, 99.9) | 93 (82, 99) | 93 (82, 99) | 95 (85, 99) | 91 (79, 98) |
| Specificity (%) (95% CI) | 67 (43, 85) | 82 (56, 96) | 29 (11, 52) | 82 (57, 96) | 76 (53, 92) | 82 (57, 96) | 82 (57, 96) |
| PPV (%) (95% CI) | 87 (74, 94) | 93 (82, 99) | 75 (62, 85) | 93 (82, 99) | 90 (77, 97) | 96 (85, 99) | 93 (81, 99) |
| NPV (%) (95% CI) | 88 (62, 98) | 93 (68, 99.9) | 86 (42, 99.7) | 82 (57, 96) | 84 (60, 97) | 82 (57, 96) | 82 (52, 94) |
| Efficiency (%) | 72 | 94 | 76 | 90 | 88 | 92 | 89 |

tile range 59–77 years). The median time from disease onset to lumbar puncture was 20 days (interquartile range 16–30 days). Overall, 11 patients had neuropathological evidence of a diagnosis other than CJD and these diagnoses were: Lewy Body disease (LBD) (2); Parkinson's disease (2); Alzheimer's disease (AD) (1); AD and cerebral arteriosclerosis (1); chronic limbic encephalitis (1); anaplastic astrocytoma WHO grade 3 (1); paraneoplastic syndrome (1); viral or post-viral encephalopathy (1); inflammatory changes on brain biopsy with no evidence of CJD (1). The remaining 10 patients had only clinical evidence of a diagnosis other than CJD and these diagnoses were: encephalopathy of unknown origin with spontaneous improvement (3); AD (1); LBD or AD (1); steroid responsive encephalopathy (1); psychiatric disorder/or epilepsy (1); mesothelioma and paraneoplastic syndrome (1); corticobasal degeneration (1); central pontine myelinolysis (1).

A group of 206 patients with neuropathologically proven sCJD where CSF was taken outwith 6 weeks of symptom onset was identified and designated as the 'sCJD > 6-week group'. This group was included in order to determine whether those patients who underwent CSF analysis within 6 weeks of symptom onset could be taken to be representative of the wider population of patients with sCJD. The male:female ratio was 107:99; the median age at disease onset was 66 years (interquartile range 60–72 years); the median total disease duration was 179 days (interquartile range 107–288 days) (one patient left the UK after diagnosis and so disease duration was unknown). The median time from disease onset to lumbar puncture was 128 days (interquartile range 69–208 days).

A group of 166 patients had a CSF sample taken outwith 6 weeks of symptom onset who had a final diagnosis other than CJD were designated as the 'non-sCJD > 6-week group'. The male: female ratio was 86:80; the median age at disease onset was 66 years (interquartile range 58–75 years). The median time from disease onset to lumbar puncture was 161 days (interquartile range 75–339 days). Neuropathological examination was performed in 63 cases and in 11 of these no neuropathological evidence of CJD was found but no alternative diagnosis could be made. In the remaining 52 patients the diagnoses were: AD (18); LBD (8); intracerebral malignancy (7); paraneoplastic syndrome (6); cerebrovascular disease (4); hypoxic brain injury (2) and one each of the following corticostrial-nigral degeneration, neuroaxonal dystrophy, normal pressure hydrocephalus, multi-focal demyelination, vasculitis, frontotemporal dementia and progressive multi-focal lecoencephalopathy. The diagnosis of CJD was excluded in the remaining 103 patients for reasons that included complete or partial recovery (33) or an alternative diagnosis was made on clinical grounds or investigations were suggestive of an alternative disorder. The diagnoses in this group were: AD (17); frontotemporal dementia (7); cerebrovascular disease (7); dementia of unknown origin (7); LBD (6); paraneoplastic syndrome (4); psychiatric disorder (3); Huntington's disease (3); encephalitis (3); intracerebral malignancy (3); vasculitis (2); drug toxicity (2) and one each of the following motor neurone disease; cerebellar syndrome; multi-system atrophy, corticobasal degeneration, tuberculosis and spinocerebellar ataxia.

Samples were initially frozen and transported to the NCJDSU on dry-ice. Samples which were insufficient for analysis, had been improperly stored or were bloodstained were not analysed. The 14-3-3 immunoassay was carried out as previously described [3]. A rabbit polyclonal anti-14-3-3-gamma or a mouse anti-14-3-3-beta monoclonal antibody was used. Tau protein in CSF was analysed using a previously described enzyme linked immunosorbant assay (ELISA) [9] according to the manufacturer's recommendations (Innogenetics, Belgium). This assay measures both the normal and hyperphosphorylated forms of CSF tau protein. Concentrations of CSF tau protein of greater than 1260 pg/ml were considered to be abnormal [8].

CSF S-100b was measured using a previously described sandwich ELISA [2]. Concentrations of CSF S-100b greater than or equal to 0.50 ng/ml were considered to be abnormal.

Genotyping for polymorphism at codon 129 of the *PRNP* gene was carried out using a previously described method [4].

The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and efficiency for each marker and for combinations of markers were calculated.

In the 'sCJD < 6-week group' the sensitivities of all three markers were similar and ranged from 96% to 98%. Sensitivities were lower in the 'sCJD > 6-week group' than in the 'sCJD < 6-week group': 86% for 14-3-3 ($p = 0.08$), 78% for tau protein ($p = 0.002$) and 92% for S-100b ($p = 0.21$). Combining the markers together resulted in a reduction of sensitivity (Table 1).

There are, however, differences in the *PRNP* codon 129 and PrP isotype distribution in the two groups of patients. Of the 31 patients in the 'sCJD < 6-week group' 30 were homozygous at *PRNP* codon 129 and one was VV at *PRNP* codon 129. In addition all of those tested for prion protein isotype ($n = 21$) were type 1. Among the 'sCJD > 6-week group' there was greater heterogeneity in *PRNP* codon 129 distribution and prion protein isotype (Table 2). Prion protein isotype testing was performed in 109 of the 'sCJD > 6-week group' patients. Of these 54 (49.5%) were type 1, 41 (37.6%) were type 2 and 14 (12.8%) had both type 1 and type 2 present.

Among the 'sCJD group' the sensitivities of all three CSF tests were lower among codon 129 heterozygotes (14-3-3 $p = 0.03$; tau $p = 0.08$; S-100b $p = 0.02$). Within the MM homozygotes there was no evidence that test sensitivities differed between the two groups for 14-3-3 ($p = 0.37$) or S-100b ($p = 0.69$). However, there was still evidence that tau was more sensitive among cases tested before 6 weeks than among those tested after 6 weeks (100% vs 80%, $p = 0.01$).

All three tests' sensitivities were lowest in those with type 2 prion protein only and highest in those with type 1 only, though the differences between type 1 and mixed type 1/type 2 cases were small (14-3-3, $p = 0.06$; tau, $p = 0.19$; S-100b, $p = 0.04$). When attention was restricted to cases with type 1 prion protein only, a similar pattern to that observed in MM cases was seen, with similar sensitivities before and after 6 weeks for 14-3-3 (90% vs 93%, $p = 0.76$) and S-100b (95% vs 96%, $p = 0.83$) but a higher sensitivity before 6 weeks than after for tau (100% vs 85%, $p = 0.07$).

**Table 2**
Sensitivity of CSF markers by time of test, codon 129 genotype and prion protein isotype.

| CSF marker sensitivity (%) | sCJD < 6-week group codon 129 status | | | sCJD > 6-week group codon 129 status | | |
|---|---|---|---|---|---|---|
| | MM ($n$ = 30) | VV ($n$ = 1) | MV ($n$ = 0) | MM ($n$ = 95) | VV ($n$ = 29) | MV ($n$ = 45) |
| 14-3-3 | 93 | 100 | – | 87 | 93 | 73 |
| Tau | 100 | 100 | – | 80 | 89 | 65 |
| S-100b | 97 | 100 | – | 95 | 100 | 82 |
| CSF marker sensitivity (%) | sCJD < 6-week group prion protein isotype | | | sCJD > 6-week group prion protein isotype | | |
| | 1 ($n$ = 21) | 2 ($n$ = 0) | 1 and 2 ($n$ = 0) | 1 ($n$ = 54) | 2 ($n$ = 41) | 1 and 2 ($n$ = 14) |
| 14-3-3 | 90 | – | – | 93 | 76 | 71 |
| Tau | 100 | – | – | 85 | 69 | 64 |
| S-100b | 95 | – | – | 96 | 83 | 93 |

Of all the markers investigated CSF tau protein had the highest specificity before 6 weeks (82%) while CSF 14-3-3 had a specificity of 67% (Table 1). Combining the markers did not improve the specificity over that of CSF tau protein alone. There were three control patients who had an elevated CSF tau protein; two patients with AD and one patient with an astrocytoma. All three of these patients also had a positive CSF 14-3-3. An additional four patients had a positive 14-3-3 and the diagnoses in these patients were encephalitis (2), paraneoplastic syndrome (1) and central pontine myelinolysis (1). The specificity for the 'sCJD > 6-week group' was 72% for CSF 14-3-3 and 84% for CSF tau protein. The specificity of both tests was less in the 'sCJD < 6-week group' when compared with the 'sCJD > 6-week group', although this difference was more marked with CSF 14-3-3 than with CSF tau protein.

CSF S-100b was a very sensitive marker for sCJD but had very poor specificity before 6 weeks (Table 1). The combination of CSF S-100b with either an elevated CSF tau protein or a positive CSF 14-3-3 did not improve the sensitivity of either marker. However inclusion of an elevated CSF S-100b did improve the specificity of CSF 14-3-3.

We found CSF protein markers to be highly sensitive in the early stages of sCJD. This is in contrast with the findings of other recent studies [7,8]. The lower sensitivities found by other authors may be related to their inclusion of not only cases of sCJD but also patients with iatrogenic and familial CJD. The sensitivity of CSF protein markers has been reported to be lower in cases of familial and iatrogenic CJD [5]. This discrepancy highlights the importance of study design when investigating these CSF proteins. The most meaningful results come from studies where neuropathologically confirmed cases of sCJD are used. In addition the most reliable and meaningful specificity data is obtained by using control patients who are initially suspected of having sCJD but are subsequently proven to have an alternative diagnosis.

The patient characteristics in the 'sCJD < 6-week group' and the 'sCJD > 6-week group' were markedly different, with a much higher prevalence of the MM1 subtype in those patients presenting within 6 weeks of symptom onset. This finding mirrors those of earlier reports of patients with the MM1 subtype having a more dramatic onset of disease and more rapid decline. This is likely to lead to such patients being investigated more promptly than those with a more insidious onset. CSF protein markers have been reported to be of higher sensitivity in patients of the MM1 phenotype [5]. There are studies which have shown that the sensitivity of CSF 14-3-3 is not affected by the timing of a lumbar puncture during disease course [5,1]. Thus the difference in sensitivity between the 'sCJD < 6-week group' and the 'sCJD > 6-week group' is probably due to the faster rate of disease progression in the former group when compared to the latter.

In this limited study we found CSF tau protein to have the greatest specificity and efficiency in distinguishing sCJD from other rapidly progressive neurodegenerative conditions. It is unclear why CSF tau protein should be of greater specificity than 14-3-3. The factors influencing the release of neuronal proteins in CJD and other conditions are not fully understood.

Our study is limited by the small numbers of samples analysed (although these are still greater than those in previous reports [7,8]) and in particular the fact that the 'non-sCJD < 6-week group' was markedly smaller than either the 'sCJD < 6-week group' and the 'sCJD > 6-week group'.

Diagnostic tests which are reliable in the early stages of sCJD are necessary for a number of reasons; to guide further investigations and appropriate management, to inform patients and their families regarding diagnosis and prognosis, and possibly in the future in order to ensure prompt treatment. However these tests should be performed only if a patient is likely to have sCJD on clinical grounds otherwise the high levels of sensitivity and specificity for sCJD are lost. Our findings show that CSF 14-3-3, S-100b and tau protein have comparable sensitivity in the early stages of sCJD and are effective tests in the early stages of sCJD.

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# *PRNP* variation in UK sporadic and variant Creutzfeldt Jakob disease highlights genetic risk factors and a novel non-synonymous polymorphism

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## Abstract

**Background:** Genetic analysis of the human prion protein gene (*PRNP*) in suspect cases of Creutzfeldt-Jakob disease (CJD) is necessary for accurate diagnosis and case classification. Previous publications on the genetic variation at the *PRNP* locus have highlighted the presence of numerous polymorphisms, in addition to the well recognised one at codon 129, with significant variability between geographically distinct populations. It is therefore of interest to consider their influence on susceptibility or the clinico-pathological disease phenotype. This study aimed to characterise the frequency and effect of *PRNP* open reading frame polymorphisms other than codon 129 in both disease and control samples sourced from the United Kingdom population.

**Methods:** DNA was extracted from blood samples and genetic data obtained by full sequence analysis of the prion protein gene or by restriction fragment length polymorphism analysis using restriction enzymes specific to the gene polymorphism under investigation.

**Results:** 147 of 166 confirmed cases of variant CJD (vCJD) in the UK have had *PRNP* codon 129 genotyping and all are methionine homozygous at codon 129; 118 have had full *PRNP* gene sequencing. Of the latter, 5 cases have shown other polymorphic loci: at codon 219 (2, 1.69%), at codon 202 (2, 1.69%), and a 24 bp deletion in the octapeptide repeat region (1, 0.85%). E219K and D202D were not found in sporadic CJD (sCJD) cases and therefore may represent genetic risk factors for vCJD.

Genetic analysis of 309 confirmed UK sCJD patients showed codon 129 genotype frequencies of MM: 59.5% (n = 184), MV: 21.4% (n = 66), and VV: 19.1% (n = 59). Thirteen (4.2%) had the A117A polymorphism, one of which also had the P68P polymorphism, four (1.3%) had a 24 bp deletion, and a single patient had a novel missense variation at codon 167. As the phenotype of this latter case is similar to sCJD and in the absence of a family history of CJD, it is unknown whether this is a form of genetic CJD, or simply a neutral polymorphism.

**Conclusions:** This analysis of *PRNP* genetic variation in UK CJD patients is the first to show a comprehensive comparison with healthy individuals (n = 970) from the same population, who were genotyped for the three most common variations (codon 129, codon 117, and 24 bp deletion). These latter two genetic variations were equally frequent in UK sCJD or vCJD cases and a normal (healthy blood donor) UK population.

## Background

At the time of this study, there were 166 confirmed cases of vCJD in the UK, and a further 44 patients in other countries. (For current UK and worldwide figures see http://www.cjd.ed.ac.uk) The widely accepted hypothesis is that vCJD was initially acquired through dietary infection with bovine produce contaminated with the infectious agent of bovine spongiform encephalopathy (BSE) [1,2]. Additionally, secondary cases have resulted from blood transfusion [3,4]. The proposed agent (designated a 'prion') has not been fully characterised but disease and infectivity are generally associated with an abnormally folded form (designated $PrP^{Sc}$) of the host encoded prion protein ($PrP^{C}$) [5]. Variant CJD shows marked differences in clinical presentation [6] and neuropathology [7] when compared with sporadic CJD (sCJD) which has a worldwide distribution affecting approximately 1-2 cases per million population per year and has an unknown aetiology. In comparison to sCJD, vCJD typically occurs in younger individuals (median ages of onset: 66 & 26 years respectively) and with a longer disease duration (median durations: 4 and 14 months respectively). There are two other forms of CJD: genetic and iatrogenic. Genetic CJD (gCJD) is an autosomal dominant inherited illness related to underlying mutations of the prion protein gene (*PRNP*). There are other genetic human prion diseases (Fatal Familial Insomnia (FFI), and Gerstmann Sträussler Scheinker syndrome (GSS)) traditionally separated from gCJD on clinico-pathological grounds, but which are related by certain common core features and an underlying causal *PRNP* mutation. Iatrogenic CJD (iCJD) results from the accidental person-to-person (medical or surgical) transmission of other forms of CJD.

Genetic analysis of the sCJD and vCJD disease cohort has an important role in accurate diagnostic classification, especially as genetic prion disease cannot always be differentiated clinically from other forms of prion disease and a family history is absent in up to about 50% of cases [8]. The gene coding for prion protein, *PRNP*, is a key target for analysis. This gene is highly conserved between mammalian species indicating an important biological role for the prion protein, but *PRNP* shows significant variation at the individual level in humans [9]. Over twenty missense and nonsense mutations have been identified in the open reading frame (ORF) that have been linked to genetic prion disease phenotypes [10]. Other polymorphic sites in *PRNP* (over twenty examples) that are not directly linked to a disease phenotype have been identified through analysis of suspected CJD cases and other populations. These polymorphisms may have disease modulating capacities and these effects may vary according to the population under investigation. The conversion of $PrP^{C}$ to $PrP^{Sc}$ is thought to involve some form of oligomerisation and the melting and refolding of the peptide chains. It is therefore possible that any changes to the amino-acid sequence could alter the thermodynamic stability of the protein and affect the kinetics of structural conversions [11].

The *PRNP* polymorphic residue at codon 129 (ATG-methionine to GTG-valine, M129V) has been studied extensively. Variation in the genotype frequencies occurs according to geographical region. In most countries studied, the MM and MV frequencies are approximately 40-50% however there is a large difference seen in Japan where the normal population frequencies are: MM 92%, MV 8%, and VV 0% [12]. Genotype data from other countries suggest a gradual increase in MM genotype frequency from West to East which may reflect the historical human migrations [12]. It has been shown that M129V may affect susceptibility to prion diseases [13], the incubation period in acquired forms [14,15] and the clinico-pathological phenotype [16]. Codon 129 homozygosity is considered a risk factor for human prion disease: while ~40% of the normal population is MM, ~70% of sCJD patients, and all clinical vCJD patients tested to date have this genotype [17]. Homozygosity has also been shown to be a risk factor, and reduces the incubation time, for both human growth hormone associated iCJD [14] and kuru [15] (an historical disease of the Fore linguistic tribe of Papua New Guinea transmitted by endocannibalism). In some circumstances, the genotype at codon 129 can determine the disease phenotype for human genetic prion cases associated with pathogenic mutations [16]. In addition, variation in sCJD clinico-pathological phenotype can be associated with specific genotypes [18].

The most significant finding with respect to codon 129 genotype and CJD is that all clinically probable and neuropathologically confirmed cases of vCJD so far analysed world-wide (n = 147 UK and 44 non-UK) have the MM genotype and, therefore, this genotype is categorised as a risk factor. However, there is evidence that other 129 genotypes are susceptible to BSE/vCJD infection and that they may develop disease after a longer incubation period than in MM individuals. It is hypothesised that MV and VV cases will occur in the future [19]. There are four reports of transmission of vCJD infection via blood transfusion from blood donors who later developed vCJD [3,4,20,21]. In three instances, the recipients developed clinical vCJD and all three were homozygous MM at codon 129. In the fourth case, there was no clinical or neuropathological evidence of vCJD in the recipient, but disease-related abnormal prion protein was found in the spleen and a lymph node. This individual was MV at codon 129 and may represent subclinical infection in a non-MM individual; it is impossible to know whether the individual would have developed clinical vCJD if they had lived longer [21]. In addition, two of three samples in an anon-

ymous appendix study in the UK that were positive for deposition of prion disease associated $PrP^{Sc}$ were genotyped as VV [22].

Variation in the DNA sequence of *PRNP* may be linked to other genetic changes separate to the prion protein itself, such as promoter elements or regions more distant to the *PRNP* gene. To understand more fully the control mechanisms of *PRNP* gene expression, such as promoter activity, extensive studies have been undertaken to examine the region of chromosome 20 adjacent to the *PRNP* locus [23-26]. Analysis of a 4.8 kb region around *PRNP*, identified 3 polymorphisms in areas that were predicted to control gene expression [24].

This study involved the genetic analysis of CJD cases referred to the National CJD Surveillance Unit during routine UK surveillance and a specific project to determine normal population *PRNP* polymorphisms in the United Kingdom.

## Methods

### *Control DNA Samples*

The Scottish Blood Donor controls (n = 778) were supplied as 0.5 ml aliquots of frozen whole blood by Dr Ian MacGregor (National Science Laboratory, Scottish National Blood Transfusion Service) from samples taken as part of a human prion disease study of blood markers (covered by Multi-Centre Research Ethics Committee for Scotland approval, reference MREC/02/10/46). There was no ethical approval for full *PRNP* sequence analysis of these samples.

DNA samples (UK DNA controls, n = 192) were purchased from the European Collection of Cell Cultures (ECACC) originating from donors in the UK cities of Oxford and Birmingham. (Catalogue references: HRC-1 and HRC-2.)

For the normal population data, the Scottish Blood Donor and UK DNA controls were genotyped for polymorphisms at M129V, and A117A, and the 24 bp deletion in the N-terminus octapeptide repeat region (the three most common polymorphic loci of *PRNP*). For the CJD cases, we had *PRNP* sequence analysis data on 118 vCJD and 309 sCJD patients.

### *Patient Samples*

Patient blood samples were taken from individuals under clinical review for suspected CJD, by venipuncture, into citrate anticoagulant treated blood collection tubes. Once received into the laboratory the blood was separated into its major constituents as follows:

1. Whole blood was spun at 450 g for 10 minutes to separate platelet rich plasma, buffy coat, and red blood cells (RBC).

2. Platelets were removed from the plasma by two washes in phosphate buffered saline (PBS) with centrifugations at 16,000 g leaving platelet poor plasma. This was stored at -80°C.

3. The buffy coat fraction was cleared of contaminating platelets by centrifugation at 180 g for 10 minutes, then the RBC were removed by lysis with distilled water and centrifugation at 180 g for 10 minutes. Finally the buffy coat fraction was washed in PBS then centrifuged at 100 g for 10 minutes before storing at -80°C.

4. The RBC were washed twice with PBS, with centrifugation at 180 g for 10 minutes each time. RBC were stored at -80°C as a 50% solution in PBS.

### *DNA Extraction*

DNA from cases and controls was prepared from 200 μl of frozen whole blood by cell lysis and column purification using the DNA Blood Mini Kit (Qiagen, UK) and stored at -20°C.

### *PCR-RFLP Analysis*

Amplification of the *PRNP* gene sequence (NCBI Accession: AL133396) by the polymerase chain reaction (PCR) involved forward primer (5'-TGA TAC CAT TGC TAT GCA CTC ATT C-3') and reverse primer (5'-GAC ACC ACC ACT AAA AGG GCT GCA G-3') at 5 pmoles each per reaction (Eurofins MWG Operon, Germany), that are specific for a 956 bp sequence. Each reaction contained 2 mM $MgCl_2$ (Qiagen, UK), 0.2 mM dNTPs (Promega, UK), and 1 Unit of Taq Polymerase (HotStarTaq, Qiagen, UK). The thermal cycling program included an annealing temperature step-down from 65°C to 60°C over ten cycles followed by 30 cycles at 60°C.

Confirmation of the codon 129 genotype was performed by restriction enzyme digestion at 37°C with NspI (New England Biolabs, UK). This enzyme cleaves the amplicon at *PRNP* codon 155 and at codon 129 only when the latter sequence codes for valine (-GTG-). This allowed for discrimination of the three genotypes: MM, MV, and VV by agarose gel electrophoresis and ethidium bromide staining, Figure 1. The presence of a 24 bp deletion in the octapeptide repeat region could be observed from the codon 129 genotyping agarose gel data due to an additional band shift for the restriction enzyme digest products. Codon 117 polymorphism genotype was determined by restriction enzyme digestion of the same *PRNP* PCR product with PvuII (New England Biolabs,

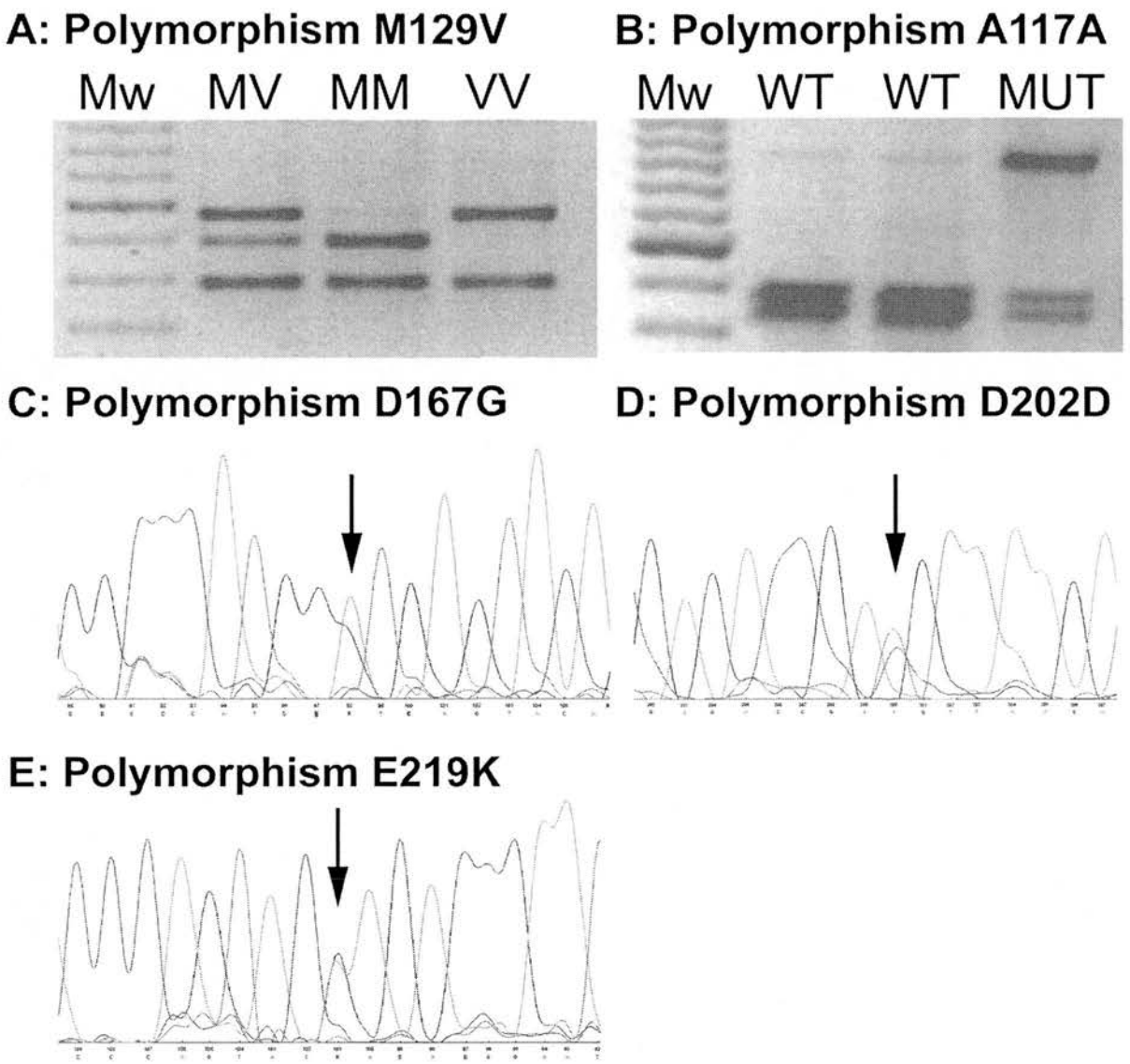


**Figure 1**
**Detection of *PRNP* polymorphisms.** Panels A and B: Genotyping by PCR amplification of *PRNP* and restriction enzyme digest. (Mw: 100 bp molecular weight ladder (dark band 600 bp); MV, MM, VV: codon 129 genotypes; WT: wild-type codon 117 genotype; MUT: heterozygous for codon 117 polymorphism) Panels C, D, and E: Electropherograms for polymorphisms detected by sequence analysis. (Arrows point to heterozygous base position.)

UK) at 37°C. This enzyme cleaves the wild-type PCR product and not the codon 117 variant. In samples heterozygous for the codon 117 polymorphism this enzyme produces digest products of sizes 499 and 457 in addition to the full length 956 bp PCR amplicon. (Figure 1)

### *Sequencing*

If consent from the patient, or relative, had been obtained for full genetic analysis then the PCR product was sequenced by fluorescent dye-primer chemistry (Thermo Sequenase Primer Cycle Sequencing Kit, Amersham Biosciences, UK) using an ALF-Express DNA Sequencer (Amersham Biosciences, UK). PCR products were purified

using a QIAquick PCR Purification Kit (Qiagen, UK) to remove excess primers and other PCR reagents. Four Cy5 labelled sequencing primers were used to give at least 2-fold coverage of the entire ORF (5'-AGG TGG CAC CCA CAG TCA GT-3'; 5'-CTA TGC ACT CAT TCA TTA TG-3'; 5'-CCT CAA GCT GGA AAA AGA TTA G-3'; 5'-CGA TAG TAA CGG TCC TCA TA-3'). Sequencing reaction products were electrophoresed through ReproGel Long Read acrylamide gels (Amersham Biosciences, UK). ALFwin Software (Amersham Biosciences, UK) was used to align the sequence data with a reference sequence and each codon of the ORF was visually checked by two individuals. This method allows for identification of novel polymorphisms rather than only checking for known ORF sequence changes. (Figure 1)

### *Statistical Analysis*

Analysis was undertaken through the use of R (v2.9.1) (R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. URL: http://www.R-project.org).

## Results

### *Variant CJD* PRNP *Sequence Data*

Table 1 shows the results of *PRNP* gene sequence analysis in vCJD cases where material and specific consent was available. 147 confirmed cases were genotyped at codon 129 and all were methionine homozygotes (MM). Complete sequence analysis of the *PRNP* ORF was performed for 118 cases and found five individuals with genetic variation. Two had a synonymous (silent) polymorphism at codon 202 (GAC-aspartic acid to GAT-aspartic acid; D202D), two had a non-synonymous change at codon 219 (GAG-glutamic acid to AAG-lysine; E219K), and one had a 24 bp deletion (classified as DelR34 according to [27]). The DelR34 and the two D202D patients fulfilled the diagnostic criteria [28] for definite vCJD. The two patients with E219K genotype did not have a post mortem and were classified as probable vCJD. The E219K mutation has not been seen in white Caucasian populations and has so far only been detected in populations from Asian and the Pacific [29]. The two individuals with the E219K genotype found in this study were not of white Caucasian origin (the specific ancestral origin is unknown).

### *Sporadic CJD Sequence Data*

Sequence variation for 309 confirmed sCJD patients is shown in Table 2. Codon 129 genotyping produced allele frequencies similar to that expected for this disease from other studies [13,18] and to the overall figures published by the UK National CJD Surveillance Unit (MM 63%, MV 19%, VV 18%; n = 647) (http://www.cjd.ed.ac.uk; Annual Report 2008). Complete sequence analysis on these patients found thirteen with the silent polymorphism at codon 117 (GCA-alanine to GCG-alanine; A117A), which included one individual with an additional silent polymorphism at codon 68 (P68P), and four cases with a 24 bp deletion in the octapeptide repeat region. Of these 17 patients 13 were classified as definite and four as probable sCJD according to the diagnostic criteria. In addition, one patient was found to have a non-synonymous polymorphism at codon 167 (GAT-aspartic acid to GGT-glycine; D167G). There was nothing in the clinical phenotype suggesting that this could be a causative mutation of gCJD and the patient was classified as probable "sporadic CJD". Neuropathological analysis confirmed sporadic CJD with type 1 prion protein isoform present, and genetic analysis showed methionine homozygosity at codon 129 of *PRNP*.

### *Control Codon 129 Genotype Data*

Codon 129 genotype frequencies are given in Table 2 for samples from the UK DNA and Scottish Blood Donor

**Table 1: *PRNP* gene sequence variation in vCJD cases**

| Codon | Number Tested | Genotype Data |
|---|---|---|
| 129 (Met/Val) | 147 | All cases MM |
| | 4 (blood transfusion associated infections) | MM (n = 3)<br>MV (n = 1)* |
| | 2 (appendix tissue) | VV (n = 2)** |
| 202 (Asp/Asp) | 118 | DD (n = 2) |
| 219 (Glu/Lys) | 118 | EK (n = 2) |
| 24 bp deletion | 118 | DelR34 (n = 1) |

* Non-clinical, non-neuropathologically confirmed case

** From anonymous screening program for vCJD associated $PrP^{Sc}$ deposition

**Table 2: *PRNP* gene sequence variation in sCJD cases and controls**

| *PRNP* Variation | 309 sCJD Patients | 192 UK DNA Controls | 778 Scottish Blood Donor Controls |
|---|---|---|---|
| Codon 129 (Met/Val) | MM (n = 184; 59.5%)<br>MV (n = 66; 21.4%)<br>VV (n = 59; 19.1%) | MM (n = 90; 46.9%)<br>MV (n = 87; 45.3%)<br>VV (n = 15; 7.8%) | MM (n = 337; 43.3%)<br>MV (n = 344; 44.2%)<br>VV (n = 97; 12.5%) |
| Codon 117 (Ala/Ala) | n = 13; 4.2%<br>(10.4% of MV/VV cases) | n = 9; 4.7%<br>(10.3% of MV/VV cases) | n = 47; 6.0%<br>(10.7% of MV/VV cases) |
| 24 bp Deletion | n = 4; 1.3%<br>Deletion Class<br>R34 (n = 3)<br>R3 (n = 1) | n = 1; 0.5%<br>Deletion Class<br>R2 (n = 1) | n = 12; 1.5%<br>Deletion Class<br>R34 (n = 12) |
| Codon 167 (Asp/Gly) | DG (n = 1; 0.32%) | (no sequence data available) | (no sequence data available) |
| Codon 68 (Pro/Pro) | PP (n = 1; 0.32%) | (no sequence data available) | (no sequence data available) |

controls. Together with the published Edinburgh/Belfast blood donors, the figures are similar across the three groups and combined (n = 1158) give the predicted UK national genotype frequencies of 44.1% (MM), 44.5% (MV), and 11.4% (VV). As there is a significant difference in age at onset between vCJD and sCJD, and that gender may influence sCJD survival [30], the Scottish Blood Donor data was stratified into four groups according to age (17-30, 31-40, 41-50, and 51-69), Figure 2, and by sex, Figure 3. Statistical analysis (Chi-Squared test) indicated that there were no age ($p = 0.5774$, 0.1270, and 0.4781) or gender ($p = 0.2228$, 0.9884, and 0.7527) effects determined by genotype at codon 129, or 117, or by the presence of a 24 bp deletion, respectively.

### *Statistical Comparison (Chi Squared Testing) of Control and Case Data*

Comparisons made between codon 129 genotype frequencies for sCJD, vCJD, and the two control groups showed statistically significant differences ($P < 0.001$, Table 3). There was no significant difference in genotype between the two control groups. There was no selection bias for the sequenced sCJD patients as there was no significant difference between that group and the larger group of UK National CJD Surveillance Unit sCJD patients that have been genotyped.

The frequencies of A117A and 24 bp deletion were not statistically different from the control data.

Analysis of the genotype frequencies of D202D and E219K could not be done as they were only found in vCJD and not in the controls, as the latter were not sequenced.

## Discussion

Analysis of *PRNP* in sCJD, vCJD and controls has highlighted the clear primary role in disease susceptibility for the codon 129 genotype and has provided a base-line for the healthy population for future vCJD susceptibility predictions. In addition, the presence of genetic variation (D202D and E219K) in vCJD that is not seen in sCJD suggests possible additional risk factors. A novel non-synonymous polymorphism (D167G) in a case of suspected sCJD has also underlined the need for *PRNP* genetic screening of patients.

For accurate diagnosis of the various forms of human CJD, and especially to identify inherited genetic forms, DNA sequence analysis of the *PRNP* open reading frame (ORF) is necessary. Aside from the detection of any pathogenic mutations, this provides data on any polymorphic residues present, important for full characterisation of non-genetic cases [18]. The majority of non-pathogenic polymorphisms are rare.

**Codon 129** genotype frequency data show the clear difference between CJD cases and controls with a reduction in the frequency of MV (21.4%) for sCJD cases compared to the controls (~45%), and 100% MM cases in vCJD. As the control genotype frequencies were not found to vary by age the case data is unlikely to be due to the significant difference in average age at onset between vCJD and sCJD. If

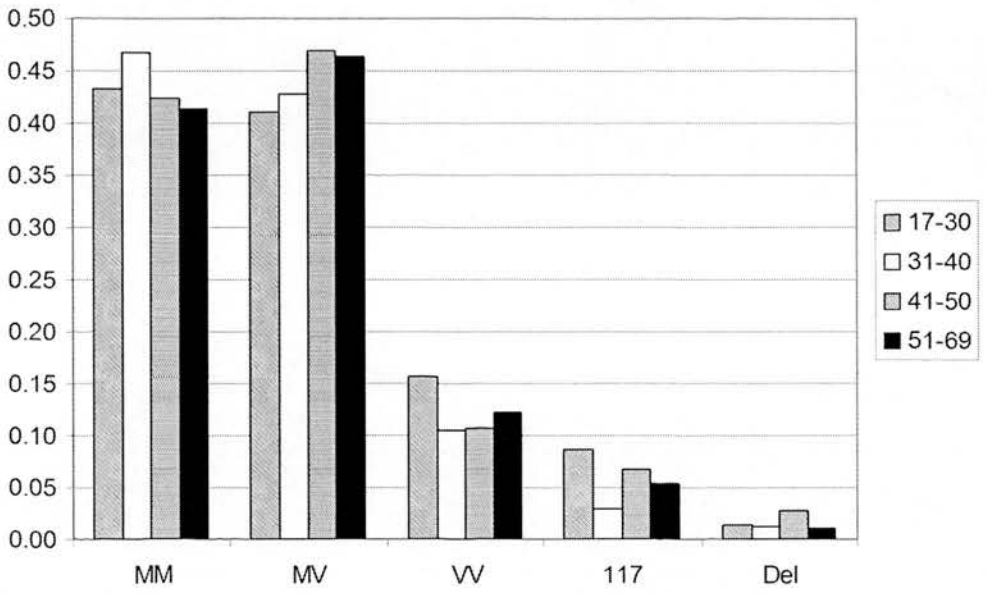


**Figure 2**
***PRNP* polymorphisms in Scottish Blood Donors**. Codon 129 and *PRNP* polymorphism frequency in relation to age of Scottish Blood Donors (n = 778).

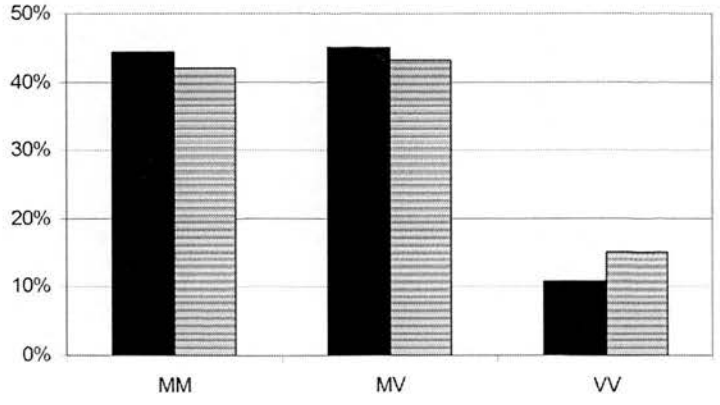


**Figure 3**
**Codon 129 and sex of Scottish Blood Donors**. Codon 129 genotype frequency in relation to sex of Scottish Blood Donors (n = 778, male = 456 (solid bars), female = 322 (hatched bars)).

**Table 3: Statistical analysis of *PRNP* polymorphism frequencies**

| | Chi-Squared Test - P value | | |
|---|---|---|---|
| **Comparison** | **Codon 129** | **Codon 117** | **24 bp Deletion** |
| Sequenced sCJD (n = 309) vs. All NCJDSU sCJD cases (n = 647) | 0.582 | NA | NA |
| UK DNA Controls (n = 192) vs. Scottish Blood Donors (n = 778) | 0.185 | 0.584 | 0.452* |
| Sequenced sCJD (n = 309) vs. UK DNA Controls (n = 192) | <0.001 | 0.975 | 0.700* |
| Sequenced sCJD (n = 309) vs. Scottish Blood Donors (n = 778) | <0.001 | 0.295 | 0.978* |
| Sequenced vCJD (n = 118) vs. Scottish Blood Donors (n = 778) | <0.001 | NA | 0.861* |

*: Chi-Squared approximation may be incorrect due to low numbers; NA: no comparison could be made

clinical vCJD continues to manifest only in MM genotype individuals then the mathematical models for predicting the total epidemic size can use the population MM frequency of 44.1% in the UK population, generated by this study.

Other than M129V the two most common *PRNP* variants in Caucasian populations are: **A117A** (frequency ~5%), and a **24 bp deletion** in the repeat expansion region (~1%). Full *PRNP* sequencing of 118 confirmed UK vCJD patients found five with *PRNP* polymorphisms: two with E219K, two with D202D, and one with a 24 bp deletion (DelR34). The codon 117 polymorphism has not been found, as this is linked to the valine allele at codon 129, and all vCJD cases to date have been 129-MM.

**D202D** is a synonymous polymorphism and therefore there is no resulting change in prion protein amino acid sequence. No data are available on normal population frequency and the only record of its appearance has been in single French cases of sCJD and iCJD related to human Growth Hormone treatment (Dr N Delasnerie-Laupretre - personal communication). There are no structural changes to the prion protein itself and so this DNA sequence alteration would not directly affect protein folding kinetics in a potentially disease causing manner. Missense DNA sequence change GAC (glutamic acid) to AAC (asparagine) at this codon (D202N) is associated with a disease phenotype of Gerstmann-Sträussler-Scheinker syndrome (GSS) a genetic form of human prion disease [31]. The D202D change may be linked with a haplotype, as yet undefined, that alters susceptibility via an alternative route such as differential expression of PrP. This may possibly be the reason why ~2% of tested vCJD cases have been found with this polymorphism. Until control population frequencies are available this possibility remains hypothetical.

**E219K** is a non-synonymous, missense mutation with a change in amino acid and, therefore, the possibility of protein structural differences which may be linked to an increase in disease susceptibility. This polymorphism has not been found in Western European populations and has only been investigated in detail, with regards to CJD, in Japan [32] and Korea [33] where approximately 12% and 8% respectively of the population carry the lysine (K) allele. These studies indicated that E219K influenced the clinical phenotype of GSS in cases with the P102L mutation [34], and found it was a protective factor to sCJD as no confirmed cases of sCJD in Japan or Korea carry the polymorphism. In the two vCJD cases found in our study with E219K, there were no clinico-pathological differences from the other vCJD cases. These patients were of non-Caucasian origin, therefore their genetic ancestry may originate from populations where E219K is more common than the UK [29]. There remains a possibility that the presence of this polymorphism may increase susceptibility to vCJD. A transgenic mouse model suggests that the lysine (K) allele $PrP^{C}$ is more susceptible than the glutamic acid (E) allele to conversion to $PrP^{Sc}$ [35].

**24 bp deletions** were found in healthy individuals, sCJD, and vCJD patients at similar frequencies. It is therefore proposed that this genetic variation does not influence the disease state or susceptibility, even though the $PrP^{C}$ formed is significantly different at the metal binding

domain of the N-terminus, as shown by other studies [36,37]. Deletion of two 24 bp repeats in this region has been linked to a familial form of CJD [38].

There have been no reported instances of the **D167G** sCJD polymorphism associated with familial human prion disease. Evidence for this event to be the cause of a genetic form of CJD in this patient is twofold:

1. The replacement of a large hydrophilic amino acid for a small hydrophobic one may disturb the physical properties of the prion protein and thus accelerate formation of the disease associated form ($PrP^{Sc}$).

2. Transgenic mouse studies propose that codon 167 may be in a region of the prion protein structure where chaperone molecules bind and accelerate disease transmission [39].

Without the additional evidence of further affected members of the family with the mutation or data on a healthy control population there is no direct proof of the association of D167G and CJD.

## Conclusions

This study has produced genotype frequencies for the most common *PRNP* genetic variants in the largest cohort of healthy individuals from the UK so far published, and in groups of patients with vCJD and sCJD. These data can act as a benchmark for studying the genotype frequency variation found in human prion disease in the UK. DNA sequence analysis of vCJD patients has revealed the extent of genetic variation within this population to include potential new risk factors, and sCJD analysis has uncovered a novel *PRNP* polymorphism.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

MTB, RGW, RSGK designed the study. MTB performed the genotyping. CP and CAH assessed the clinical presentation of vCJD and sCJD patients with *PRNP* polymorphisms. All authors helped draft and approve the final manuscript.

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