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Magnetic Resonance Imaging in the diagnosis of Creutzfeldt-Jakob disease

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Declaration

I hereby declare that this thesis has been composed by myself, and that the work submitted is my own. Certain parts of this work were undertaken in collaboration with colleagues, and this is acknowledged in the text. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others. This work has not been submitted for any other degree or professional qualification.

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Abstract

The diagnosis of Creutzfeldt-Jakob disease (CJD) is dependent on an assessment of clinical features together with specialist investigations including electroencephalogram (EEG), magnetic resonance imaging (MRI) of the brain and the cerebrospinal fluid (CSF) tests 14-3-3 immunoassay and real-time quaking induced conversion (RT-QuIC).There has been an evolution in the diagnosis of CJD with time as new tests have been developed and this includes MRI, which has proved very useful in the diagnosis of all forms of CJD, including sporadic CJD (sCJD) and variant CJD (vCJD). MRI signal abnormalities in sCJD have a relatively high diagnostic sensitivity and specificity, although this varies in different studies in relation to the precise details of the study population.

The primary aim of this thesis: To examine the overall role of MRI brain scan in the diagnosis of sCJD.

Other, specific, aims:

- How accurate is local reporting of the MRI findings in CJD and has this changed with time?
- Is there variation in the utility of MRI in the diagnosis of CJD according to codon 129 genotype and prion protein isotype
- Is there evidence that MRI in sCJD and vCJD is more likely to show characteristic features at different stages of the clinical illness and what is the role of serial scans?
- Is there a relationship between specific MRI appearances and the clinical presentation in sCJD?
- Is the MRI of use in identifying cases of methionine-valine (MV) vCJD?

- Are there scans in non-CJD cases that show features suggestive or characteristic of CJD, and are these cases distinguishable from true cases on the basis of clinical features or other investigations?

- Are there cases of CJD in which the scans show no characteristic features, and are there any parameters that distinguish these cases, for example scan quality or timing?

In order to assess the study aims, 462 cases of sCJD referred the National CJD Research and Surveillance Unit (NCJDRSU), identified as having an MRI brains scan performed during their illness, were analysed retrospectively (2010-2015). 54 non-CJD cases (referred to NCJDRSU, with an initial suspicion of CJD, but who turned out to have other illnesses) were included for comparative analyses (2010-2015). In addition, 25 cases of vCJD with brain MRI DWI sequences (1999-2015) were identified and these scans were analysed. The scans were reviewed by a single Neuroradiologist, experienced in the assessment of MRI, for the presence or absence of features associated with these diseases. The Neuroradiologist was aware of the suspicion of CJD but was blinded to the specific diagnosis in each case.

This thesis determines the role of MRI brain scan in the diagnosis of sCJD and provides further validation of the current European diagnostic criteria (2017) for the condition. The results show that brain MRI, with the inclusion of DWI sequences, is a highly sensitive, specific and reliable test for the diagnosis of sCJD if applied in the appropriate clinical circumstances and can improve the diagnostic classification of sCJD in life.

Lay Summary

Creutzfeldt-Jakob Disease, or 'CJD', is a rare and invariably fatal brain condition. There are different forms of the disease including a type that occurs by chance, known as sporadic CJD (sCJD), and acquired forms, the most notable of which is known as variant CJD (vCJD).

sCJD is the most common form of the disease and tends to present with a rapidly progressive dementia (a general term for a group of brain diseases that cause memory and thinking problems) and muscle twitching. The condition gets worse and death occurs within several months of when the symptoms first start. The underlying cause of CJD is unknown, but the different types are linked by a change in shape of a naturally occurring protein within the brain, called the prion protein. The change in shape of the prion protein starts a sequence of events which result in brain cell death leading to a number of neurological symptoms.

Unfortunately, there is no cure for CJD and the diagnosis can only definitively be made by examining brain tissue at post-mortem. However, there are a number of investigations that can assist with making a diagnosis of CJD in life with varying degrees of certainty. These include an MRI scan of the brain, specialised spinal fluid tests collected by way of a lumbar puncture and an EEG ('electroencephalogram') to measure abnormalities in brain waves. Of these tests, the MRI scan of the brain is one of the most useful as it is relatively easy to do and available in most hospitals. The MRI scan can show abnormalities that can help make a diagnosis of CJD as well as exclude other conditions with similar symptoms.

There have been a number of studies that have investigated the use of MRI in the diagnosis of CJD, for instance how often are signal abnormalities present on scans in individuals with CJD and whether these abnormalities occur in other conditions which are not CJD. The results of these studies vary depending on the types of patients that have been assessed.

It is therefore important to determine the overall role of MRI in the diagnosis of CJD. This thesis aims to assess this in the context of a large national study as well as addressing a number of additional scientific questions that are relevant to the clinical diagnosis of CJD.

The main aim of this thesis was to determine the use of MRI in the clinical diagnosis of sCJD.

Other, specific, aims:

- To review the accuracy of local reporting of MRI findings in CJD and if this has changed with time.
- To assess whether presenting symptoms influence the MRI result.
- To ascertain whether the timing of the MRI scan during the illness influences the outcome of the test and if there is a role for doing repeat scans.

In order to access the study aims, 462 cases of sCJD referred the NCJDRSU, identified as having an MRI brains scan performed during their illness, were analysed retrospectively (2010-2015). 54 non-CJD cases (referred to NCJDRSU, with an initial suspicion of CJD, but who turned out to have other illnesses) were included for comparative analyses (2010-2015). In addition, 25 cases of vCJD with brain MRI DWI sequences (1999-2015) were identified and these scans were analysed. The scans were reviewed by a single Neuroradiologist, experienced in the assessment of MRI, for the presence or absence of features associated with these diseases. The Neuroradiologist was aware of the suspicion of CJD but was blinded to the specific diagnosis in each case.

This thesis determines the role of MRI brain scan in the diagnosis of sCJD and provides further validation of the current European diagnostic criteria (2017) for the condition. The results show that brain MRI is a highly sensitive, specific and reliable test for the diagnosis of sCJD if applied in the appropriate clinical circumstances and can improve the diagnostic classification of sCJD in life.

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Glossary

AD	Alzheimer's disease
ALS	Amyotrophic Lateral Sclerosis
BSE	Bovine spongiform encephalopathy
CJD	Creutzfeldt-Jakob disease
CNS	Central nervous system
CSF	Cerebral spinal fluid
DWI	Diffusion weighted imaging
DTI	Diffusion tensor imaging
EEG	Electroencephalogram
FFI	Fatal familial insomnia
FLAIR	Fluid attenuated inversion imaging
gCJD	Genetic Creutzfeldt-Jakob disease
GSS	Gerstmann Sträussler Scheinker
hGH	Human growth hormone
iCJD	Iatrogenic Creutzfeldt-Jakob disease
LBD	Lewy body disease
M	Methionine
MRI	Magnetic resonance imaging
NCJDRSU	National Research and Surveillance Unit
PRNP	Human prion protein gene
PrP^{c}	Normal soluble prion protein
PrP	Prion protein
PrP^{Sc}	Abnormal insoluble prion protein
SBO	Specified bovine offals
sCJD	Sporadic Creutzfeldt-Jakob disease
TME	Transmissible mink encephalopathy
TSE	Transmissible spongiform encephalopathy

UK	United Kingdom
vCJD	Variant Creutzfeldt-Jakob disease
V	Valine
VPSPr	Variably protease sensitive prionopathy
WCC	White cell count

Contents

Chapter 1 Introduction

1.1 Prion diseases

Prion diseases, also known as transmissible spongiform encephalopathies (TSE), are a group of invariably fatal neurodegenerative disorders affecting humans and a variety of other mammalian species. Their name reflects the underlying molecular disorder in these diseases: a post-translational structural change of a normal protein, prion protein (designated PrP^{C}), to an abnormal form (designated PrP^{Sc})(1). They have also been termed 'transmissible spongiform encephalopathies' due to their transmissibility (both experimentally, and, in some cases, naturally) and also due to the distinctive spongiform changes identified on pathological examination of the brain.

Prion diseases are characterised pathologically by the deposition of the abnormally folded, relatively protease resistant, prion protein (PrP^{Sc}) in tissues (2). Other neuropathological features include neuronal loss, astrocytic gliosis and spongiform change.

Prion diseases have been divided into three main forms, according to causation: Genetic, Sporadic and acquired (3). Genetic forms reflect an underlying mutation in the prion protein gene (in humans, designated *PRNP*), acquired human disease occurs in iatrogenic and zoonotic forms (4), but the commonest human prion disease occurs sporadically with uncertain cause (5).

1.2 Molecular Underpinning & Pathogenesis

The normal prion protein (PrP^{c}) is encoded by the prion protein gene (*PRNP*) on human chromosome 20 (6), with a similar corresponding prion protein gene in animals. It is found on the outer surface of plasma membranes of cells, anchored by a glycosylated phosphatidyl tail (7) and is expressed in numerous cellular tissues, with a high concentration found within the central nervous system (8). The normal function of the prion protein is not fully understood, but it forms a pivotal role in the development of prion diseases (9-11).

The abnormal protease resistant isoform, PrP^{Sc} (Sc denoting the scrapie isoform of the protein) is structurally different form the normal prion protein (see figure 1). This results in different physico-chemical properties, and, importantly, an ability to interact with normal PrP^{C} causing further conversion to PrP^{Sc}; thus, triggering a cascade reaction (1, 12, 13) . The abnormal prion protein is relatively insoluble and protease resistant, and tends to aggregate, forming amyloid structures, which are deposited in tissue (1, 12). Clinical disease results from neuronal degeneration/loss, astrocyte gliosis and spongiform change (2, 3). The pathogenesis of prion disease is not fully understood – the precise relationship between the prion protein changes and neuronal death is uncertain.

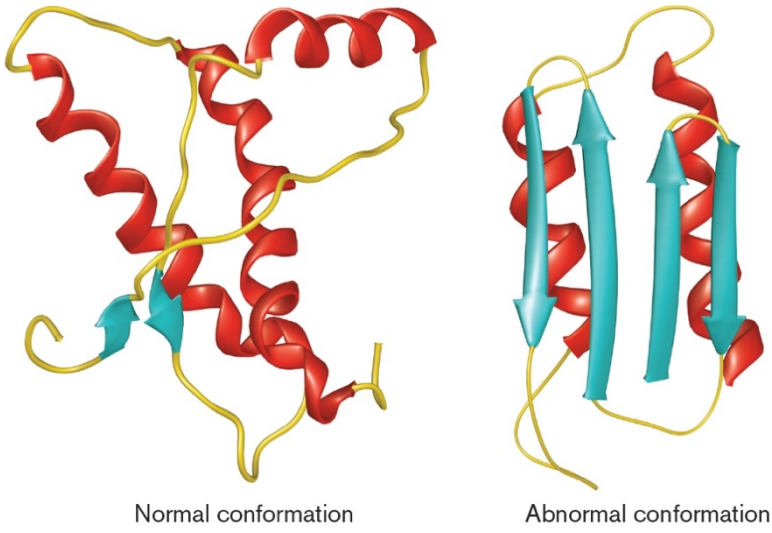


Figure 1: A representation of the normal and disease-related forms of prion protein, illustrating less alpha-helical and more beta-sheeted structure in PrP^{Sc} than in PrP^{C}

1.2.1 Codon 129

The human prion protein (PrP) has a common polymorphism at codon 129, coding for either methionine (M) or valine (V). This polymorphism has important effects on the susceptibility of prion diseases, the incubation period (in acquired forms) and on the clinicopathological phenotype (14, 15). There are three possible genotypes related to this polymorphism: methionine homozygosity (MM), valine homozygosity (VV) or methionine valine heterozygosity (MV). There is variation in genotype frequencies observed in populations according to geographical region. In the UK population, the genotype distribution has been reported as 39% MM, 50% MV and 11% VV (16).

MM homozygosity at codon 129 is considered a risk factor in the development of human prion diseases and is present in approximately 70% of sporadic CJD and in the majority of variant CJD cases (16, 17). The majority of iatrogenic cases of CJD are homozygous at codon 129 and the presence of this genotype is associated with a reduced incubation time in this disease (4). Kuru, an acquired CJD limited to the region of Papa New Guinea, also appears to be influenced by codon 129 genotype, with MM cases being associated with an earlier age of onset and a shorter disease duration, when compared to MV cases (18, 19).

1.2.2 Molecular typing of PrP^{Sc}

As well as polymorphism at codon 129, the specific isoform of PrP^{Sc} deposited in the brain appears to influence the clinicopathological features of human prion disease (20). There are two major isoforms of PrP^{Sc}, which in the most commonly used nomenclature are termed type 1 and type 2 (21). These isoforms can be distinguished on the basis of physiochemical properties including differences in fragment size seen on Western blotting following proteolytic degradation (21). Type 1 PrP^{Sc} is a core fragment with an N-terminus at glycine 92 and has a molecular weight around 21kDa. Type 2 PrP^{sc} is a core fragment with an N-terminus at serine 97 with a molecular weight of around 19kDa (22). The western blot also

demonstrates the three possible glycoforms of PrP (diglycosylated, monoglycosylated and unglycosyated), which are used in protein typing (23).

1.3 The transmissible agent

The transmissible nature of prion diseases was first recognised in 1935 with the experimental inoculation of a healthy ewe using scrapie infected spinal tissue (24). The transmissibility of the human diseases came years later with the inoculation of chimpanzees with kuru (1966), followed by sporadic CJD (1968), Gerstmann-Straussler-Scheinker disease (1981), and fatal familial insomnia (FFI) (1995)(25). These conditions thus became grouped by their transmissible and pathological affinities, although the underlying infectious agent continued to evade detection. In 1952, a theory that these diseases were caused by a 'slow-virus' emerged and gained popularity amongst the scientific community (26). However, no virus was ever isolated to support this theory.

In the 1970s, an American Scientist, Stanley B. Prusiner, proposed the infectious agent to be a self-replicating protein. He coined the term 'Proteinacaeous infectious particle' or 'Prion', which was devoid of nucleic acid (27). There was much controversy around this novel theory, with many in the scientific community continuing to believe in the concept of a slow virus. Further experiments revealed a remarkable resistance of the infectious agent to conventional materials and methods that inactivated viruses and other microorganisms (28). Conversely, methods that denature or destroy proteins prevented transmission of the agent, strongly supporting the now increasingly accepted protein-only hypothesis as the causative agent (29). Stanley Prusiner was awarded the Nobel prize for Physiology or Medicine in 1997 for this discovery (30).

1.4 Prion diseases in Animals

1.4.1 Scrapie

Scrapie is the most common naturally occurring TSE of animals, affecting sheep, goats and mouflon. The term derives from the tendency of the affected animal to scrape against bushes and trees. It was first recognised in England in 1730 with subsequent reporting in other countries across the world (31). In France it is known as 'la tremblante' (trembling disease), 'traberkrankheit' (trotting disease) in Germany, and 'rida' (tremor) in Iceland (31)

The transmissibility of scrapie has been well established since the 1930s (28, 32, 33), but the exact mechanism of the natural spread of the disease remains obscure (33). It shares a property with chronic wasting disease (CWD) of cervids, in that they both are readily transmissible within herds (although this is more marked in CWD) (34, 35). As with other TSEs, the neuropathological triad of neuronal loss, astrocyte gliosis and spongiform change occurs in scrapie (36).

Despite being the first TSE to be recognised in animals, it was the emergence of bovine spongiform encephalopathy (BSE) in the 1980s that brought general international prominence to this unique set of disorders.

1.4.2 Bovine spongiform encephalopathy

In November 1986, the first ever confirmed case of BSE emerged in the United Kingdom (UK) which subsequently developed into a major epidemic over the following decade. Around 180,000 cattle developed this previously unrecognised TSE, with a likely 1-3 million subclinical cases slaughtered for human consumption (37). The disease has occurred in a number of other European countries although the reported incidence is much lower than the UK (38). The duration of the clinical course is typically 1-2 months, with commonly observed symptoms including apprehension, ataxia, decreased milk yield and weight loss (39).

A number of epidemiological studies have indicated that BSE was transmitted in the cattle population from the use of cattle feed containing meat and bonemeal from infected animals (40, 41). The original source of the epizootic remains unknown, but it is postulated to have arisen from either a scrapie source or sporadic/hereditary disease of cattle amplified by feeding practices (40-42)

The British Government made BSE a notifiable disease in June 1988, followed soon after by the introduction of a statutory ban on the feeding of ruminant-derived protein to ruminants. In November 1989, a ban on the use of specified high-risk bovine offal (SBO) for human consumption was introduced (43). By late 1992, the BSE epizootic reached its peak incidence before rapidly declining; almost certainly as a result of the statutory measures introduced from 1989 (41, 44).

Regardless of the actions taken to reduce the BSE epizootic and thus minimize the theoretical risk of BSE transmission, it was known that a proportion of the human population had been exposed to contaminated meat products. The potential of transmission of BSE to humans via this route had been discussed from the onset of the epidemic(45, 46) In April 1996, ten years after the emergence of the first case of BSE, a novel form of CJD was reported in 10 patients in the UK (47). These cases were distinguished from sporadic CJD by younger age of onset, disease duration and a clinical complex dominated by neuropsychiatric features. They also exhibited certain neuropathologic features, most notably amyloid plaques staining for PrP, rarely seen in sCJD (3), although known to occur in other human prion diseases, such as kuru and Gerstmann-Sträussler-Scheinker disease (GSS). The disease became known as new variant CJD (vCJD).

The temporal relationship between the BSE epidemic and the rise of vCJD, coupled with laboratory transmission studies in macaque monkeys and wild-type and transgenic mice, supports the hypothesis that vCJD represents transmission of BSE infection to humans (48-50). BSE is now very rare, with only sporadically occurring cases being detected by active surveillance.

1.4.3 Chronic wasting disease

Chronic wasting disease (CWD) is a TSE originally described in 1967 in captive North American mule deer (51). It was initially recognised by biologists as a clinical wasting syndrome and was first recognised as a spongiform encephalopathy in 1977 (52). As the name suggests, the most prominent clinical sign is progressive weight loss often associated with behavioural changes followed by excessive salivation, polyuria, polydipsia and death (52). Since its recognition, species other than mule deer have been found to display the disease, including Rocky Mountain elk, white tailed deer, moose, and reindeer. The transmission of CWD is highly efficient, although the exact mechanism of spread remains unknown. Exposure to salvia, faeces and placenta from affected animals, however, has been postulated to be the mechanism of transmission as all these tissues contain infective material (51).

CWD was first diagnosed in Europe in March 2016 in a free-ranging reindeer from the Nordfjella mountain area in Norway (53). This was followed by further cases of infected moose and wild reindeer in Selbu, 300km North of the original case, suggesting geographical dissemination of the disease (54). To date, 24 cases of CWD have been identified in Norway, comprising 19 free-ranging reindeer, four moose and one red deer (55)

The histopathological changes in the reindeer cases are reminiscent of the highly contagious CWD found in North America. Conversely, three of the moose cases and only one red deer case, which were noted to be older animals, showed molecular and IHC phenotypes differing from the classic North American cases, suggesting a novel strain of CWD termed atypical CWD (54). In 2018, an older moose who died naturally in Finland was diagnosed with CWD with histopathology similar to that of the atypical CWD cases in Norway (56).

The increasing prevalence of CWD in both North America and Europe, have caused concern that this TSE may also transmit to other animals, including humans. To date, epidemiologically studies have found no evidence of CWD transmission to humans (57). There have been two studies on squirrel monkeys providing evidence that transmission of CWD prions is possible under experimental conditions (58). Furthermore, a number of in vitro experiments have provided evidence that CWD can convert human prion protein into a misfolded state (59). Transmission to macaques – a monkey genetically closer to humans

than squirrel monkeys, was unsuccessful in one laboratory (60), but successful transmission in macaques exposed to skeletal muscle tissue from a CWD case in 2019 has caused concern (61).

1.4.4 Transmissible Mink Encephalopathy

Transmissible Mink Encephalotomy (TME) is a rare sporadic disease occurring in ranch raised mink. It was first recognised in 1947 in Minnesota and Wisconsin (62) but has been known to occur in outbreaks in Idaho, Russia, Canada, Finland and Germany (63). Only adult minks appear to be affected by TME and the incubation period is believed to be approximately 6-12 months with symptom onset to death ranging between 2-8 weeks (63).

The underlying source of TME outbreaks is unknown. Evidence points towards the ingestion of scrapie infected feed as a potential cause, although experimental transmission of scrapie to mink via the oral route have been unsuccessful (62).

The clinical features of TME are characterised by behavioural disturbance including confusion, aimless circling and aggression, followed by abnormal gait, compulsive biting, somnolence and death (63).

The pathological findings of TME are similar to other spongiform encephalopathies with spongiform degeneration of the cerebral cortex, telencephalon, mesencephalon and diencepdorhalon (63).

1.4.5 Camel prion disease

In 2018, camel prion disease (CPD) was detected in dromedary species (camelus dromedarius) in an abattoir in South Eastern Algeria (64). The camels displayed clinical symptoms including weight loss and behavioural changes, and neurological signs such as tremor, aggressiveness and hyperactivity. The disease progressed over a period of 3-8 months with the development of ataxia and recumbency followed by death (64). Anecdotal evidence

from breeders and those working in the abattoir suggested that the disease may have been present since the 1980s with a speculative prevalence of 3.1% between 2015 and 2016. The neuropathology in the identified cases demonstrated spongiform change, gliosis and neuronal loss similar to that in other prion diseases (64).

The origin of CPD is unknown and at the current time there is no evidence to suggest that it results from transmission of a prion disease from another species. The PrP^{Sc} by a chemical signature in CPD differs from that of BSE and classical scrapie (64).

1.5 Human prion diseases

The human prion diseases have been divided into three main forms, according to causation: Genetic, Sporadic and acquired.

1.5.1 The history of CJD – what's in a name?

The term CJD is somewhat of a misnomer. In 1920 Hans Creutzfeldt, a German neurologist reported a case of a 22-year-old female with a six-year history of a progressive and fatal neurological disorder (65). In the following few years, Alfons Jakob, a Hamburg neuropathologist, published five cases of rapidly progressive dementia felt to be similar to Creutzfeldt's original case (66-68). In 1922, the name 'CJ Krankheit' was proposed by Walter Spielmeger, a colleague of Creutzfeldt and Jakob, in recognition of their work and in order that the publication appeared (69).

Creutzfeldt's case was very different from those Jakob described and, in fact, is unlikely to be a case of CJD based upon modern clinical and histological criteria (70). Furthermore, the re-examination of Jakob's five cases indicated that only his third and fifth cases fulfilled the criteria for sporadic CJD as they are now defined (71).

The sequence of eponyms is often debated with some believing that is should be known as 'Jakob's disease' or perhaps 'JC disease' as it often appears in German literature. However,

the term CJD has become deeply entrenched in both scientific and public domains and is therefore unlikely to change.

1.5.2 Kuru

Kuru is a human TSE geographically restricted to the Okapa mountainous area of Papua New Guinea (72). It was first described by Western medicine in the 1950's, but cases were likely occurring for several decades before this. The term "Kuru" derives from the Fore word for "shiver" or "trembling", referring to the characteristic body tremor associated with the disease (73). Although the cause of Kuru was initially unknown, extensive epidemiological studies concluded that the disease resulted from the practice of ritualistic cannibalisation in which the affected communities engaged in the consumption of dead relatives as a mark of respect and mourning (74). This resulted in exposure to a variety of tissues including highly infectious brain material. Whilst it was rare for adult males to follow this practice, women and children engaged more frequently, which explains the observed sex and differential age incidence. It is proposed that Kuru initially arose following the cannibalisation of an individual with either sporadic or familial CJD within the Fore region, given other tribes with similar cannibalistic practices in Papua New Guinea were unaffected (75).

The Kuru epidemic has gradually declined since the late 1950s due to the cessation of cannibalisation with the last recorded death in 2005 (76).

Clinically, cases of Kuru were remarkably homogenous with a progressive cerebellar syndrome, often with a prodromal phase characterised by headache and limb pains (75). In contrast to sCJD, cognitive impairment is often absent or occurs late in Kuru, and the mean duration of illness is longer at 12 months. The cardinal neuropathological features of these cases are the presence of PrP -positive amyloid plaques found concentrated in the cerebellum in around 80% of cases, as well as neuronal loss and spongiform changes (77). It was recognised that these pathological features were similar to those in scrapie, a disease demonstrating transmissibility back in 1936. This observation paved the way for transmission studies in Kuru, and in 1966 the first human TSE was confirmed following intracerebral inoculation of Kuru brain tissue in primates by Gajdusek and colleagues (78).

This was a monumental breakthrough and proved for the first time that a transmissible agent caused human prion diseases; however, the underlying mechanism that caused the disease to spread would remain elusive for years to come. This scientific study has been crucial to our current understanding of other prion diseases and highlighted the need for disease surveillance for these conditions to determine an underlying aetiology.

1.5.3 Sporadic CJD

Sporadic CJD accounts for the majority (85%) of cases of human prion disease. The cause is unclear, with cases appearing sporadically throughout the world. Extensive epidemiological studies have failed to show causative links with animal diseases, with the only risk factors identified being age and the *PRNP*-129 polymorphism (16). However, the prevailing hypothesis is that sCJD either arises from a somatic mutation of *PRNP* or is due to spontaneous change of PrP^{C} to PrP^{Sc}, which then accumulates as more normal protein is converted by interaction with the abnormal form (1).

It typically presents with a rapidly progressive dementia with additional neurological features including predominant ataxia and myoclonus. The peak incidence is in the seventh decade, with younger (20-40s) or older (>80) cases being much less common (79). In most cases rapid clinical progression ensues, leading to akinetic mutism and eventual death. The median disease duration is around 4 months (80).

There are a range of atypical presentations, including those with a long disease duration and slower progression of cognitive decline resembling that of Alzheimer's disease, cases presenting with cerebellar ataxia, cases presenting with pure visual symptoms and those with a stroke-like onset (21, 81, 82). The variation in the clinical phenotype of sCJD is related, at least in part, to the codon 129 genotype and the biochemical properties of PrP^{Sc} deposited within the brain (21). The combination of the different codon 129 genotypes and the two types of PrP^{Sc}, discussed earlier in this chapter, allows classification of sCJD into six subtypes as defined by the Parchi classification system (20). The neuropathological findings provide additional classification as to whether features predominantly affect thalamic or cortical regions (MM2-thalamic and MM2-cortical respectively) (see table 1).

Those with a "typical" clinical phenotype of advanced age at onset, rapidly progressive dementia, prominent ataxia and myoclonus and a short illness duration are represented in the MM1 and MV1 subgroups, accounting for approximately 60% of cases. Those with an "atypical" phenotype defined in terms of illness duration, clinical presentation, investigation results and neuropathology, are composed from the other four subtypes (20). The occurrence of type 1 and 2 isoforms of PrP^{Sc} are not mutually exclusive and certain brain samples and individuals can contain both type 1 and type 2 isoforms. In 2009 Parchi *et al* proposed a revised subclassification system for sCJD that identifies six additional mixed subtypes to those mentioned above (83).

At neuropathology, spongiform change, astrocytosis and neuronal loss are seen, with amyloid plaques present in a small minority. The mechanisms underlying the relationship between molecular and clinical findings is yet to be determined (3).

Table 1: Typical clinical features of sCJD subtypes adapted from Parchi et al 2011)

sCJD subtype	Percentage of cases (%)	Clinical features
MM1 or MV1	70	Rapidly progressive dementia, early myoclonus, ataxia at onset in 50% and visual impairment in 30%
MM2-C (cortical)	2	Dementia, myoclonus and pyramidal signs. Ataxia rare.
MM2-T (thalamic)	2	Ataxia, insomnia and psychomotor hyperactivity ('sporadic fatal insomnia')
MV2	9	Progressive dementia with ataxia. Long duration (>2 years) in some cases
VV1	1	Progressive dementia followed by myoclonus and pyramidal signs
VV2	16	Ataxia at onset, late dementia

1.5.4 Variant CJD

Variant CJD (vCJD) was first described in 1996 following the identification of ten cases in the United Kingdom (UK) with distinctive clinical and pathological features to sCJD (47). It was hypothesised that this novel form of CJD was caused by human infection with BSE; an epidemic predominantly confined to the UK (43). The European collaborative surveillance system failed to identify similar cases at that time in other European countries (38). Subsequent laboratory transmission studies in wild-type and transgenic mice indicated that the infectious agent in vCJD is indistinguishable from the BSE agent, strongly supporting the hypothesis that this condition represents BSE transmission to the human population via dietary exposure (48, 49, 84).

To date, a total of 179 cases of definite or probable variant CJD have been identified in the UK and a small number in other countries. Several legislative measures introduced in the UK, including the Specified Bovine Offal (SBO) ban in 1989 and the "over 30 months rule" in 1996, significantly reduced the exposure to humans and, as a result, there has not been a reported case of vCJD in an individual born after 1989 (85).

vCJD has characteristically affected individuals in younger age groups with a mean age of death of 29 years (range 14-74) (86). The initial clinical presentation typically consists of a neuropsychiatric profile such as depression, anxiety and withdrawal. In two-thirds of cases, painful sensory symptoms occur affecting the limbs, face and trunk (86, 87). Nearly all develop cerebellar ataxia, and chorea, dystonia or myoclonus are observed in the majority of cases. As the condition progresses, clear cognitive impairment ensues terminating in a bed-bound, akinetic and mute state (87). The mean duration of illness is 14 months (range 6-84), in contrast to that observed in sCJD with a mean duration of 4 months (range 1-74).

The annual number of deaths from vCJD peaked in 2000 and declined significantly thereafter (85). Until recently all tested cases of definite and probable vCJD had been methionine homozygote (MM) at codon 129 of *PRNP*, but in 2016 a neuropathologically confirmed case with an MV genotype was identified (88). The possibility of a further outbreak of vCJD, has been predicted experimentally and, if this should occur, the numbers of cases will likely be relatively limited and probably less than that of the primary epidemic in the MM population. The UK population were exposed to a significant level of BSE infectivity in the food chain in

the 1980s and early 1990s and the relatively small scale of the vCJD epidemic may imply a significant barrier to infection between bovines and humans.

The prevalence of vCJD infection in the general UK population has been studied by examining routine appendicectomy samples by immunocytochemistry. 19 of 33,115 samples were positive for abnormal PrP, leading to an estimated prevalence of 1 in 4000 in the UK population(89). A recent study examined appendix tissue from before 1980, presumed to be prior to the start of the BSE epidemic, and in those born after 1996, after which human exposure to BSE was believed to be minimal. Somewhat surprisingly, 7 of 29,516 samples were positive for the abnormal PrP suggesting either a more prolonged period of human exposure to BSE or that, perhaps, positivity may not imply infection with vCJD (90).

There has been a consistent and gradual rise in sCJD mortality rates in the UK and in many other countries where CJD surveillance is undertaken. This may relate to improved case ascertainment as a result of increased awareness and more sensitive diagnostic investigations as well as changes in population demographics. Another possibility for the rising mortality rates, is that cases of vCJD are being misclassified as sCJD. If this were to be a contributory factor, it would be expected that the UK, where the majority of human exposure to BSE occurred, would display higher mortality rates of CJD than countries with no recorded BSE cases, but this is not the case (91).

Secondary transmission of vCJD has been recognised with three cases of vCJD linked to the transfusion of non-leucodepleted red blood cells from donors who themselves went on to die from vCJD (92). In addition, PrP^{Sc} was discovered in the spleen of an individual who died of a non–neurological disorder 5 years after receiving a blood transfusion from a donor who subsequently died of vCJD, suggesting pre-clinical infection. This asymptomatic individual was a codon 129 heterozygote and laboratory transmission studies using spleen tissue demonstrated prion infectivity with characteristics similar to primary cases (93).

1.5.5 Iatrogenic CJD

Iatrogenic transmission of CJD occurs by the accidental inoculation of tissue containing prions from a variety of medical procedures. Although, as described above, blood and blood product transmission has been reported in vCJD, these cases tend not to be classified under

iatrogenic CJD (iCJD) and the modes of transmission have not been reported in the other forms of human prion diseases. The first documented case of human iCJD was in 1974 and involved the development of CJD 18 months following the transplant of a corneal graft from a donor who had died from sCJD (94). Shortly following this, two further cases of iCJD were reported linked to depth EEG electrodes that had been implanted previously in a patient, for a period of two days, who was subsequently confirmed to have died from CJD (4).

In retrospect, transmission of CJD via neurosurgical instruments likely occurred back in the 1950s in three cases of individuals operated on with the same surgical instruments used on a previous CJD case (4). Further iatrogenic routes have since been reported including human cadaveric pituitary derived growth hormone (pit-hGH) and dura mater grafting (95). To date, there have been approximately 400 cases of iCJD (96). The clinical presentation of iCJD is determined by the route of exposure. Cases that result from intracerebral or optic transmission manifest clinically as sCJD with rapidly progressive dementia (96). Whereas cases from a peripheral exposure, notably pit-hGH tend to present with progressive cerebellar ataxia with relatively mild or late cognitive impairment similar to Kuru (95).

1.5.6 Genetic CJD

Inherited or genetic CJD (gCJD) occur as a result of pathogenic mutations in the *PRNP* gene and account for 10-15% of all prion diseases (97). They are transmitted in an autosomal dominant fashion and over 30 different mutations have been recognised (98). Historically, the clinical phenotypes have been divided into Gerstmann-Sträussler-Scheinker disease (GSS), fatal familial insomnia (FFI) and genetic (previously familial) CJD (13).

The clinicopathological features of the genetic prion diseases show striking heterogeneity, including age of onset, duration of disease and neuropathological findings (98). The most common *PRNP* mutation world-wide is E200K, accounting for approximately 70% of cases of genetic CJD (99). The clinical phenotype of the E200K mutation can be similar to classical sCJD with rapid cognitive decline, myoclonus and pyramidal and cerebellar signs. Age of onset is slightly younger than that of sCJD with a median age of 58 and a mean duration of 7 months (100). GSS disease is most commonly associated with the P102L-129M mutation

(101), traditionally presenting with a slowly progressive ataxia with cognitive impairment appearing later on in the disease process. Symptom onset is often in the fourth decade and disease duration can range from months to 6 years (99).

FFI is a disease characterised by relentless insomnia, prominent autonomic disfunction and myoclonus. This phenotype is associated with the D178N-129M mutation and has a median age of onset of 49 and a disease duration of approximately 12 months (99). The rare MM2T subtype of sCJD has a remarkably similar clinicopathological phenotype to FFI and, because of this, is known as sporadic Fatal Insomnia (sFI) (102). Interestingly, in contrast to what is observed is other neurodegenerative disorders, sFI is rarer than its genetic counterpart (103).

1.5.7 Variably Protease Sensitive Prionopathy (VPSPr)

In 2008, Gambetti and colleagues reported a novel human prion disease originally termed protease-sensitive prionopathy (PSPr), on the basis of 11 cases identified in the National Prion Disease Pathology Surveillance Centre in Ohio (104). The affected individuals had a younger age of onset compared to typical sCJD as well as a longer disease duration. Further investigation of individuals with the PSPr phenotype, resulted in a change of the name to Variably Protease Sensitive Prionopathy (VPSPr). Since its discovery both prospective and retrospective review of atypical sCJD cases have identified VPSPr in other countries, including the UK. This novel type of human prion disease appears to disproportionately affect codon 129 VV individuals with MV and MM1 cases rarely occurring (104). The combination of long disease duration and reported atypical clinical features in cases of VPSPr can make it difficult to distinguish from other forms of neurodegenerative disorders (105)

The evidence at the current time suggests that VPSPr is a sporadic human prion disease given the lack of iatrogenic risk factors and mutations. It may be that it is a second type of sCJD although this is yet to be determined. The diagnosis of VPSPr is reliant on neuropathology and critically the PrP biochemistry with no formal diagnostic criteria established as of yet.

1.5.8 Other protein misfolding disorders

In addition to prion diseases, there are a number of other disorders associated with protein misfolding. Conditions including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's are all associated with the accumulation of oligomers of A-beta, tau and alpha-synuclein (106). Similarly, alpha-synuclein deposits have been identified in grafted foetal neurons that were transplanted into two PD patients (107), leading to the hypothesis that there may be the potential of seeding of protein from abnormal to normal tissue in this condition. As well as this, beta-amyloid deposits have been identified in the brains of human growth hormone recipients, and not in age-matched controls (108), leading to concerns about equivalences between prion diseases and other neurodegenerative disorders (109). The public health implications of this are uncertain, and there may be a distinction between the seeding of protein and the transmission of disease.

1.6 Surveillance of CJD in the UK

National surveillance of CJD in the UK was established in May 1990 in response to a recommendation in the Southwood Committee report of the working party on BSE (110). The primary aim of this surveillance system, undertaken by the National CJD Research and Surveillance Unit (NCJDRSU), was to identify any possible changes in the pattern of CJD that may indicate the transmission of BSE to the human population. Before the establishment of the NCJDRSU, data on CJD had been retrospectively gathered between 1970 – 1979 and prospectively between 1980 – 1984 as part of MRC funded research funded project in England and Wales. Between 1984 to April 1990, cases within the UK were retrospectively identified on the basis of death certificates.

In 1993, a collaborative surveillance system within Europe was set up in order to share data on the epidemiology of CJD as well as criteria for the definition of cases.

1.7 Investigations and diagnostic classification for sCJD

A definite diagnosis of sCJD requires neuropathological examination of the brain, often performed at autopsy. Although a brain biopsy is a means of obtaining a definite diagnosis in life, it is an invasive and potentially dangerous investigation that is infrequently performed (111). Instead, ante-mortem diagnosis of sCJD is dependent on an assessment of clinical features; the exclusion of alternative, potentially treatable diseases; and the use of specialist investigations including electroencephalogram (EEG), MRI brain scan and the cerebrospinal fluid (CSF) tests 14-3-3 immunoassay and real-time quaking induced conversion (RT-QuIC). These investigations are useful in the appropriate clinical context and are included in the current European diagnostic criteria for the condition (see table 2).

Table 2: Diagnostic criteria for surveillance of sporadic CJD from 1 January 2017 (http://www.cjd.ed.ac.uk/documents/criteria.pdf)

1.1	**DEFINITE:**		
	Progressive neurological syndrome AND		
	Neuropathologically or immunohistochemically or biochemically confirmed		
1.2	**PROBABLE:**		
		1.2.1	I + two of II and typical EEG*
	OR	1.2.2	I + two of II and typical MRI brain scan**
	OR	1.2.3	I + two of II and positive CSF 14-3-3
	OR	1.2.4	Progressive neurological syndrome and positive RT-QuIC in CSF or other tissues
1.3	**POSSIBLE:**		
	I + two of II + duration < 2 years		
I	Rapidly progressive cognitive impairment		
II	A Myoclonus B Visual or cerebellar problems C Pyramidal or extrapyramidal features D Akinetic mutism		

*Generalized periodic complexes
**High signal in caudate/putamen on MRI brain scan or at least two cortical regions (temporal, parietal, occipital) either on DWI or FLAIR

1.7.1 Electroencephalogram

The characteristic Electroencephalogram (EEG) in sCJD shows periodic, triphasic sharp wave complexes (PSWC) at a rate of 1 per second (see figure 2). The underlying pathogenesis of PSWC is unknown, but it has been reported to resemble the EEG pattern observed in preterm newborns (112, 113). This observation has led to speculation that cortical degeneration from sCJD may erode the normal physiological sleep architecture, replacing it with activity from an underlying pacemaker which is involved with the ascending reticulothalmocortical activating system (114).

The sensitivity of EEG is dependent on the codon 129 genotype, PrP^{Sc} protein subtype and the stage of illness at which it is performed. In one study, the overall sensitivity and specificity of EEG in sCJD was 44% and 92% respectively (115). A 'typical' EEG pattern is more often found in MM1 and MV1 cases, whereas it is rarely detected in less common subtypes such as VV2 and MM2-Thalamic (116). PSWC are not pathognomonic of sCJD and have bene reported to occur in other neurodegenerative conditions including end stage Alzheimer's disease and dementia with Lewy bodies (117).

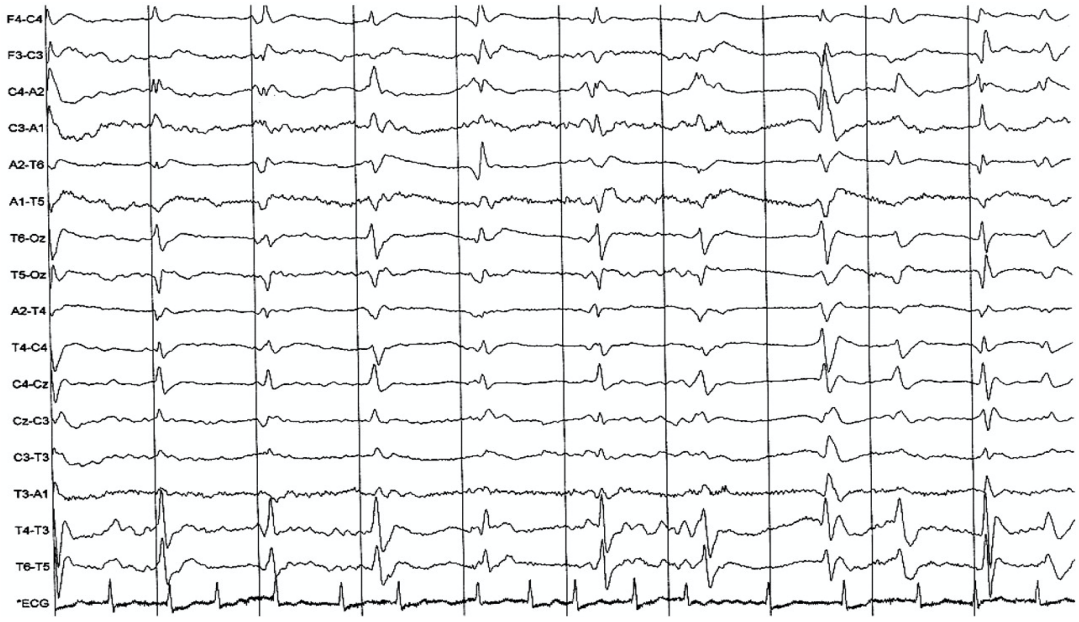


Figure 2: Typical EEG in sCJD demonstrating periodic sharp wave complexes

1.7.2 CSF examination and specialist tests

Routine cerebral spinal fluid (CSF) examination is usually normal in sCJD and the presence of a high CSF white cell count (WCC) should prompt consideration of an alternative diagnosis (118). A mildly raised CSF protein is present in approximately a third of sCJD cases, but levels over 1 g/dl are rarely found (118). There have been a number of CSF biomarkers which have been found to be elevated in sCJD including 14-3-3 and S100b proteins. Similar to the EEG, the detection of these biomarkers in isolation is of limited value but in the appropriate clinical context can be diagnostically useful. In one study a positive 14-3-3 test had a reported sensitivity and specificity of sCJD of 86% and 68% respectively (115). When a positive 14-3-3 test was combined with an elevated S100b level, a reported sensitivity of 95% and specificity of 94% was obtained (119). The detection of CSF 14-3-3 varies with codon 129 genotype, PrP^{Sc} type, and the stage of illness at which the lumbar puncture is performed; with studies reporting it is more likely to be positive in the later stages of illness (116, 120). Given that CSF 14-3-3 protein is a marker for neuronal damage it can also be elevated in a number of other neurological conditions, including viral encephalitis, stroke and paraneoplastic processes (120).

CSF RT-QuIC is a relatively new test which has demonstrated a remarkably high sensitivity and specificity for sCJD in international collaborative studies (sensitivity 85.7% and specificity 100%) (121). The technique exploits the ability of PrP^{Sc} to induce PrP^{C} to misfold to form aggregates of PrP^{Sc} fibrils (122). The test involves the use of recombinant prion protein as a substrate which is induced to aggregate by the addition of CSF containing PrP^{Sc}. The formation of these aggregates is monitored in real time by using a fluorescent dye, Thioflavin T (Tht), which binds to aggregated PrP^{Sc} causing a rapid change in the ThT emission spectrum (122) (see figure 3).

CSF RT-QuIC has a high sensitivity across the various subtypes of sCJD and its positivity does not appear to be influenced by age of disease onset, duration of disease or timing of CSF collection (121). The sensitivity of RT-QuIC is lower in the rarer subtypes with VV1 and MM2-C having reported sensitivities of 75% and 78% respectively (123).

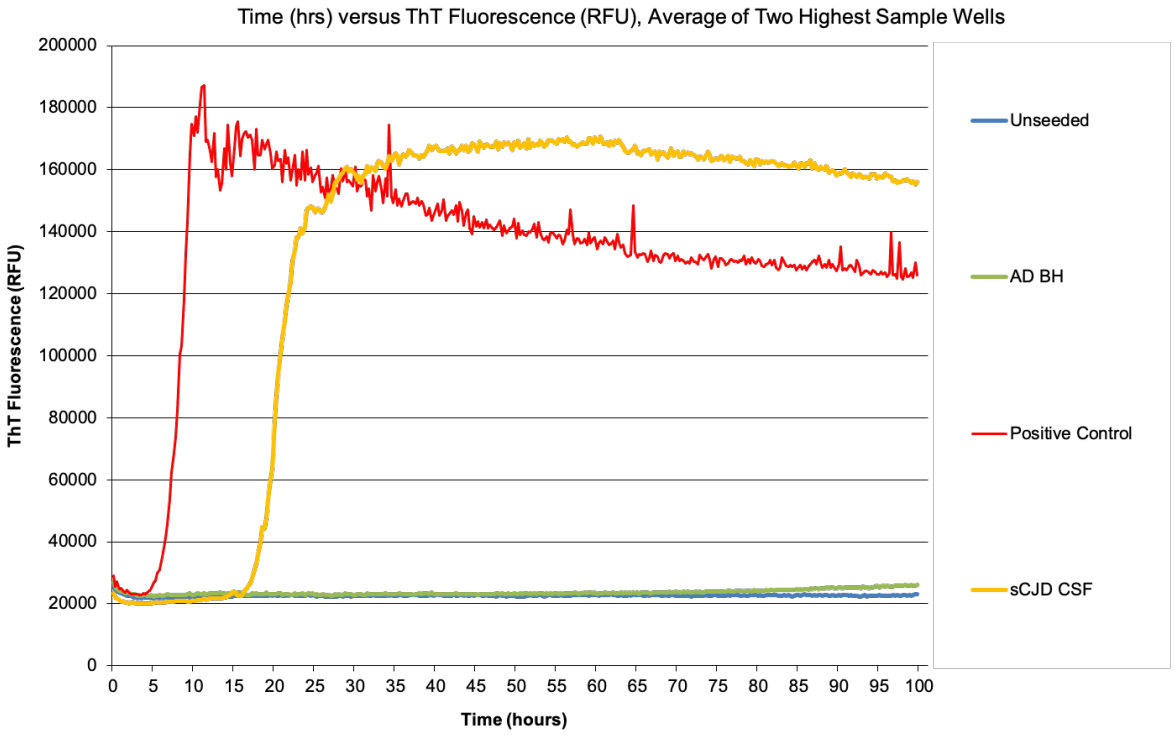


Figure 3: RT-QuIC responses from reactions seeded with CSF from a sCJD case (yellow) alongside a positive control (red). The RT-QuIC from an unseeded reaction (blue) and a reaction seeded with brain homogenate from Alzheimer's disease (AD) are also shown for neg controls. (Image courtesy of Dr Neil McKenzie, University of Edinburgh, Edinburgh, UK).

1.7.3 RT-QuIC olfactory mucosa brushings and skin biopsy

The RT-QuIC technique has been modified to detect prion-seeding activity in other tissues containing PrP^{Sc} including the olfactory epithelium (124) and skin (125). RT-QuIC analysis of olfactory mucosa (OM) nasal brushing has been demonstrated to have a comparable specificity and improved sensitivity compared to CSF analysis (124). A recent study combined RT-QuIC analysis on both OM nasal brushings and CSF in patients with suspected CJD, producing an overall sensitivity and specificity of 100% for the diagnosis of sCJD (126). OM RT-QuIC is limited, however, by the technical expertise required to collect appropriate specimens.

1.7.4 Magnetic Resonance Imaging

MRI brain is one of the most useful, non-invasive investigations in the diagnosis of sCJD (115, 127-129). It is widely available and most patients with a significant neurological illness will have an MRI scan performed. Although initially undertaken to exclude alternative diagnoses, recent advances in MRI technology, including more sensitive sequences, have established MRI as an extremely important diagnostic test for sCJD (see figure 4).

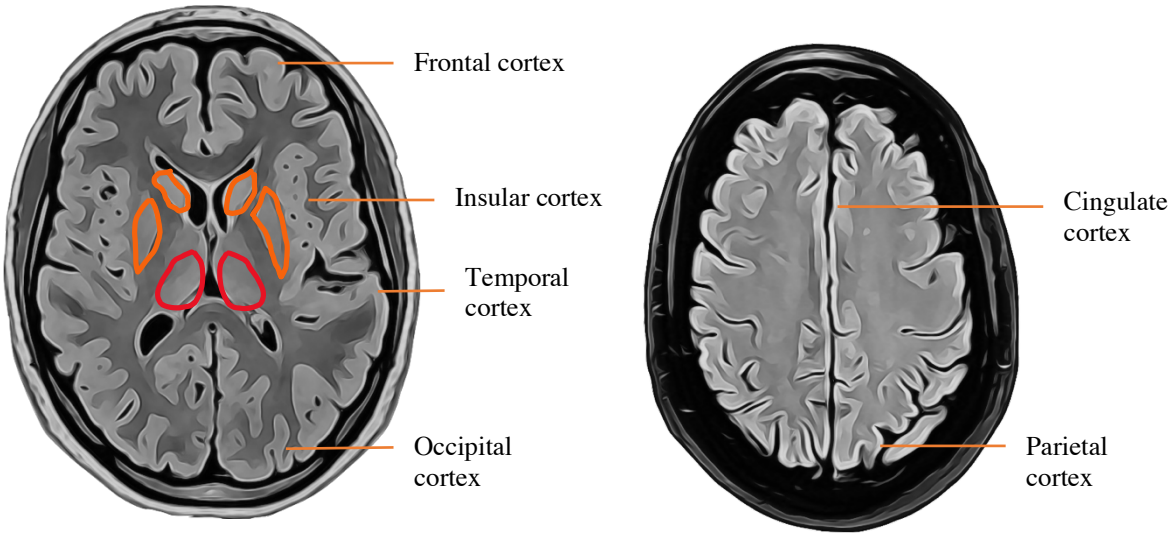


Figure 4: Illustration of cross-sectional MRI brain anatomy showing basal ganglia (highlighted orange), thalamus (highlighted red) and the main cortical regions as labelled.

The characteristic MRI appearances in sCJD include hyperintensities of the basal ganglia (caudate and putamen) and/or cerebral cortex on either FLAIR or DWI sequences (see figure 5) (115, 127-130). DWI has emerged as the most sensitive sequence for the detection of these signal abnormalities, which are present in approximately 80% of cases, either in combination or in isolation (129, 131-135).

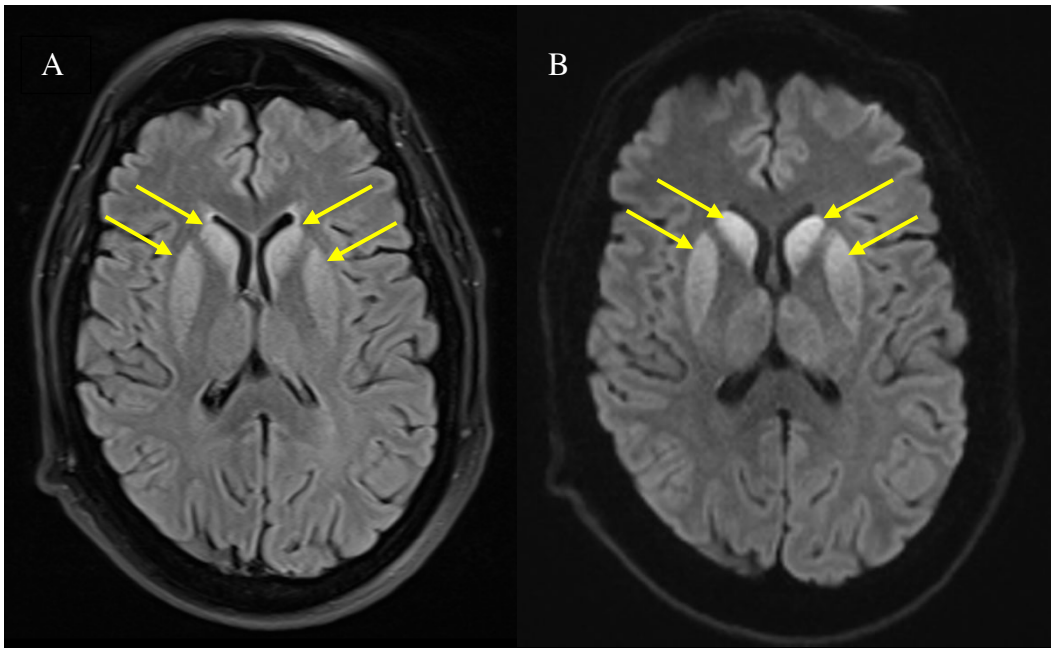


Figure 5: Axial MRI brain showing characteristic basal ganglia high signal on FLAIR (A) (arrows) with more conspicuous high signal on DWI sequences (B).

The seminal study that supported the introduction of MRI abnormalities in the diagnostic criteria for sCJD was carried out in 2009 as an international collaboration and reported both a sensitivity and specificity of 83% with MRI in the diagnosis of sCJD (115). In other studies that used patients with rapidly progressive dementia as controls, MRI with DWI sequences had sensitivities that ranged between 83 and 94%, higher than that of a typical EEG and comparable to CSF 14-3-3 (134-137). The current European diagnostic criteria for sCJD defines a supportive MRI scan as having high signal in the basal ganglia and/or more than two specific cortical regions (parietal, temporal, occipital).

In the vast majority of scans, the caudate and putamen signal abnormalities are symmetrical, with asymmetry occurring in 10-20% of cases (127). Hyperintensities of the cortex, commonly referred to as 'cortical ribboning', involve all lobes, particularly frontal, parietal and temporal regions, whilst isolated limbic involvement is rare and the perirolandic cortex is usually spared (134, 138-141).

MRI abnormalities may vary with the clinical presentation and molecular subtype of CJD (116, 129, 133, 142). For instance, cases with basal ganglia high signal on T2-weighted imaging are more likely to have early dementia and shorter disease duration (129). In a study of 211 patients with neuropathologically confirmed sCJD, basal ganglia high signal occurred more frequently with MV2, VV2 and MM1 subtypes. Widespread cortical involvement was more commonly seen in VV1 and MV1, with thalamic high signal occuring more often in VV2 and MV2 (see figure 6) (133).

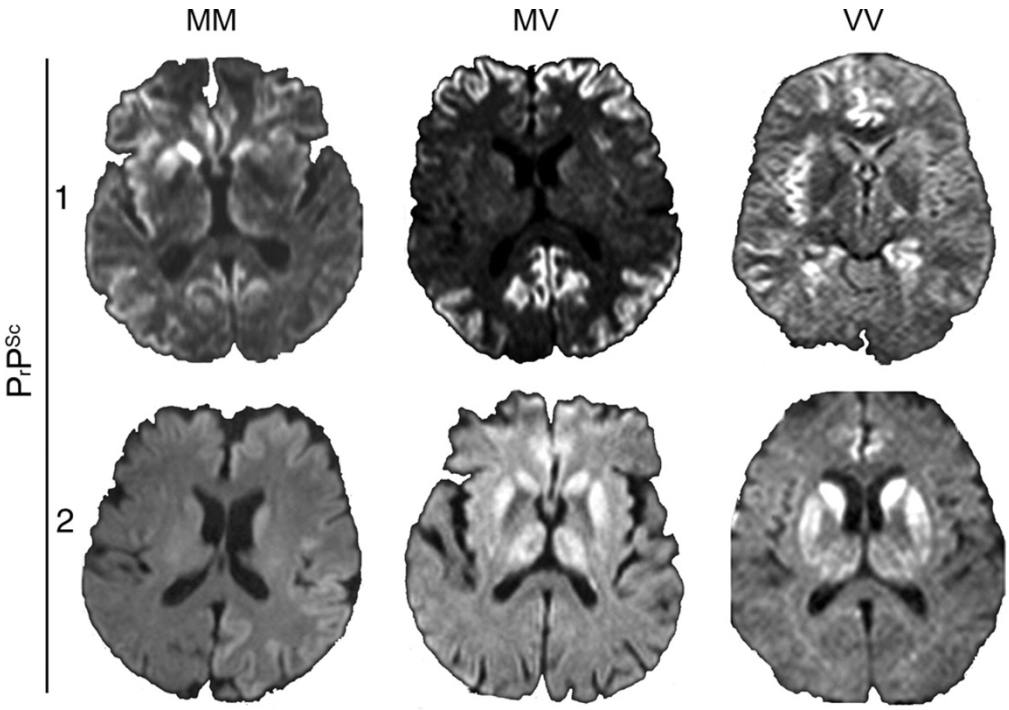


Figure 6: Typical MRI appearances by sCJD subtype (adapted from Meissner et al 2009) (133)

The MRI signal abnormalities associated with sCJD are not entirely specific for the condition, with basal ganglia high signal known to occur in cerebral hypoxia, Wilson's disease, acquired toxic/metabolic disorders, encephalitis and carbon monoxide poisoning(143) . Cortical high signal has also been reported to occur in cerebral hypoxia, stroke as well as hypoglycaemia and seizures (144) (see figure 7). The majority of these conditions, however, can be distinguished from sCJD on the basis of the clinical features.

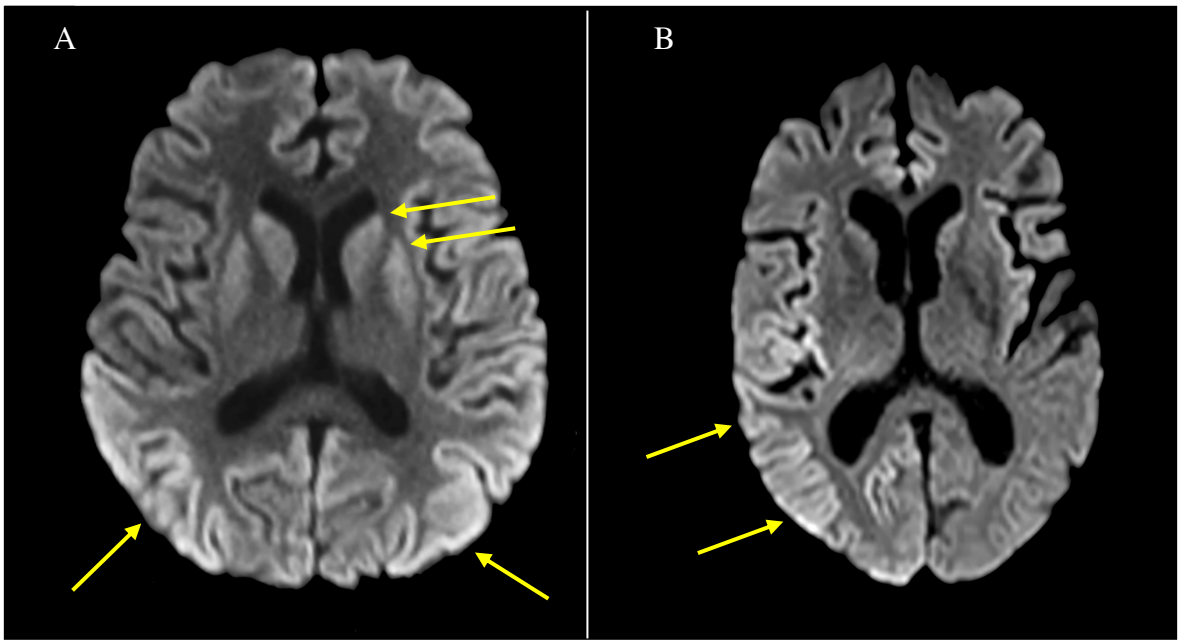


Figure 7: Differential diagnosis of basal ganglia and cortical high signal (A) DWI sequences showing symmetrical high signal of basal ganglia and cortex (arrows) in hypoxic brain injury. (B) DWI sequences showing right sided cortical high signal (arrows) following a generalised tonic-clonic seizure

1.8 Investigations and diagnostic classification for vCJD

The diagnosis of vCJD has important implications for clinical management as well for public health given the transmissibility of the disease. Definite diagnosis relies on neuropathological confirmation either by cerebral biopsy or necropsy, although standard clinical diagnostic criteria have been established and validated, allowing for reasonably accurate diagnosis of most cases in life (see table 3).

Table 3: Diagnostic criteria for surveillance of variant CJD (http://www.cjd.ed.ac.uk/documents/criteria.pdf)

DEFINITE:

Progressive neuropsychiatric disorder AND Neuropathologically confirmation of vCJD[e]

1.2 **PROBABLE:**

1.2.1 I + 4/5 of II **+** IIIA **+** IIIB

OR 1.2.2 I + IVA[d]

1.3 **POSSIBLE:**

I + 4/5 of II + IIIA

I A Progressive neuropsychiatric disorder
B Duration of illness > 6 months
C Routine investigations do not suggest an alternative diagnosis
D No history of potential iatrogenic exposure
E No evidence of a familial form of TSE

II A Early psychiatric symptoms[a]
B Persistent painful sensory symptoms[b]
C Ataxia
D Myoclonus or chorea or dystonia
E Dementia

III A EEG does not show the typical appearance of sporadic CJD[c] in the early stages of illness
B Bilateral pulvinar high signal on MRI scan

IV A Positive tonsil biopsy[d]

a. Depression, anxiety, apathy, withdrawal, delusions.

b. This includes both frank pain and/or dysesthesia.

c. The typical appearance of the EEG in sporadic CJD consists of generalised triphasic periodic complexes at approximately one per second. These may occasionally be seen in the late stages of vCJD.

d. Tonsil biopsy is **not** recommended routinely, nor in cases with EEG appearances typical of sporadic CJD, but may be useful in suspect cases in which the clinical features are compatible with vCJD and MRI does not show bilateral pulvinar high signal.

e. spongiform change and extensive PrP deposition with florid plaques throughout the cerebrum and cerebellum.

1.8.1 Electroencephalogram

The periodic sharp wave complexes (PSWCs) that are characteristic of sCJD are usually not seen in vCJD, expect in very rare circumstances in the late stages of the illness (145) (146). Instead, the EEG usually shows non-specific slow wave activity. The presence of PWSCs can therefore be helpful in the differentiation of sCJD from vCJD (147).

1.8.2 Tonsil biopsy

Unique to vCJD, compared to the other human prion diseases, is that PrP^{Sc} accumulation and infectivity can be found outside the central nervous system, in lymphoreticular tissue (148-150). As a result, examination of tonsillar tissue for PrP^{Sc}, obtained via a biopsy, can be a very useful test in vCJD (150). It is, however, an invasive procedure that is not without risk.

1.8.3 CSF examination and specialist tests

Similar to sCJD, the basic CSF constituents are usually normal in vCJD although occasionally there may be a mild elevation in CSF protein (147). The CSF 14-3-3 immunoassay is not a sensitive test in vCJD with fewer than half of cases having a positive result (151). Levels of CSF phosphorylated tau can be increased in the CSF in vCJD, and this may be of potential diagnostic value, although it lacks specificity (152). The current form of the CSF RT-QuIC assay does not produce a positive result in the CSF of patients with vCJD (153).

Recently, an innovative technique known as protein misfolding cyclic amplification (PMCA) has been modified to detect the abnormal PrP^{Sc} in vCJD in CSF with sensitivities between 97-100% and specificity of 100% (154) (155). This technique also detected the vCJD PrP^{Sc} in the CSF of the recently identified heterozygous vCJD case (155).

1.8.4 Blood and urine tests

PMCA has also been utilised to detect the abnormal PrP^{Sc} in vCJD in blood and urine from clinical cases with high sensitivities and 100% specificity (156, 157). PMCA has also been able to detect vCJD PrP^{Sc} in blood from two pre-symptomatic blood donors, taken 1.3 and 2.6 years prior to symptom onset (157). PMCA may therefore not only be used as a diagnostic test in vCJD, but also as a possible screening tool in transfusion medicine.

1.8.5 Magnetic Resonance Imaging

The utility of MRI brain scan in the diagnosis of vCJD was reported shortly after the condition was first described (158) eventually becoming a crucial component of the diagnostic criteria for this condition. The MRI characteristically demonstrates symmetrical hyperintensity in the posterior thalamus (relative to the grey matter of the caudate head) termed the 'pulvinar sign'(159) (see figure 1).

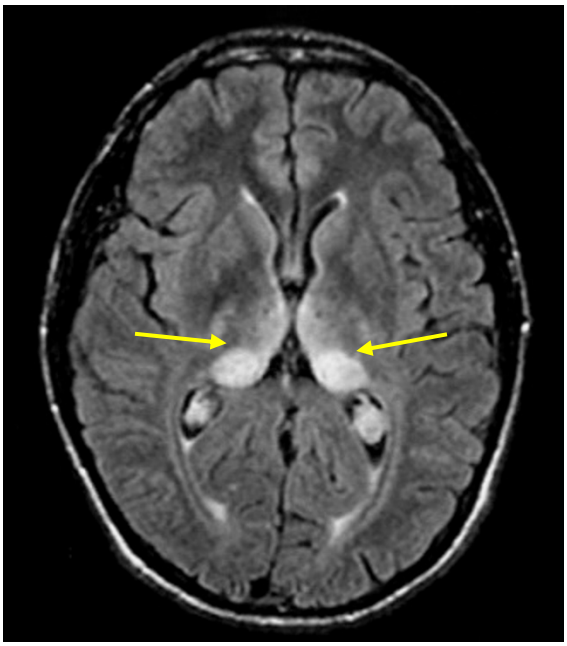
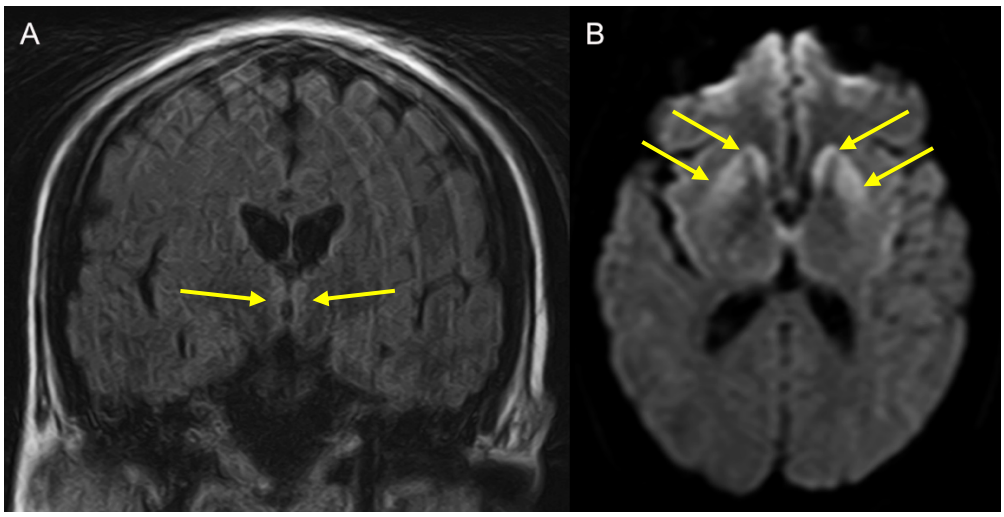


Figure 8: MRI brain in variant CJD. Axial FLAIR at the level of the basal ganglia demonstrating bilateral high signal in the pulvinar nuclei of the posterior thalamus.

This finding is rare in other forms of the disease and has a reported sensitivity of 78-90% and a specificity of 100% for vCJD in the appropriate clinical context (159). In addition to pulvinar signal changes, abnormalities in the mediodorsal thalamic nucleus, caudate nucleus, the putamen, and the periaqueductal grey matter have also been observed. Cortical high signal is a rare finding in vCJD in contradistinction to sCJD (127).

To date, a total of 178 cases of probable and definite vCJD have been identified in the UK and a further 54 cases worldwide in 11 other countries (85). Of the 161 UK cases of vCJD that had genetic analysis data, 160 were MM at Codon 129 in the prion protein gene, which may partly account for the observed similarity of MRI appearances amongst these cases compared to other human prion diseases. The one remaining case with genetic analysis data was heterozygous (MV) at Codon 129 and was neuropathologically confirmed at post-mortem (88). The MRI in this case demonstrated high signal on DWI in the basal ganglia, hypothalami and medial thalami but not in the pulvinar nuclei. As such, the radiological features were deemed to be more suggestive of sCJD (88). The FLAIR images in this case were degraded by movement artefact and were of limited diagnostic quality, albeit they did not identify obvious pulvinar high signal (see figure 9).

Figure 9: MRI brain of heterozygous (MV) variant CJD case. (A) Coronal FLAIR



showing medial thalami hyperintensity only. (B) Axial diffusion-weighted image demonstrating hyperintensity in the basal ganglia, medial thalami and bilateral insular cortices.

The seminal study assessing MR appearances in vCJD determined FLAIR imaging to be the most sensitive sequence for detecting the pulvinar sign when compared with either T2-weighted or Proton Density (PD) weighted imaging (see figure 10) (159). This study was

limited however by a small number of cases with DWI sequences (2 of 110 MRI scans), given it was a relatively new sequence during the peak incidence of vCJD and therefore not routinely available in many hospitals across the UK. As a result, very few studies have commented on the appearances of DWI in vCJD (160-162) and none have looked at whether it is a more sensitive sequence for detection of pulvinar hyperintensity.

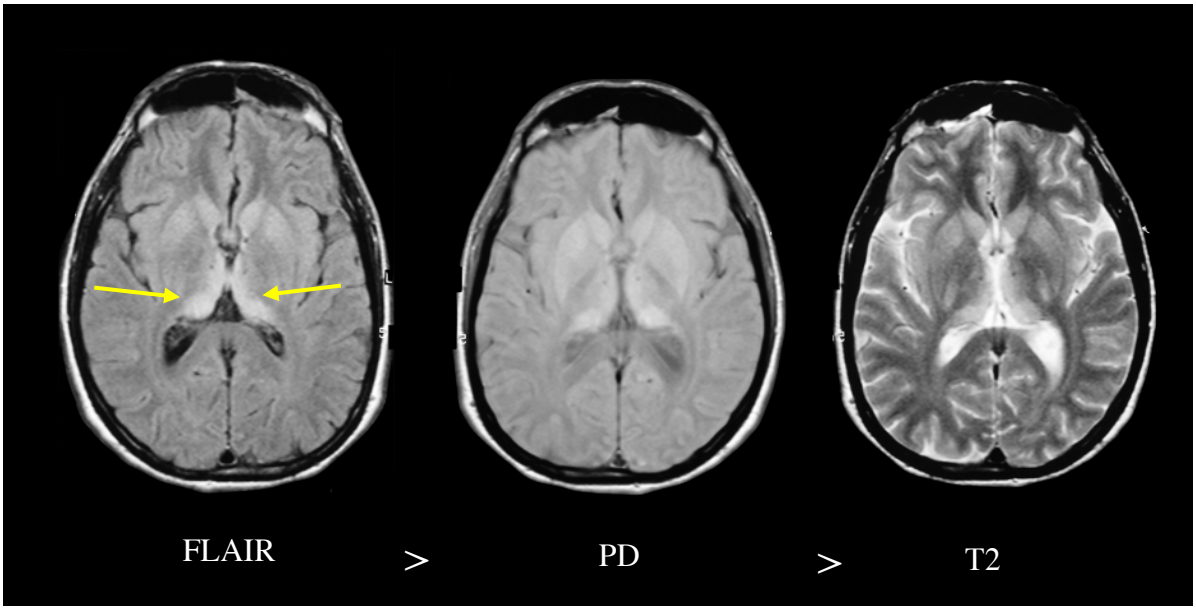


Figure 10: Effect of MR sequence on visibility of the pulvinar sign in vCJD with pulvinar high signal (arrows) being more conspicuous on FLAIR imaging compared to either PD-weighted imaging and T2-weighted imaging

1.9 Rationale for current study

MRI changes in sCJD have a relatively high diagnostic sensitivity and specificity, although this varies in different studies in relation to the precise details of the study population. The largest study was carried out in 2009 as an international collaboration and this supported the introduction of MRI brain scan in the diagnostic criteria for this condition. This was not, however, a systematic study including all cases serially and there have been technical developments in MRI since the study was carried out.

It is important to determine the overall sensitivity and specificity of MRI brain scan in a consecutive and unselected series of sCJD cases and whether these parameters have evolved

with time. The time at which MRI brain scan shows diagnostic changes in relation to the course of the illness should also be examined as this may be of clinical relevance to the diagnostic process. There is variation in the clinical presentation of sCJD and there is limited available information on whether the early clinical features in sCJD correlate with a diagnostic scan and whether these features relate to specific scan appearances, for example the pattern of cortical signal change. In addition, there is evidence that the scan appearance may vary in relation to codon 129 genotype and brain prion protein isotype.

Studies have underlined MRI brain scan as a crucial component of the diagnostic criteria for vCJD. However, MRI techniques have developed in the past 20 years since the first description of vCJD with improved resolution and the introduction of new sequences, including diffusion weighted imaging (DWI). The latter of which has increased sensitivity for the identification of cortical high signal. An important question is whether the MRI changes originally described as typical of this condition need to be revised in the light of these technical developments.

As mentioned in this chapter, a case of vCJD has recently been identified in an individual who is MV heterozygous at codon 129 of the prion protein gene (*PRNP*) in contrast to all tested previous definite and probable cases which have had an MM genotype. The scan in this case was reported as showing changes suggestive of sCJD on the basis of DWI sequences, whereas a previous possible vCJD case had an equivocal scan. The MRI scans in these cases need to be reviewed to identify if the scan appearances are comparable and if there are any distinctive radiological features in either case. Furthermore, vCJD cases with DWI need to be identified and reviewed in order to describe the image appearances and overall sensitivity of this sequence in the diagnosis of the condition.

There is evidence that the signal changes that support the diagnosis of CJD are commonly missed by reporting radiologists in local hospitals. This may reflect the rarity of CJD and the evolution of knowledge about the utility of MRI brain scan in its diagnosis. It is important to determine the accuracy of local reporting of MRI brain scan in CJD and whether this has changed with time.

Work plan

1. Identify the proportion of cases of sCJD and non-CJD cases undergoing an MRI brain scan from 2010 to June 30th 2016 and for vCJD from 1994 with those with DWI sequences. Obtain details of hospital reports of these scans if available and enter into a database.
2. Identify the cases with scans reported by the Unit specialist radiologist and establish a database with the details of the scan reports, including available scan sequences and scan quality.
3. Identify the dates of the scans and relate this to the date of onset and death in each case.
4. Add the mode of clinical onset in each case.
5. Identify the codon 129 genotype and CJD subtype in cases in which this information is available.

Study aims

The primary aim of this thesis: To examine the overall role of MRI brain scan in the diagnosis of sCJD.

Other, specific, aims:

- How accurate is local reporting of the MRI findings in CJD and has this changed with time?
- Is there variation in the utility of MRI in the diagnosis of CJD according to codon 129 genotype and prion protein isotype?
- Is there evidence that MRI in sCJD and vCJD is more likely to show characteristic features at different stages of the clinical illness and what is the role of serial scans?
- Is there a relationship between specific MRI appearances and the clinical presentation in sCJD?
- Is the MRI of use in identifying cases of MV vCJD?

- Are there scans in non-CJD cases that show features suggestive or characteristic of CJD, and are these cases distinguishable from true cases on the basis of clinical features or other investigations?

- Are there cases of CJD in which the scans show no characteristic features, and are there any parameters that distinguish these cases, for example scan quality or timing?

Summary

The resources of the NCJDRSU provide the opportunity to assess the utility of MRI brain scan in the diagnosis of CJD in the context of a long-term systematic national study. The detailed information available on cases will also allow a range of additional scientific questions to be answered that are relevant to the clinical diagnosis of CJD.

Chapter 2 Methodology

2.1.1 Introduction

The National CJD Research and Surveillance Unit (NCJDRSU) has systematic information on all diagnosed cases of CJD in the UK since May 1990. This includes clinical data and copies of hospital notes including MRI scan reports in the majority of cases. The Unit database contains information of date of onset and death and mode of clinical presentation in each case together with data on codon 129 genotype and prion protein isotype if available.

Efforts have been made to review MRI scans in all referred cases of CJD, in the early years by obtaining hard copies of scans, in later years by obtaining CDs and latterly by directly reviewing scans via the PACS system.

Data on a range of parameters in CJD cases and cases referred as suspected CJD, but with an alternative final diagnosis, are stored on a database at the Unit. This includes data on MRI brain scan, including whether an MRI scan was carried out, the date of the scan, whether the scan has been reviewed at the Unit and whether the scan was diagnostic of CJD. In addition, paper copies of details of scan reports by the Unit specialist radiologists are available and this includes a description of the regional distribution of changes including basal ganglia, thalamic and cortical signal changes.

The timing of MRI brain scan in relation to clinical onset and death are available in the great majority of cases. In a minority of case information is available on the results of serial scans. The scans were reviewed by a Neuroradiologist, experienced in the assessment of MRI in CJD, for the presence or absence of features associated with these diseases. The Neuroradiologist was aware of the suspicion of CJD but was blinded to the specific diagnosis (i.e. sporadic, variant and non-CJD).

In the assessment of vCJD in this study, given the majority of probable cases of were classified as such on the presence of a supportive MRI scan (positive pulvinar sign), it was decided to analyse these cases separately to those confirmed neuropathologically so as not to confound results. Only vCJD cases with DWI sequences were reviewed.

2.1.2 Study population

462 cases of sCJD referred to the NCJDRSU, identified as having an MRI brain scan performed during their illness, were analysed retrospectively (2010-2015). 54 non-CJD cases (referred to NCJDRSU, with an initial suspicion of CJD, but who turned out to have other illnesses) were included for comparative analyses (2010-2015). In addition, 25 cases of vCJD with brain MRI DWI sequences (1999-2015) were identified and these scans were analysed.

The above data was extracted from the NCJDRSU database by Ms Jan Mackenzie (NCJDRSU surveillance co-ordinator). A database was created using Microsoft Excel (version 16.44).

The sCJD group consisted of 212 males and 250 females. In 245 cases, the diagnosis of sCJD was confirmed at autopsy ('definite' cases) and in 208 by using the established clinical diagnostic criteria during the study period ('probable' cases) (see table 4).

It is important to note that the diagnostic criteria during the study period differs from the current diagnostic criteria for sCJD as mentioned in the introduction. The former diagnostic criteria did not allow for the use of CSF-RT-QuIC nor for the provision of specific cortical regions of high signal as supportive investigations for sCJD.

Of the 'probable' sCJD cases, 84 fulfilled the above diagnostic criteria on the use of MRI scan and as such, 'definite' cases and 'definite' and 'probable' cases were analysed separately. The 54 non-CJD controls consisted of; 27 with autopsy data excluding CJD and 27 with either an alternative clinical diagnosis identified or those that demonstrated sustained clinical improvement in their conditions.

Table 4: Diagnostic criteria used to classify sporadic CJD during study period (http://www.cjd.ed.ac.uk/documents/criteria.pdf)

1.1 **DEFINITE:**

Neuropathologically confirmed sporadic CJD

1.2 **PROBABLE:**

1.2.1 I + two of II and typical EEG*

OR 1.2.2 I + two of II and typical MRI brain scan**

OR 1.2.3 I + two of II and positive CSF 14-3-3

1.3 **POSSIBLE:**

I + two of II + duration < 2 years

I Rapidly progressive cognitive impairment

II A Myoclonus
B Visual or cerebellar problems
C Pyramidal or extrapyramidal features
D Akinetic mutism

*Generalized periodic complexes
**MRI brain high signal abnormality in caudate/putamen either on DWI or FLAIR

2.1.3 MRI data analysis

MRI containing FLAIR and/or DWI sequences were included in this study. Of these, the majority contained T1/T2-weighted sequences.

Using a standardised protocol (see appendix 2), FLAIR and DWI sequences were assessed for the presence or absence of hyperintensity observed within the cerebral cortex, the caudate head, putamen, globus pallidus and posterior and dorsomedial thalamic nuclei. The cortical areas assessed included frontal, cingulate, temporal, parietal and occipital. The MRI was also

classified according to the likely CJD subtype (variant/sporadic/undetermined), based on the overall radiological findings.

Image quality was scored subjectively based on the presence and degree of movement artefact as well as the ability to clearly delineate the boundaries between grey-matter and white-matter structures. DWI is less affected by movement artefact compared to other sequences, owing to a shorter acquisition time, and as such image quality was assessed across all images and not just DWI alone. Conversely, DWI sequences can be degraded as a result of susceptibility artefact from the skull base, limiting its utility in this region. Scan quality was graded from 1 to 6, with 5 and 6 considered to be of non-diagnostic quality (1=excellent, 2=good, 3= average, 4= sufficient, 5= insufficient, 6= poor).

A total of 678 MRI scans were reviewed by the NCJDRSU Neuroradiologist from 516 consecutive patients. 410 patients had one scan, 105 had two, 18 had three and 1 had four. A total of 1210 imaging sequences were assessed (619 DWI and 591 FLAIR). An additional 95 scans, from 95 patients, were reviewed by one of the Research Registrars, the data for which was analysed separately. 82 examinations were analysed by both the Neuroradiologist and Research Registrar with high interobserver agreement for 1) basal ganglia +/- cortex 2) cortex alone or 3) no changes (concordance 87.8%, Kappa=0.703, p=0.000). The majority of images were Digital Imaging and Communication in Medicine (DICOM) data (98.4%).

MRI local reporting data was obtained from 576/678 scans (85%) by reviewing scanned hospital records stored within the NCJDRSU database. This data was added to the Excel database.

In variant CJD, MRI was performed in 176 cases, of which 27 were identified as having DWI sequences. Two cases were excluded from analysis as their DWI sequences were of too poor image quality. Of the 25 remaining, 15 were neuropathologically confirmed and 10 classified as probable. The latter group were predefined on the basis of an appropriate clinical history together with positive MR imaging or positive tonsil biopsy or a combination of both.

2.1.4 Statistical analyses

Data are presented as median (range) or frequency (%). Continuous variables were analysed using 2-tailed Mann-Whitney U test (for 2 group comparisons). Categorical variables were compared using Fisher exact test. Statistical significance was defined as $p < 0.05$. Interobserver agreement was assessed using Kappa.

To access for variation in MRI signal distribution amongst the molecular subtypes of sCJD, a univariate logistic regression analyses was undertaken. This compared the proportion of cases with high signal in each region of interest against the same proportion in the rest of the cases not belonging to that subgroup (MM1 vs other subgroups, MM2 vs other subgroups, MV1 vs other subgroups, MV2 vs other subgroups, VV1 vs other subgroups and VV2 vs other subgroups). Odds ratios (ORs) were calculated from the logistic regression analyses (using odd ratio Chi-squared test) with 95% confidence intervals provided.

In order to determine a potential relationship between MRI appearance and mode of clinical presentation, a two-way analysis of variance (ANOVA) was undertaken looking for an association/effect of specific MRI appearances on mode of clinical presentation, followed by post hoc t-test analysis. Statistical analyses were performed using SPSS (version 25).

Chapter 3 MRI brain scan in the diagnosis of sCJD

3.1 Aim

The primary aim of this chapter was to determine the overall role of MRI brain scan in the diagnosis of sCJD. Analyses of the neuropathologically confirmed sCJD cases and non-CJD controls (definite sCJD and non-CJD) are presented initially, followed by an analysis of all sCJD cases (definite and probable) and non-CJD controls (confirmed neuropathologically or on the basis of clinical features and investigations).

Other specific aims in this chapter include:

- Are there scans in non-CJD cases that show features suggestive or characteristic of CJD, and are these cases distinguishable from true cases on the basis of clinical features or other investigations?

- Are there cases of CJD in which the scans show no characteristic features, and are there any parameters that distinguish these cases, for example scan quality or timing?

3.2 Results of definite sCJD and non-CJD cases

3.2.1 Clinical characteristics of definite sCJD and non-CJD cases

Here, the sensitivity and specificity for MRI in the diagnosis of sCJD was calculated from patients with neuropathologically confirmed sporadic CJD (definite sCJD n=254) compared to controls with an alternative diagnosis confirmed by autopsy (definite non-CJD cases) (n=27). Figure 11 illustrates the final diagnosis in these non-CJD patients.

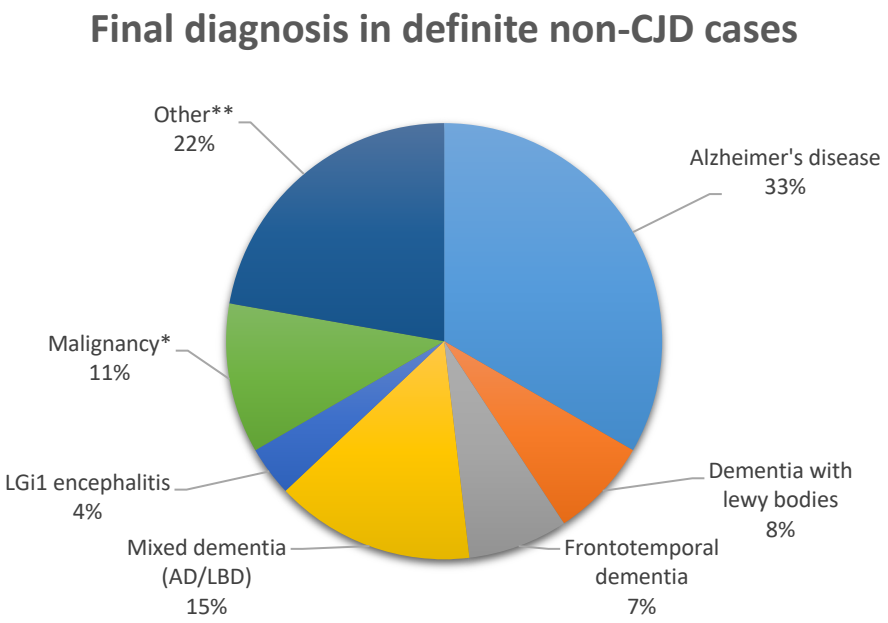


Figure 11: Diagnosis of non-CJD cases confirmed on autopsy

**** Malignancy = two cases with Diffuse B cell Lymphoma and one case with Chronic Myeloid leukaemia***

***** Other = four cases with no evidence of CJD at autopsy, one with non-necrotising granulomatous disease and one with multi-focal necrotising granulomatous disease.***

FLAIR sequences were available for 216 cases and 26 controls, DWI in 227 cases and 21 controls. Both sequences were available in 188 cases and 10 controls. The time to first MRI was 3.18 months in cases and 4.26 in controls. The timing of first MRI expressed as a percentage of disease duration was 67% in sCJD and 63% in controls. Codon 129 data was available in 226 sCJD cases (MM=139, MV=42, VV=45) and in 10 controls (MM=8, MV=0, VV=2). The patient characteristics are defined in table 5.

Table 5: Patient characteristics of definite CJD and non-CJD cases

	n	Sex (M:F)	Median age (range)	Median duration (months) (range)	Median Time to 1st MR (months)	Time of MR (% disease duration) (range)	FLAIR (n)	DWI (n)
Definite sCJD	254	110:144	68 (25-85)	4.53 (0.87-62.03)	3.18 (0.1-23.45)	67% (4-96)	216	227
Not CJD	27	13:14	73.5 (58-89)	8 (2.13-59.87)	4.26 (1.03-33.61)	63% (15-91)	26	21
p-value			0.0039	0.0003	0.0268			

3.2.2 MRI signal abnormalities in definite sCJD cases

The distribution of signal abnormalities in the definite sCJD cases between FLAIR and DWI is shown in figure 12.

3.2.2.1 Basal ganglia

High signal of the basal ganglia was found in 173 (68.1%) of sCJD cases (on either FLAIR, DWI or both). Bilateral involvement of the basal ganglia was observed in 108 (62.4%) cases, with asymmetric high signal found in 65 (37.6%) cases. 28 patients had FLAIR sequences only, with basal ganglia high signal identified in 9 of these (32.1%). In 38 cases with only DWI available, basal ganglia involvement was observed in 25 (65.8%). When both sequences were available (n=188), basal ganglia high signal was found in 138 (73.4%) on DWI compared to 71 (37.8%) on FLAIR.

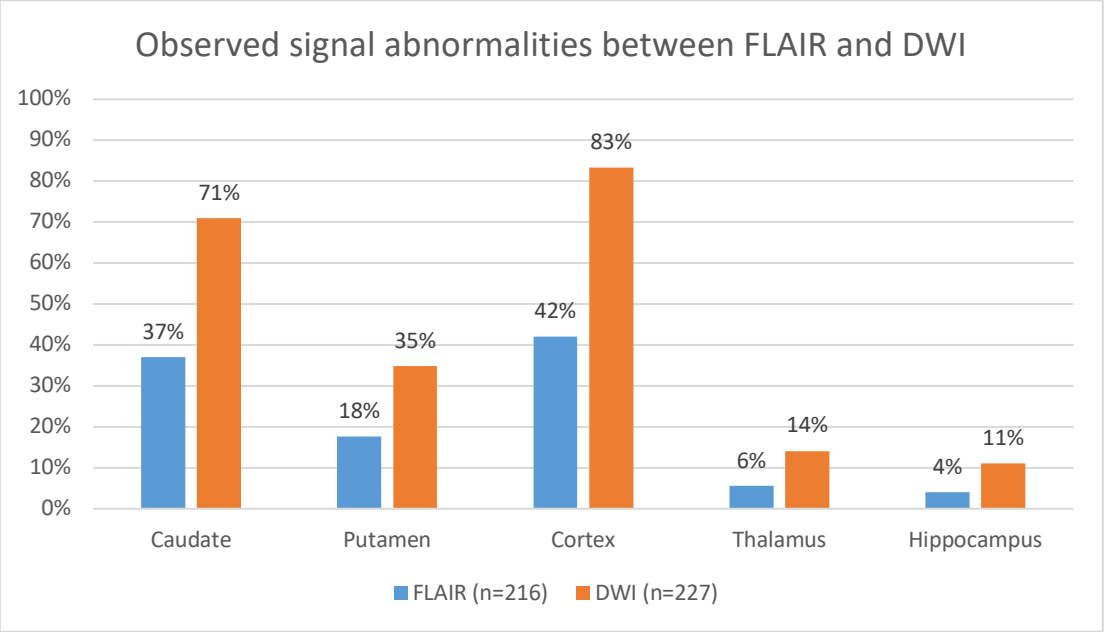


Figure 12: Distribution of signal abnormalities in definite sCJD cases (FLAIR and DWI)

3.2.2.2 Cortex

High signal of the cortex was observed in 203 (79.9%) cases on either FLAIR, DWI or both. 152 of these cases had associated basal ganglia involvement and in 51 cases the cortical high signal was identified in isolation. Of the 227 DWI images, 189 (83.3%) demonstrated at least one region of cortical high signal compared to 91/216 (42.1%) FLAIR images. Figure 13 outlines the regions of cortex involvement between FLAIR and DWI.

When excluding cortical regions susceptible to signal artefact (cingulate, frontal and insular regions) 162 cases demonstrated at least one other area of cortical involvement (occipital, parietal or temporal) on a combination of FLAIR (21.3% of images) and DWI (66.5% of images).

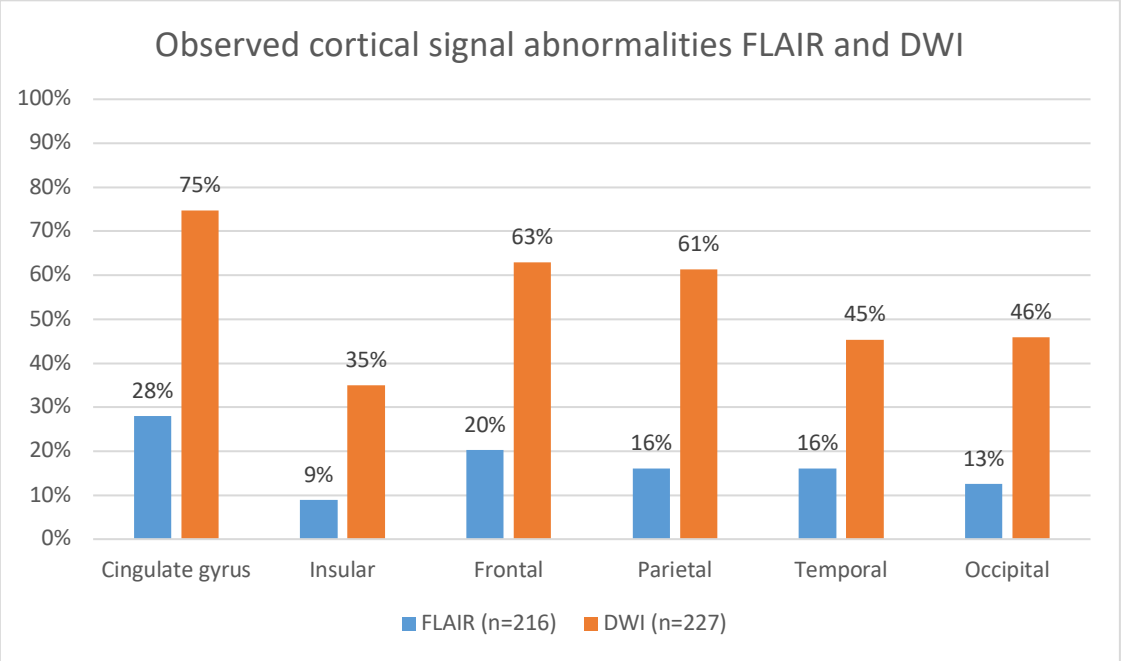


Figure 13: Distribution of cortical signal abnormalities in definite sCJD cases (FLAIR and DWI)

3.2.2.3 Thalamus

Thalamic high signal (anterolateral +/- dorsomedial +/- pulvinar) was observed in 36 cases in total (14.17%) on a combination of FLAIR (5.6% of all images) and DWI (14.1% of all images). Of those with thalamic high signal with both FLAIR and DWI sequences available (n=28), the thalamic high signal was observed in 96.4% on DWI compared to 32.1% on FLAIR. Figure 14 demonstrates the areas of thalamic involvement.

When both sequences were available (n=188), 2 cases demonstrated anterolateral thalamic high signal on FLAIR, compared to 11 on DWI. Dorsomedial involvement was seen in 7 cases on FLAIR and in 22 on DWI and likewise pulvinar involvement was seen in 7 cases on FLAIR and in 16 on DWI. Aside from 2 cases, all those that demonstrating thalamic high signal on FLAIR had corresponding involvement on DWI.

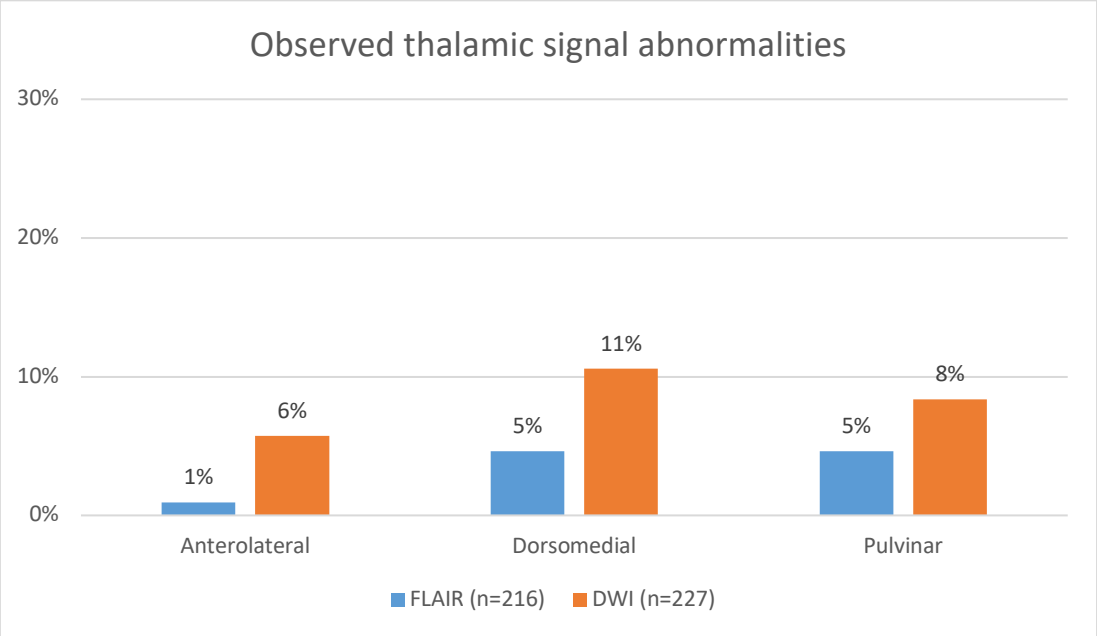


Figure 14: Distribution of thalamic signal abnormalities in definite sCJD cases (FLAIR and DWI)

3.2.2.4 Pulvinar sign

There were two sCJD cases with pulvinar signs; one with a pulvinar sign on FLAIR and DWI and the other only on FLAIR imaging only (figure 15). Both were MV2 cases and both had associated basal ganglia high signal in the absence of cortical involvement.

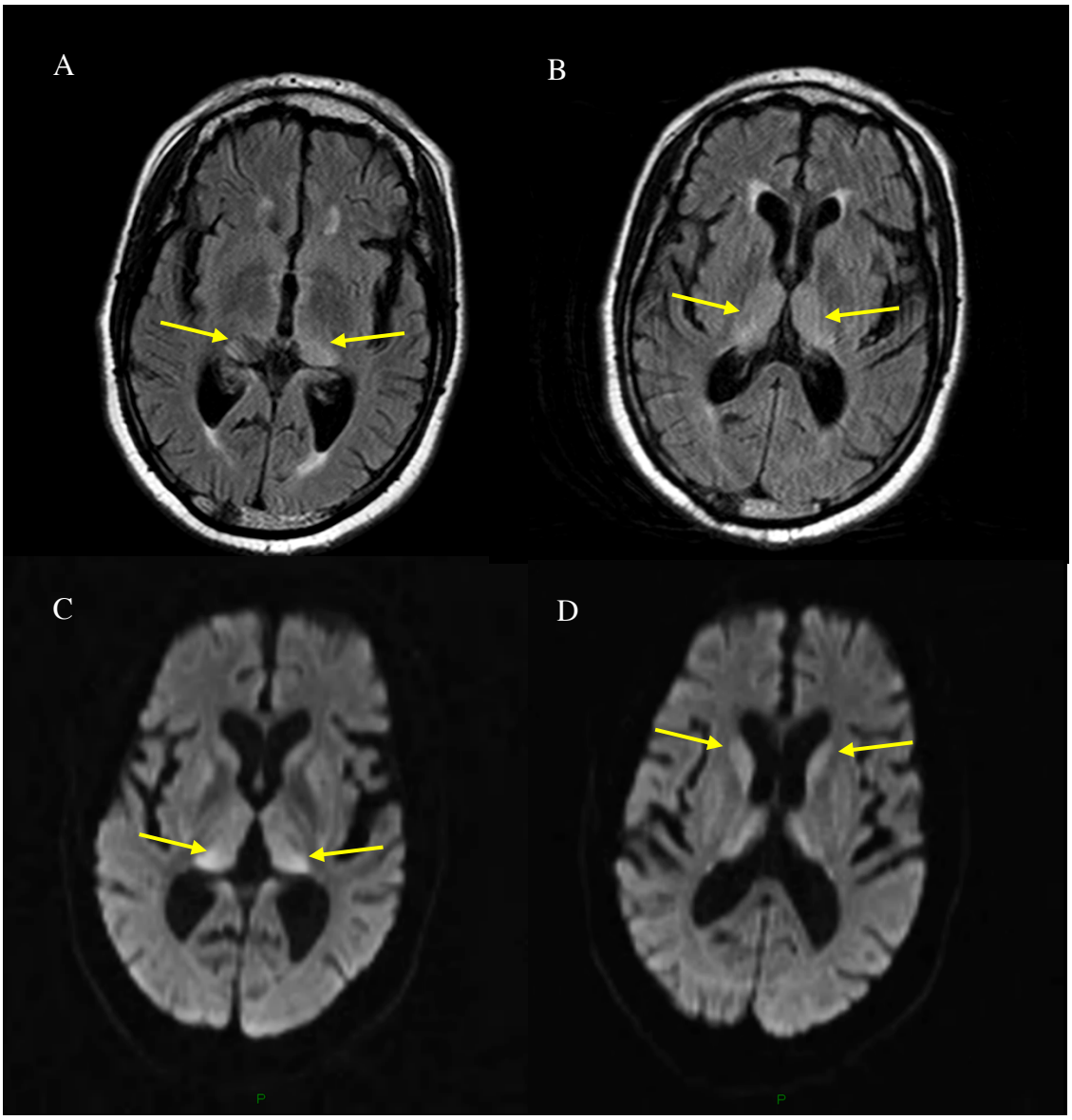


Figure 15: Positive pulvinar sign in definite MV2 sCJD patient. (A) Axial FLAIR sequences (A+B) showing positive pulvinar sign (A) (arrows) with (B) associated dorsomedial thalamic high signal (arrows). (B) DWI sequences (C+D) demonstrating more conspicuous pulvinar high signal (arrows) with (D) associated dorsomedial thalamic and bilateral caudate head high signal (arrows).

3.2.3 MRI signal abnormalities in non-CJD cases

3.2.3.1 Basal ganglia

Basal ganglia high signal was found in 2 non-CJD cases (11.1%), one with Lewy body dementia and one with mixed Alzheimer's and Lewy body pathology. In both cases, the caudate heads were involved (one with symmetrical involvement). One of these studies lacked DWI and the other demonstrated basal ganglia high signal on FLAIR only with no corresponding DWI abnormalities (see figure 16).

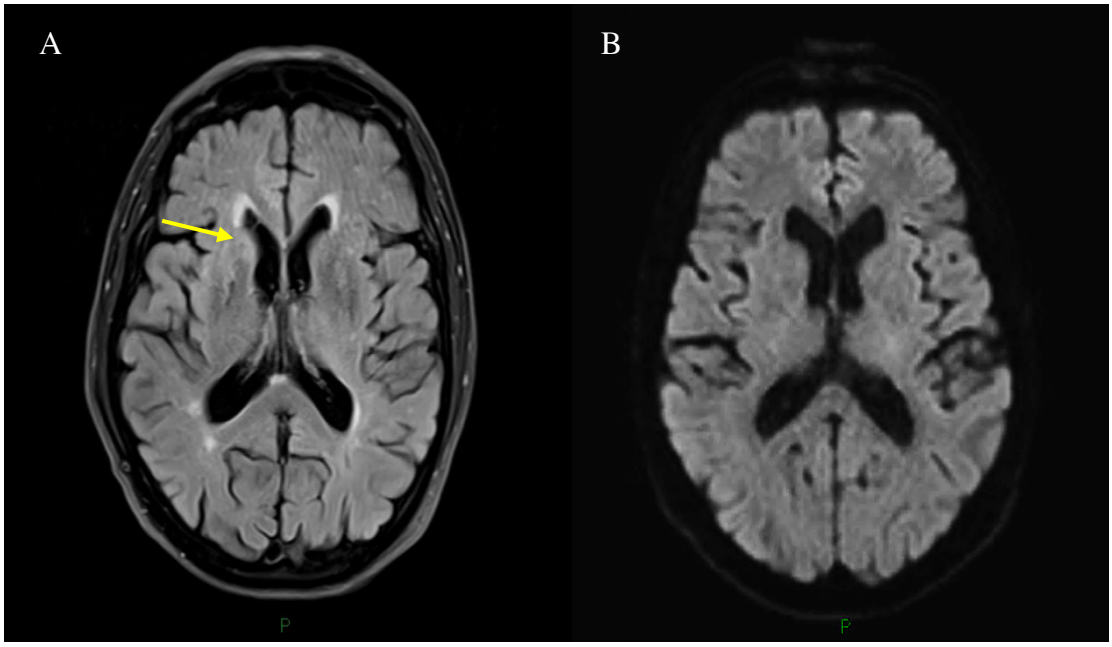


Figure 16: Axial MRI brain of a 74-year-old with autopsy confirmed Lewy body dementia. The FLAIR (A) shows subtle right caudate head signal abnormality with no associated high signal on DWI (B)

3.2.3.2 Cortex

Cortical high signal was found in 6 (22%) non-CJD cases (2 on FLAIR only and 4 DWI only) (see figure 17). None of these cases had associated basal ganglia or thalamic high signal. The cortical regions involved were limited to one region in 3 cases (multifocal necrotising granulomatous disease, dementia with Lewy body and one case with no evidence of CJD at autopsy) and to two regions in 3 cases (mixed Alzheimer's and Lewy body pathology,

autoimmune encephalopathy and Alzheimer's disease). Figure 18 shows the relative regions of cortical involvement. Only 2 controls demonstrated cortical signal abnormalities when excluding regions prone to signal artefact (frontal, cingulate and insular cortices).

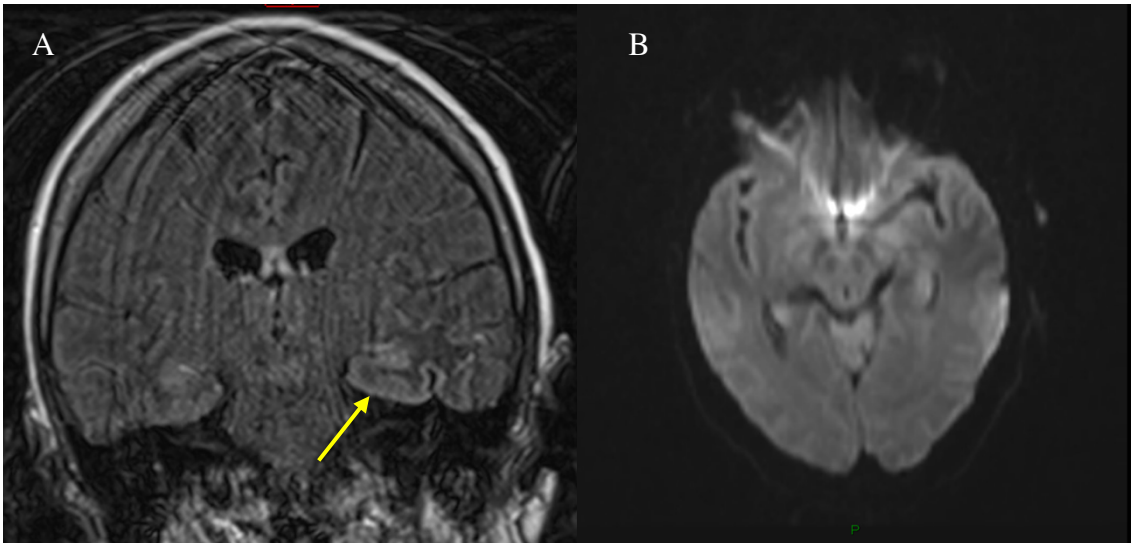


Figure 17: MRI brain signal abnormalities resembling that of sCJD in a 73-year-old with neuropathologically confirmed multi-focal granulomatous disease. Coronal FLAIR (A) shows apparent high signal affecting the left temporal cortex (arrow) in the context of image degradation by movement artefact. There was no associated cortical high signal evident on the DWI (B).

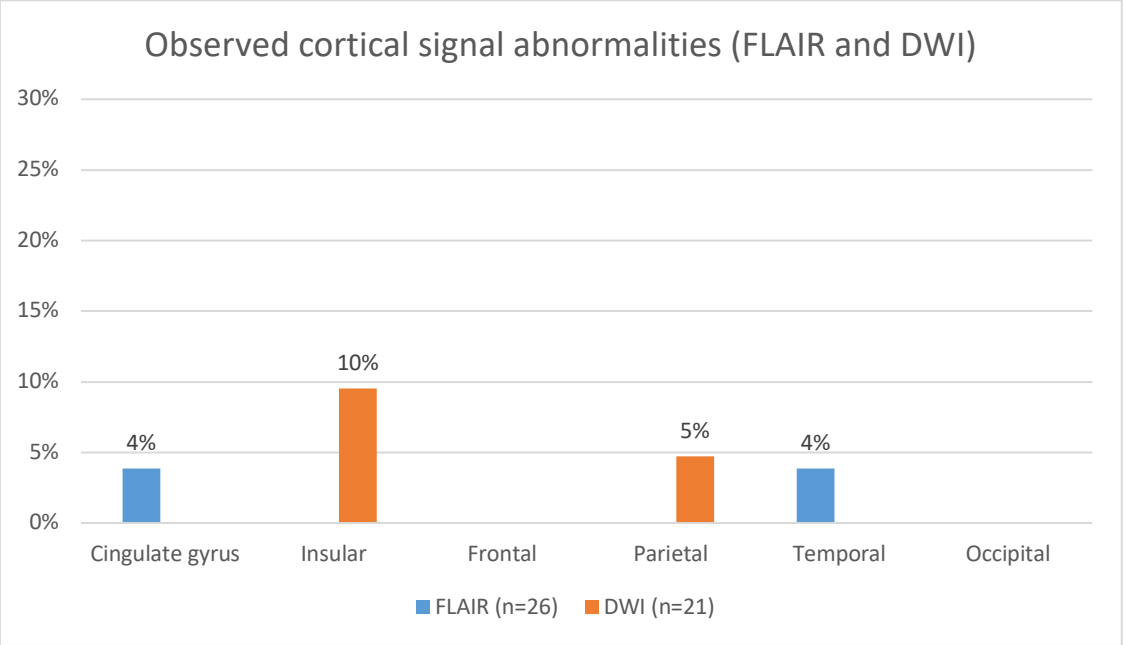


Figure 18: Distribution of cortical signal abnormalities in definite non-CJD cases (FLAIR and DWI)

3.2.3.3 Thalamus

Thalamic signal abnormality was present in two non-CJD cases. One was proven to have chronic myeloid leukaemia on autopsy, involving the dorsomedial and pulvinar regions on FLAIR imaging only, with no associated signal abnormalities on DWI. The signal changes were initially patchy and progressed over serial imaging to become more confluent. The imaging was atypical for vCJD in that there was associated periventricular and pontine high signal (see figure 19). The other case was a patient with Alzheimer's disease that had isolated pulvinar high signal on DWI only.

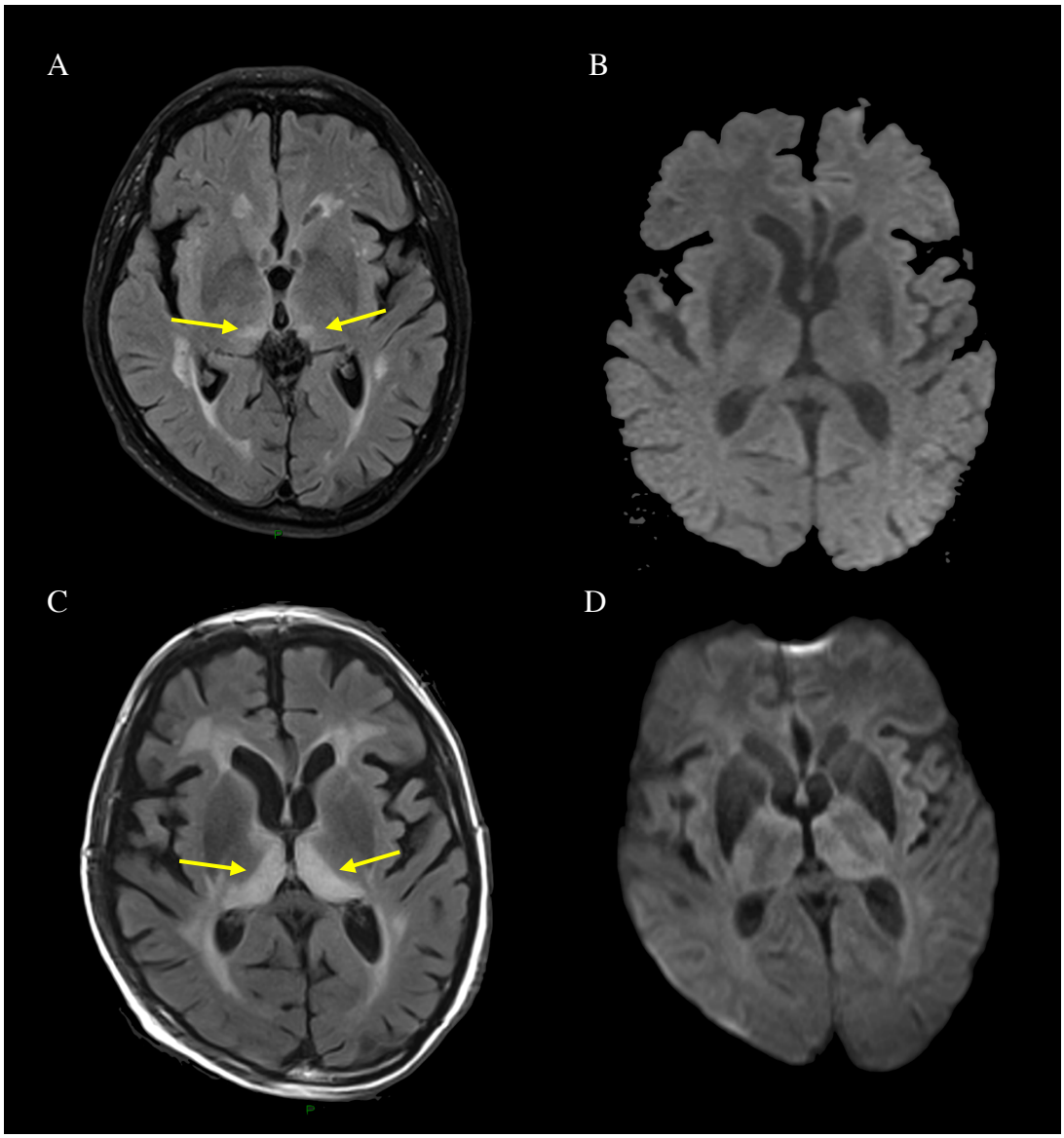


Figure 19: Thalamic signal abnormalities in a patient with autopsy proven chronic myeloid leukaemia. First MRI (A+B) showing thalamic high signal on axial FLAIR (A) predominantly involving the right thalamus (pulvinar and dorsomedial regions (arrows) with no corresponding DWI (B) signal abnormalities. Second MRI performed 5 months later (C + D) now demonstrating global bilateral thalamic high signal on FLAIR (C) (arrows), again with no associated DWI signal abnormalities.

3.2.4 Diagnostic utility of MRI for sCJD (definite sCJD and non-CJD cases)

The combined results of brain imaging in both CJD and non-CJD cases are shown in table 6. 88% of patients with CJD had CJD-associated signal abnormalities on imaging (basal ganglia and/or any cortical signal abnormalities) compared to 30% of non-CJD controls (p=<0.0001). When excluding cortical areas prone to signal artefact, only 15% of non-CJD cases had CJD-associated signal abnormalities, which were predominantly observed on FLAIR sequences only. 60% of the CJD cases had a combination of basal ganglia and cortical signal abnormalities, whereas none of non-CJD cases had this combination.

Table 6: MRI brain results in definite sCJD and non-CJD cases

Diagnosis	No BG or cortex (%)	BG alone (%)	Cortex alone (%)	BG and cortex (%)	Either BG and/or any cortex (%)	Either BG and/or >2 cortical areas**
CJD	30/254 (12)	21/254 (8)	51/254 (20)	152/254 (60)	224/254 (88)	213/254 (84)
Non-CJD	18/27* (87)	2/27 (7)	6/27 (22)	0/27	8/27 (30)	2/27 (11)
Fisher's exact test	-	>0.9999	0.8023	<0.0001	<0.0001	<0.0001
Sensitivity	-	8%	20%	60%	88%	84%
Specificity	-	93%	78%	100%	70%	93%

*1/27 non-CJD case had isolated thalamic high signal

** from temporal, occipital or parietal regions

3.2.4.1 Sensitivity and specificity of FLAIR and/or DWI sequences

The sensitivity and specificity of MRI in the diagnosis of sCJD, based on the current diagnostic criteria (high signal in basal ganglia and/or at least 2 cortical regions from occipital-parietal-temporal) is highlighted in table 7.

Table 7: Sensitivity, specificity and predictive values of MRI for sCJD (FLAIR and/or DWI)

Statistic	Value	95% CI
Sensitivity	83.86%	78.75% to 88.16%
Specificity	92.59%	75.71% to 99.09%
Positive Predictive Value (*)	99.07%	96.56% to 99.75%
Negative Predictive Value (*)	37.88%	31.12% to 45.15%

3.2.4.2 Sensitivity and specificity of DWI sequences

The sensitivity and specificity of MRI on the basis of DWI sequences alone were calculated in a sub-group of patients with this sequence available (227 sCJD and 21 non-CJD cases). The results are highlighted in table 8.

Table 8: Sensitivity, specificity and predictive values of MRI in sCJD (DWI alone)

Statistic	Value	95% CI
Sensitivity	94.25%	90.36% to 96.90%%
Specificity	100%	84.56% to 100.00%
Positive Predictive Value (*)	100.00%	97.98% to 99.86%
Negative Predictive Value (*)	62.86%	39.91% to 54.95%

3.3 Results of definite and probable sCJD and all non-CJD cases

3.3.1 Clinical characteristics of sCJD and non-CJD cases

Here, the sensitivity and specificity for MRI was calculated from patients with sCJD (254 definite and 208 probable) compared to non-CJD controls with an alternative diagnosis either confirmed neuropathologically (n=27) or on the basis of clinical features and investigations (n=27). Figure 20 illustrates the final diagnosis in the non-CJD patients.

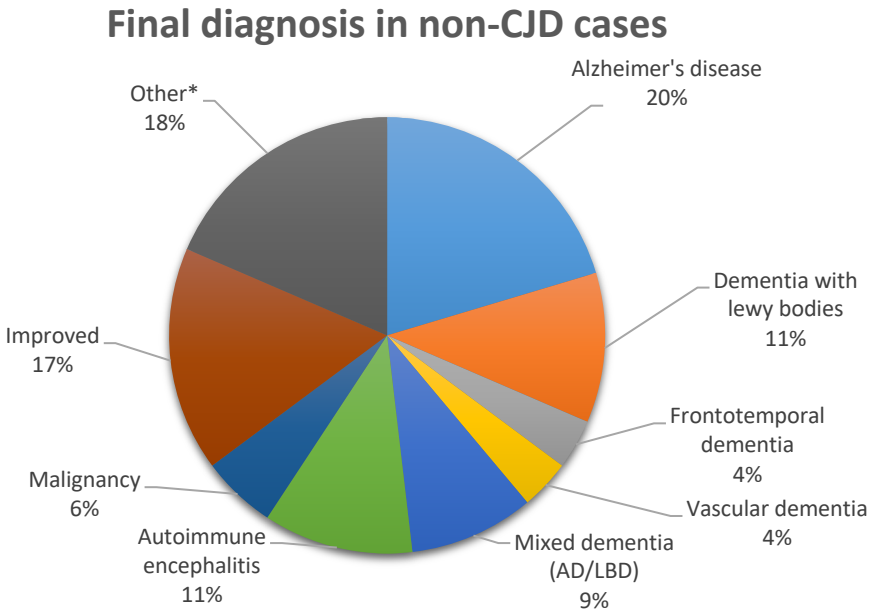


Figure 20: Diagnosis of non-CJD cases (confirmed on either autopsy or on clinical features)

**other (Granulomatous disease (2), metabolic encephalopathy (2), vasculitis (1) autopsy negative for CJD but missing final diagnosis (1) and those not felt to have CJD but final diagnosis unknown (2).*

FLAIR sequences were available for 395 cases and 51 controls, DWI in 414 cases and 38 controls. Both sequences were available in 347 cases and 37 controls. The time to first MRI was 4.14 months in cases and 10.44 in controls. The timing of first MRI expressed as a percentage of disease duration was 67% in sCJD and 63% in controls. Codon 129 data was

available in 226 sCJD cases (MM=139, MV=42, VV=45) and in 14 controls (MM=11, MV=1, VV=2). The patient characteristics are defined in table 9.

Table 9: Patient characteristics of definite and probable sCJD and non-CJD cases

	n	Sex (M:F)	Median age (range)	Median duration (months) (range)	Median Time to 1st MR (months)	Time of MR (% disease duration) (range)	FLAIR (n)	DWI (n)
sCJD	462	212:250	68 (25-88)	4.14 (0.87-62.03)	2.75 (0.35-23.45)	67% (3-98)	395	414
Controls	54	31:23	70.5 (52-89)	10.44 (2.13-59.87)	4.42 (1.03-34.65)	63% (6-91)	51	38
P-Value			0.0112	0.0013	0.0189			

3.3.2 MRI signal abnormalities in definite and probable sCJD cases

The distribution of signal abnormalities between FLAIR and DWI is shown in figure 21.

3.3.2.1 Basal ganglia

Hyperintensity of the basal ganglia was present in 335 of 462 (72.5%) CJD cases (on either FLAIR, DWI or both). Bilateral involvement of the basal ganglia was observed in 186 (55.5%) cases, with asymmetric high signal in 149 (44.5%) cases.

There were 48 CJD cases with only FLAIR sequences available on MR study, of these 18 (37.5%) had basal ganglia signal abnormalities. There were 67 cases with only DWI available, of whom basal ganglia high signal was observed in 49 (72.9%) studies. When both

sequences were available (n=347), basal ganglia high signal change was identified in 259 scans (73.4%) on DWI compared to 140 scans (40.3%) on FLAIR.

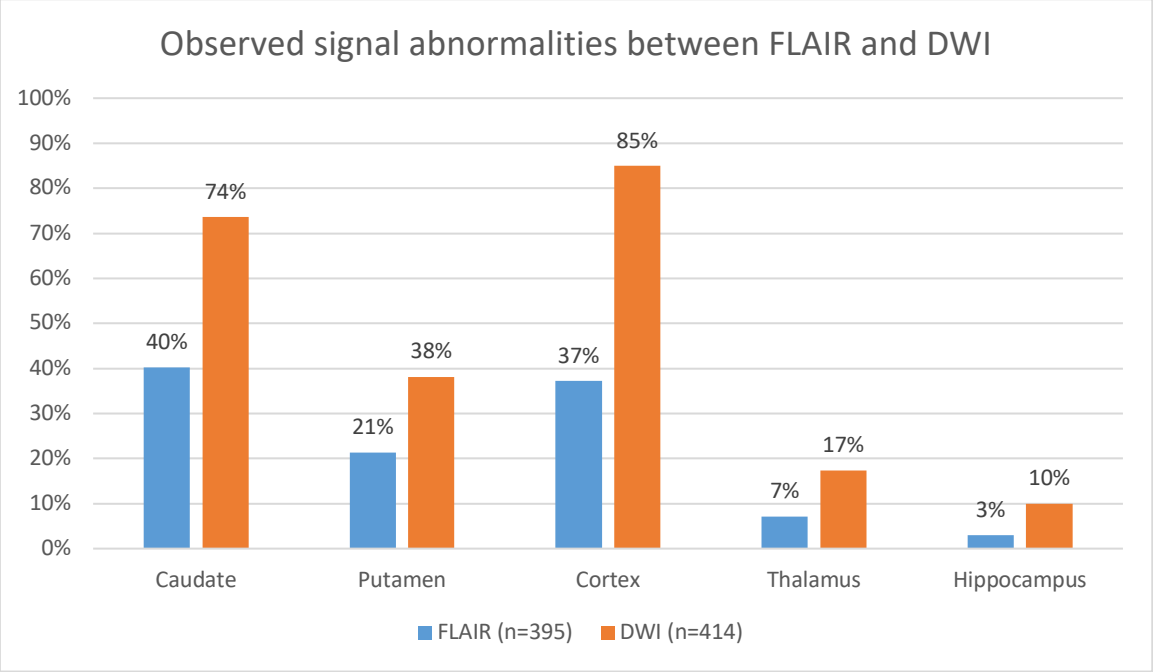


Figure 21: Distribution of basal ganglia signal abnormalities in definite and probable sCJD cases (FLAIR and DWI)

3.3.2.2 Cortex

High signal of the cortex was observed in 378 (82%) cases on either FLAIR, DWI or both. 294 of these cases (78%) had associated basal ganglia involvement and in 84 cases (22%) the cortical high signal was identified in isolation. When excluding cortical regions susceptible to signal artefact (cingulate, frontal and insular regions), a total of 303 cases (66%) demonstrated at least one other area of cortical signal abnormality (occipital, parietal or temporal). Figure 22 outlines the regions of cortex involvement between FLAIR and DWI.

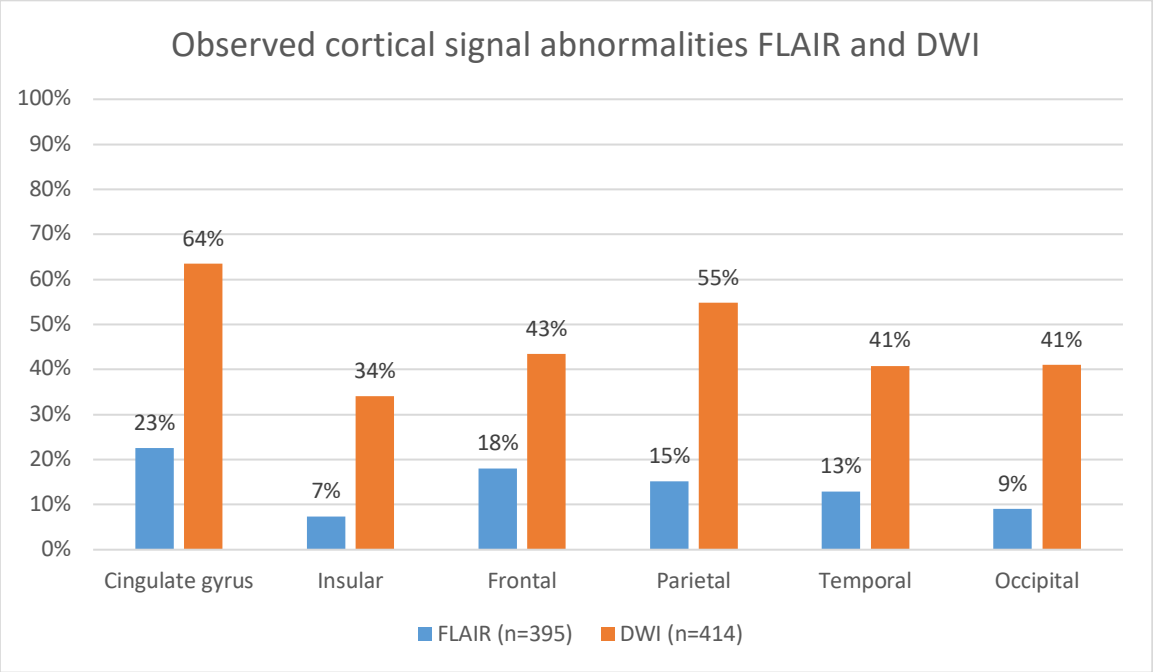


Figure 22: Distribution of cortical signal abnormalities in definite and probable sCJD cases (FLAIR and DWI)

3.3.2.3 Thalamus

Thalamic high signal (anterolateral +/- dorsomedial +/- pulvinar) was observed in 79 cases in total (17%) on a combination of FLAIR (7% of images) and DWI (17% of images). Isolated thalamic high signal was observed in 2 cases (0.4%). Of those with thalamic high signal with both FLAIR and DWI sequences available (n=63), the thalamic high signal was observed in 90% on DWI compared to 44% on FLAIR.

3.3.2.4 Pulvinar sign:

There were four sporadic CJD cases with positive pulvinar signs; three on DWI only and the other on both FLAIR and DWI. All had associated basal ganglia high signal in the absence of cortical involvement. Two were definite cases (both MV2 subtypes) and the remaining two were probable cases (one with genotype VV, the other unknown). Figure 23 demonstrates the distribution of thalamic involvement.

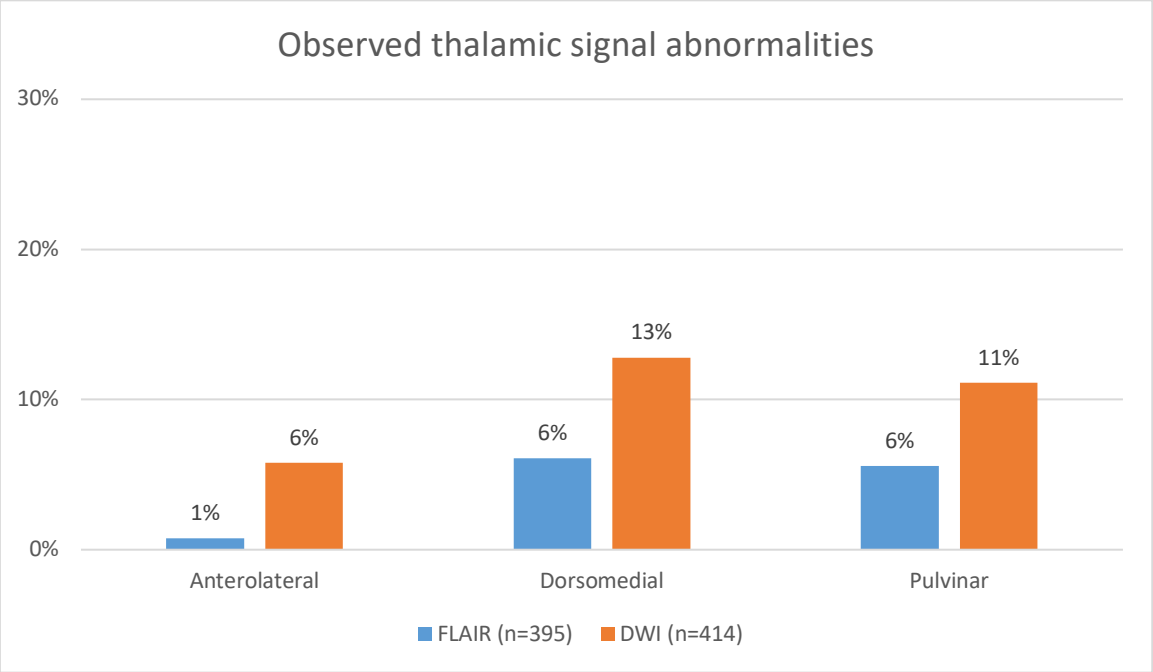


Figure 23: Distribution of thalamic signal abnormalities in definite and probable sCJD cases (FLAIR and DWI)

3.3.3 MRI signal abnormalities in definite and probable non-CJD cases

The distribution of signal abnormalities between FLAIR and DWI is shown in figure 24.

3.3.3.1 Basal ganglia

Basal ganglia high signal was found in 5 of 54 (9%) non-CJD cases, one with Lewy body dementia, one with mixed Alzheimer's and Lewy body pathology, one with VGKC encephalitis and one that showed sustained clinical improvement in their condition. Four cases demonstrated caudate head involvement (three with symmetrical changes) and one case had asymmetric caudate and putamen high signal. Of the three cases which had FLAIR and DWI sequences available, only one had corresponding basal ganglia signal abnormalities on the DWI.

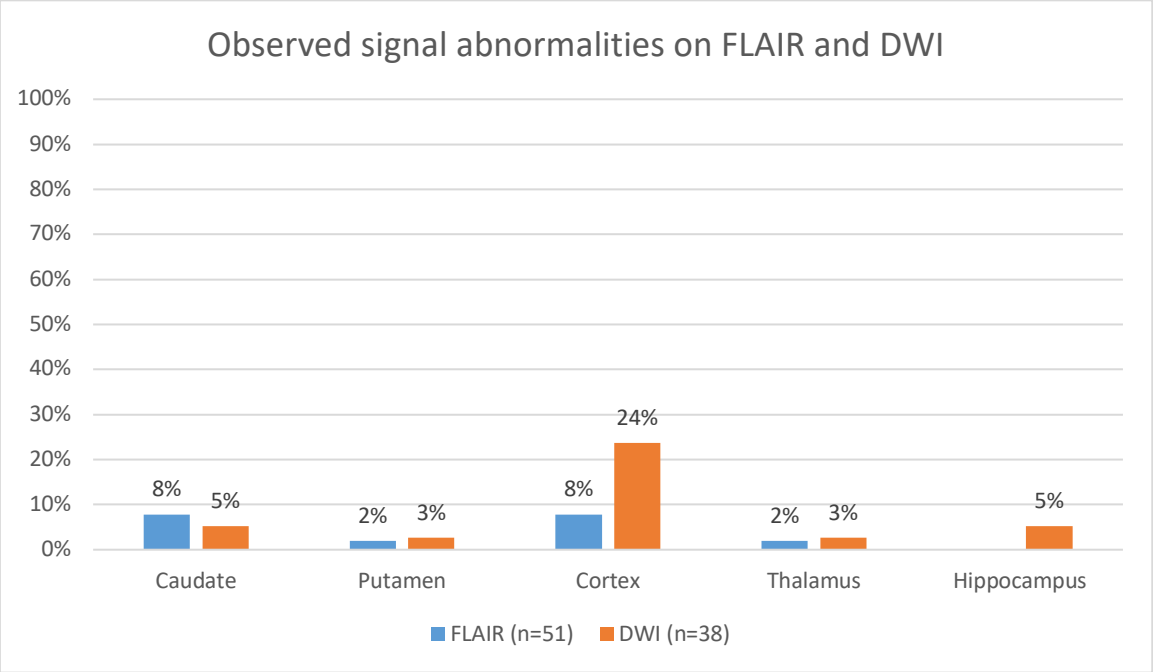


Figure 24: Distribution of basal ganglia signal abnormalities in definite and probable non-CJD cases (FLAIR and DWI)

3.3.3.2 Cortex

Cortical signal change was present in 11 (20%) non-CJD cases (2 on FLAIR only, 8 on DWI only and 1 on both). Two of these cases had associated basal ganglia involvement (one on FLAIR only and one DWI only). The cortical regions involved were limited to one region in 3 cases (multifocal necrotising granulomatous disease, dementia with Lewy body and one case with no evidence of CJD at autopsy). 6 cases had two regions of cortical involvement (one mixed Alzheimer's and Lewy body pathology, two autoimmune encephalitis, one Alzheimer's disease, one inflammatory CSF and one who improved), one case who improved had 3 regions and one with Huntington's disease had 5.

Figure 25 shows the relative areas of cortical involvement. 4 controls demonstrated cortical high signal when excluding areas prone to signal artefact (frontal, cingulate and insular cortices).

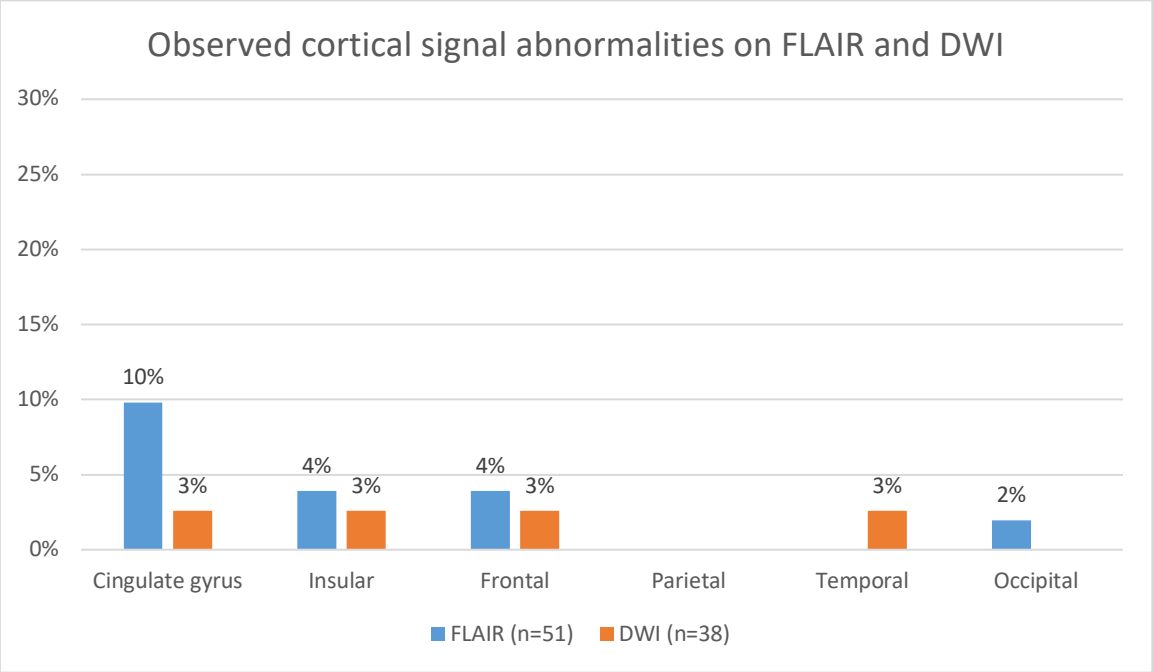


Figure 25: Distribution of basal ganglia signal abnormalities in definite and probable non-CJD cases (FLAIR and DWI)

3.3.3.3 Thalamus

Thalamic high signal was present in two non-CJD cases confirmed on autopsy, one proven to have chronic myeloid leukaemia (see figure 19) and the other Alzheimer's disease.

3.3.4 Diagnostic utility of MRI for sCJD (definite and probable sCJD and non-CJD cases)

The results of MRI brain imaging in both CJD and non-CJD cases are shown in table 10. 92% of patients with sCJD had high signal in the basal ganglia or the cortex compared to 28% of non-CJD controls (p=<0.0001). 85% of the CJD cases had either basal ganglia high signal and/or more than 2 regions of cortical high signal (from occipital, parietal or temporal regions), whereas only 9% of non-CJD cases had this combination.

Table 10: MRI brain results in definite sCJD and non-CJD cases

Diagnosis	No BG or cortex (%)	BG alone (%)	Cortex alone (%)	BG and cortex (%)	Either BG or cortex (%)	Either BG or ≥ 2 cortical regions**
CJD	38/462 (8)	41/462 (8)	84/462 (18)	294/462 (64)	425/462 (92)	392/462 (85)
Not CJD	39/54* (72)	5/54 (7)	9/54 (19)	2/54 (2)	14/54 (28)	5/54 (9)
Fisher's exact test (p-value)	-	>0.9999	>0.9999	<0.0001	<0.0001	<0.0001
Sensitivity	-	9%	18%	64%	92%	85%
Specificity	-	93%	81%	98%	72%	91%

* 1/54 non-CJD case had isolated thalamic high signal

** >2 cortical areas of high signal from temporal, occipital or parietal regions (unpaired t-test, two-tailed p-value)

3.3.4.1 Sensitivity and specificity of FLAIR and/or DWI sequences

The sensitivity and specificity of MRI findings for sporadic CJD, based on the current diagnostic criteria (high signal on DWI or FLAIR in basal ganglia or at least 2 cortical regions from occipital-parietal-temporal) are highlighted in table 11.

Table 11: Sensitivity, specificity and predictive values of MRI for the diagnosis of sCJD (all definite and probable sCJD and non-CJD cases) (FLAIR and/or DWI)

Statistic	Value	95% CI
Sensitivity	84.85%	81.25% to 87.99%
Specificity	90.74%	79.70% to 96.92%
Positive Predictive Value (*)	98.74%	97.14% to 99.45%
Negative Predictive Value (*)	41.18%	35.69% to 46.89%

3.3.4.2 Sensitivity and specificity of DWI sequences

The sensitivity and specificity of MRI on the basis of DWI sequences alone were calculated in a sub-group of patients with this sequence available (414 sCJD and 38 non-CJD cases). The results are highlighted in table 12.

Table 12: Sensitivity, specificity and predictive values of MRI for the diagnosis of sCJD (all definite and probable sCJD and non-CJD cases) (DWI alone)

Statistic	Value	95% CI
Sensitivity	90.34%.	87.08% to 93.01%
Specificity	94.74% *	82.25% to 99.36%
Positive Predictive Value (*)	99.47%	97.98% to 99.86%
Negative Predictive Value (*)	47.37%	39.91% to 54.95%

* one non-CJD case (VGKC encephalitis) had imaging features suggestive of sCJD, but this case was distinguished from sCJD on the basis of a CSF pleocytosis.

3.3.4.3 Sensitivity and specificity of CSF RT-QuIC

The sensitivity and specificity of CSF RT-QuIC (available in 334 sCJD cases and 16 non-CJD cases) is shown in table 13.

Table 13: Sensitivity, specificity and predictive values of CSF RT-QuIC for the diagnosis of sCJD (definite and probable sCJD and non-CJD cases)

Statistic	Value	95% CI
Sensitivity	85.33%	81.07% to 88.95%
Specificity	100.00%	79.41% to 100.00%
Positive Predictive Value (*)	100.00%	
Negative Predictive Value (*)	24.62%	20.13% to 29.72%

3.3.5 Non-CJD cases with MRI scans resembling sCJD

Of the 54 non-CJD cases, 5 (9%) had MRI features resembling that of sCJD (referred to here as 'false positive' scans). Two cases had dementia with Lewy body on autopsy, one of whom had additional minor Alzheimer's disease pathology. One case had Huntington's disease confirmed by genetic testing, one had voltage-gated potassium channel (VGKC) (Caspr-2) encephalitis and one case showed sustained improvement in their condition (see table 14).

The criteria for referral of suspect cases to the NCJDRSU have always been defined loosely - in order to increase the chance of identifying cases with unusual or atypical clinical phenotypes. Whilst the general reasons for case referral have been recorded, this has not been done with sufficient granularity to give a reliable figure for cases referred solely on the basis of an abnormal MRI scan, if any. However, based on the available data and my experience working in the NCJDRSU, it is unlikely that many non-CJD cases were referred on MRI

appearances alone. While this would represent a degree of selection bias in the non-CJD cases, it is unlikely to be of significant magnitude.

Table 14: Final diagnosis of non-CJD cases with MRI scans resembling sCJD

	Definite diagnosis	Clinical and investigation evidence
DLB	2	0
HD	0	1
VGKC encephalitis (Caspr2)	0	1
Sustained Improvement	0	1

DLB, Dementia with Lewy body disease; HD, Huntington's disease; VGKC; Voltage-gated potassium channel antibody encephalitis

3.3.5.1 MRI signal abnormalities in false positive scans

Basal ganglia signal abnormalities were present in 4/5 cases. Three cases had FLAIR and DWI sequences available, two of these had no associated basal ganglia high signal on DWI (see figure 26) and one did (see figure 27). One case with basal ganglia high signal had associated cortical high signal over both insular regions, again on FLAIR imaging only.

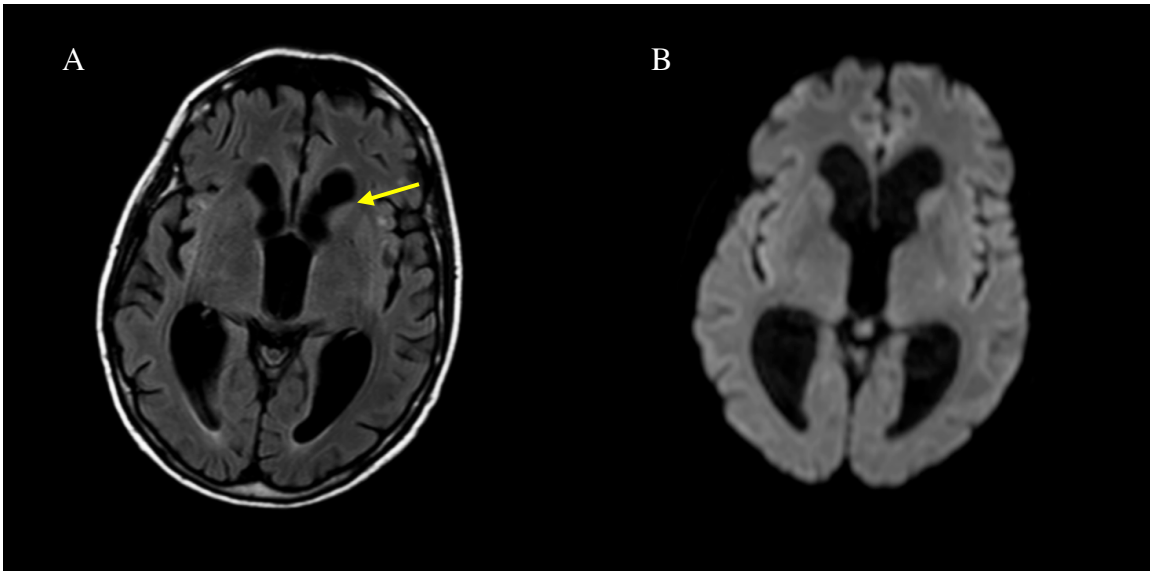


Figure 26: MRI signal abnormalities resembling sCJD in a 74-year-old with neuropathologically confirmed Alzheimer's disease. The FLAIR (A) demonstrates subtle signal abnormality affecting the left caudate head, with no corresponding signal abnormality on the DWI

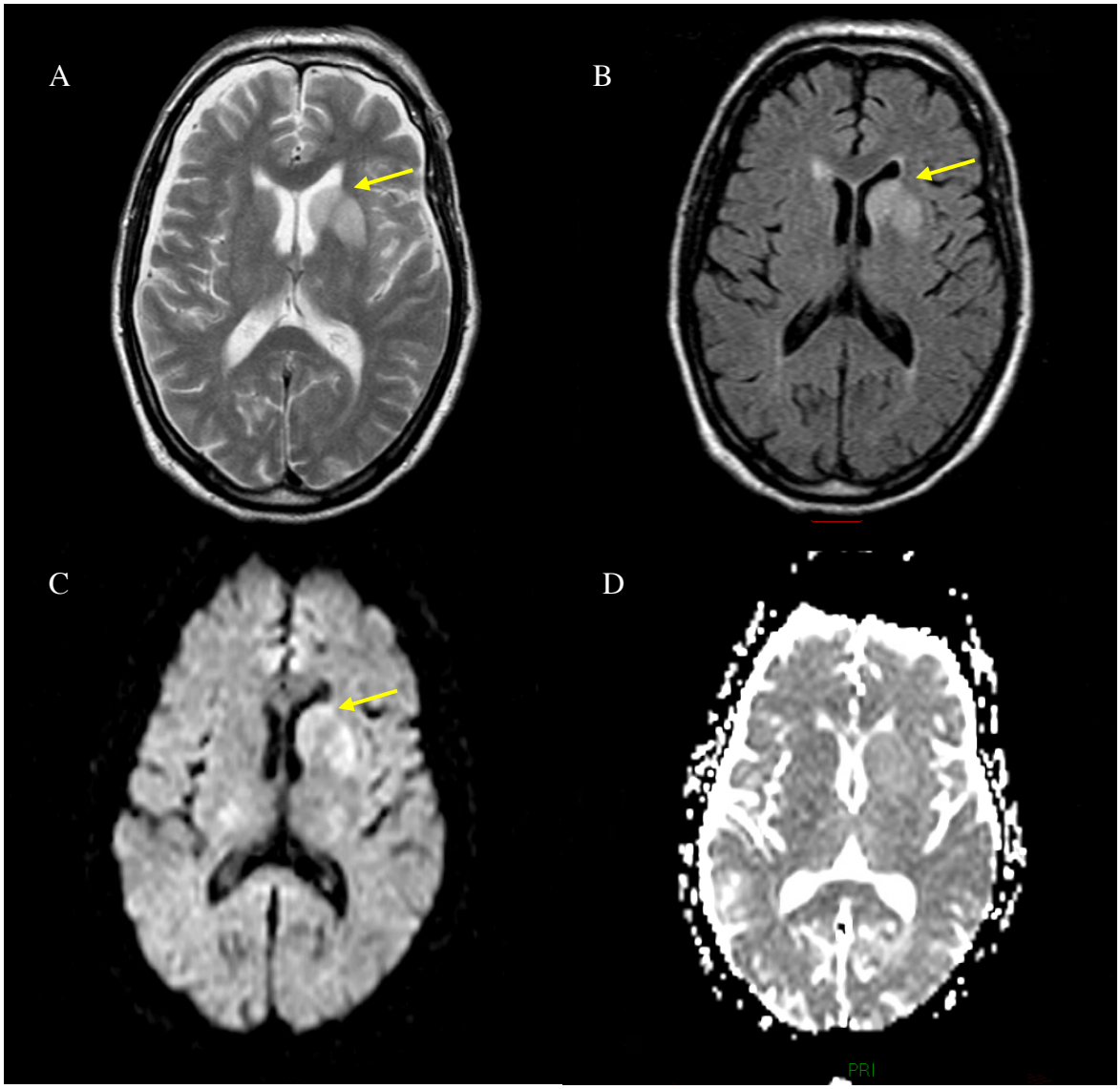


Figure 27: MRI signal abnormalities resembling sCJD in in a 68-year-old patient with voltage gated potassium channel (Caspr-2) autoimmune encephalitis with an inflammatory CSF. The axial T2 (A) and FLAIR (B) sequences show asymmetric left basal ganglia high signal with corresponding high signal on DWI (C). As opposed to sCJD there is no associated low signal on the apparent diffusion coefficient map (D) indicating absence of true restricted diffusion. There is also swelling of the affected caudate head, a feature that does not occur in CJD.

Isolated cortical signal abnormalities occurred in a case of Huntington's disease on a combination of FLAIR and DWI sequences; predominantly affecting regions prone to signal artefact (frontal and cingulate regions). If discounting these regions, the cortical

abnormalities were limited to bilateral temporal cortices on FLAIR only and one occipital region on DWI only (figure 28).

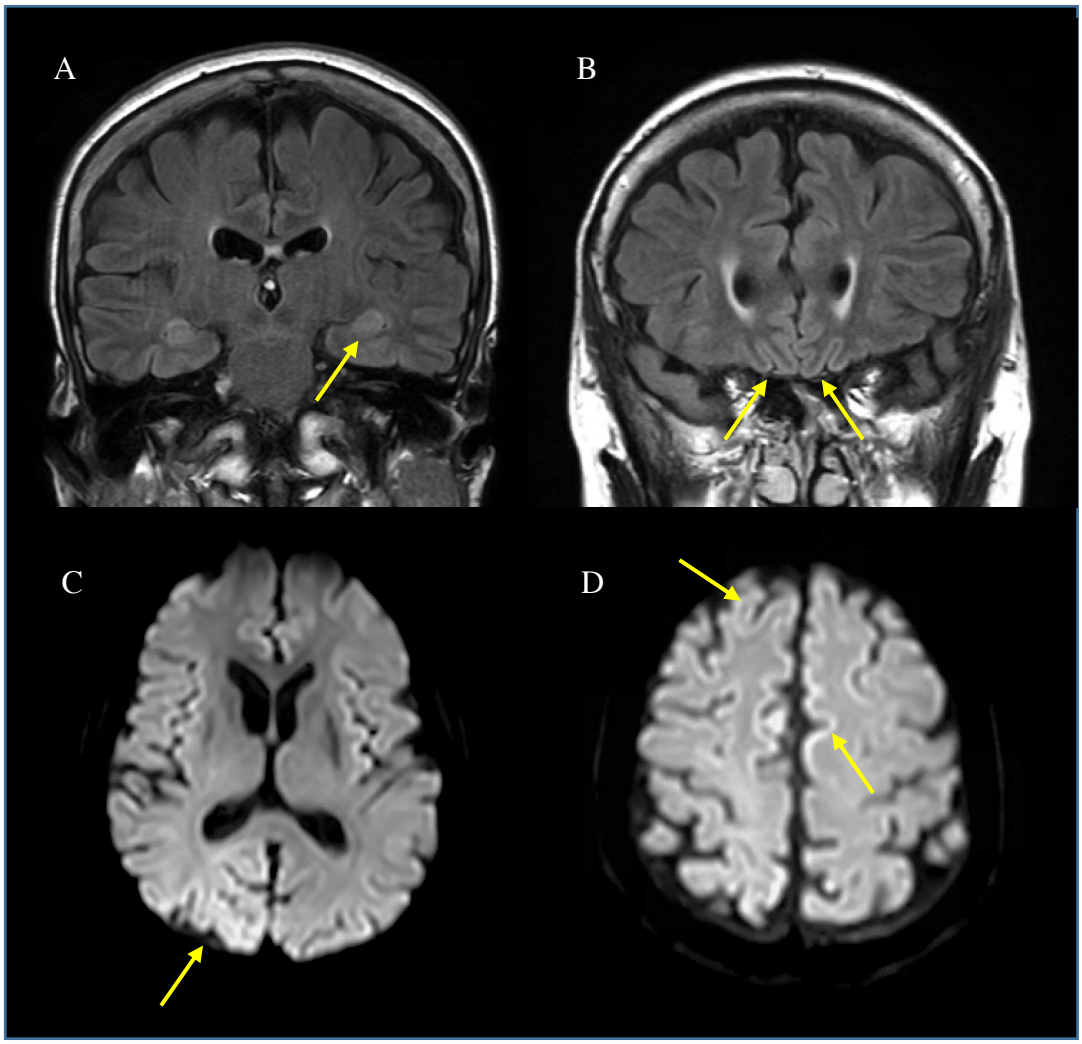


Figure 28: MRI brain abnormalities similar to sCJD in a 61-year-old with genetically confirmed Huntington's disease. Coronal FLAIR demonstrates bilateral signal abnormalities in the hippocampi (A) and frontal cortices (B) (arrows). Axial DWI shows associated high signal in the right occipital cortex (C) as well as both frontal and cingulate cortices (D)

3.3.6 CJD cases with no CJD associated signal abnormalities

38 of 462 sCJD cases (8%) had MRI scans that failed to show characteristic CJD abnormalities (referred to here has 'false negative' scans). There was no significant difference in median age of onset between cases with negative imaging (median 71) to those with positive imaging (median 68) (p= 0.08).

3.3.6.1 MRI sequences

Only 15 of the 38 cases with negative scans had DWI sequences (39%) and in only 13 cases were there a combination of FLAIR and DWI sequences performed (34%).

3.3.6.2 MRI quality

There was a significant difference in median scan quality between the negative MRI group (Median = 3 (graded 'average')) and those with positive scans (median = 2 (graded 'good')) (p= 0.0006).

3.3.6.3 Timing of MRI scan

There was no significant difference in the timing of MRI scan between the negative group and the positive group measured in months from symptom onset to scan (median 2.4 and 2.8 respectively, p=0.51). A significant difference was observed, however, when comparing the timing of the scan as represented as a percentage of total disease duration (median percentage of negative = 55% and positive = 68%, p=0.0025).

3.3.6.4 Serial MRI scans

18 of the 38 cases with an initial negative MRI had serial imaging; 14 of whom (78%) became 'positive' on subsequent imaging and 4 remained 'negative'. Of the 14 positive serial studies, 8 (57%) were positive on the basis of the DWI alone. When including serial imaging, the sensitivity of MRI increased from 85% to 87%.

The median timing of a positive MR in the serial imaging subgroup (represented as a percentage of disease duration) was not significantly different to those with positive imaging on initial study (median percentage = 71% and 68% respectively, p=0.55).

Of those that remained negative, all had DWI on initial imaging, but only 2 had DWI on subsequent imaging. The study quality in all these cases deteriorated on serial imaging.

3.3.6.5 CSF RT-QuIC

CSF RT-QuIC was available in 20 of the 38 cases with initial negative MRI scans and was positive in 19 (95%) of cases. The one case with a negative RT-QuIC result had positive serial imaging. There were no cases in this analysis with both negative imaging (with the inclusion of serial scans) and negative CSF RT-QuIC.

3.4 Discussion

In clinical practice, the diagnosis of sCJD is not based on MRI findings in isolation but instead requires to be interpreted in an appropriate clinical context. It is paramount to exclude other potential diagnoses and if clinical features and investigations are incompatible with CJD, then the diagnostic criteria for the condition should not be applied. Instead, under the current clinical diagnostic criteria, the role of MRI is to elevate the classification of possible sCJD to probable. Therefore, the sensitives and specificities of MRI findings discussed in this chapter are used in this context.

There were a small number of controls in this study that had MRI findings suggestive of sCJD. Almost half of these cases were neurodegenerative disorders, which can be difficult to distinguish from sCJD, especially when they present with rapid cognitive impairment. The majority of these non-CJD cases had signal abnormalities observed on FLAIR sequences only with no corresponding signal abnormalities on DWI. The most important differential diagnoses of sCJD are probably those with a central nervous system inflammatory condition as they may be treatable. There was one case of VGKC encephalitis in which a diagnosis of sCJD was suspected, where high signal was observed in the basal ganglia on both FLAIR and DWI sequences. There were some factors however, that distinguished this case from CJD. Firstly, the imaging was atypical in that the affected caudate head was swollen, a finding that does not occur in CJD. As well as this, the high signal on DWI was not associated with low signal on ADC mapping, implying the absence of restricted diffusion. This is a finding that is not typical in sCJD – and its absence has been reported as a useful tool in discriminating sCJD from other causes of rapidly progressive dementia (135). This thesis did not formally assess ADC mapping alongside DWI sequences, and it may be of interest to explore such an analysis in a similar cohort in future studies to review the combined utility of these sequences

further. CSF analysis can also help in differentiating CJD from non-CJD patients. In the case above, an inflammatory CSF was present with a pleocytosis, which rarely occurs in CJD and when it does, is certainly limited to less than 20 white cells (118).

This is the largest single-centre study in Europe examining the MRI findings in a series of consecutive and unselected sCJD cases and controls. It determines the role of MRI in the clinical diagnosis of CJD and provides further validation of the current diagnostic MRI criteria in line with other studies (115, 163).

High signal abnormalities of the basal ganglia and/or cortex were the most frequent abnormalities observed in sCJD and were present in most cases, particularly on DWI sequences. CJD-associated MRI abnormalities occurred in a number of non-CJD cases and, based on the current MRI diagnostic criteria, the sensitivity and specificity of these radiological features for the diagnosis of sCJD were 85% and 91% respectively. The overall sensitivity increased to 87% when serial scans were considered. Sensitivities were similar in the neuropathologically confirmed group (84%), but with a slightly increased specificity of 93%. The relatively small number of neuropathologically confirmed controls in the latter group makes this observed specificity less reliable, and the analysis of the aggregate of all cases is probably the most informative and reliable measure of the overall diagnostic utility of MRI. It is important to note that the specificity in this study was based on a very relative control group, consisting of patients with suspected CJD referred by local clinicians to a national referral centre, in which the diagnosis was subsequently excluded by autopsy or in which there was a definite alterative clinical diagnosis made.

The seminal study that supported the introduction of MRI abnormalities in the diagnostic criteria for sCJD was carried out in 2009 as an international collaboration and reported both a sensitivity and specificity of 83% with MRI in the diagnosis of sCJD (115). This was not, however, a systematic study including non-selected, consecutively occurring cases. Furthermore, the number of cases with DWI sequences, known to have the highest sensitivity for the identification of signal abnormalities associated with CJD, was lower (69%).

The review in this thesis expands on previous similar imaging studies in CJD by determining the clinical utility of MRI in a large consecutive and unselected series of patients with suspected CJD, referred by clinicians across a single country comprising a population of

around 60 million people. As a result, the study sample, and analysis thereof, is both representative of and relevant to patients encountered in clinical practice.

This study also benefits from consistent data collection on a rare disease due to the national referral system to NCJDRSU; where attempts are made to review every suspected case of CJD in person by a clinician from this unit. MRI scans were examined by a single neuroradiologist experienced in the assessment of MRI in CJD so avoiding interobserver variability. Furthermore, there was a high percentage of sCJD scans with DWI sequences available (90%).

In this thesis, 92% of sCJD cases and 28% of non-CJD controls had high signal abnormalities in the basal ganglia and/or at least one area of cortex (sensitivity 92% and specificity 72%). These results contrast with a recent study by Bizzi et al that looked at the utility of DWI in a large series of sCJD cases and controls; based on high signal in at least one region of the cortex or in subcortical structures (164). The brain regions that were analysed consisted of frontal, parietal, temporal and occipital cortical regions and subcortical regions of the caudate, putamen and the thalamus. Using these proposed MRI criteria, the study found that DWI had a sensitivity of 92% and a specificity of 98%, which was comparable to the diagnostic utility of CSF RT-QuIC in the same cohort (164). This is a surprising outcome, particularly given the inclusion of high signal in the frontal cortices; a region prone to artefactual high signal in healthy individuals (165) and the presence of which would be predicted to decrease the specificity of MRI. Indeed, the analysis in this thesis identified high signal of the frontal cortices in 3% of non-CJD cases on DWI sequences, with higher frequencies reported in other studies (ranging between 9-15%) (115, 136).

In contrast, when the current diagnostic MRI criteria were applied to a subgroup in this thesis on the basis of DWI sequences alone, an increased sensitivity of 90% and specificity of 95% was achieved. Rather unexpectedly, this sensitivity was superior to CSF-RT-QuIC within this cohort. The increased specificity was largely due to non-CJD cases having high signal changes restricted to FLAIR sequences only, with no associated signal abnormalities on the DWI. Indeed, only the case of VGKC encephalitis had radiological features suggestive of sCJD on FLAIR and DWI sequences. This case was distinguished from CJD on the basis of an inflammatory CSF and positive VGKC antibodies. This is an important point, as the diagnosis of CJD should not be made on the basis of MRI findings in isolation and rather it

needs to be interpreted alongside the clinical features and other investigations. Therefore, when applying the MRI findings on DWI sequences in conjunction with the appropriate clinical circumstances, the specificity for diagnosis of sCJD was 100%.

The relatively smaller number of controls with DWI sequences (70%) makes the observed specificity less reliable in this subgroup analysis, although all but one of the controls with abnormal high signal changes on FLAIR had DWI sequences available.

Certainly, the high number of sCJD cases compared to non-CJD controls (90% vs 10% respectively) was a general limitation in this study, which may overestimate specificity. The control group consisted of patients referred to the NCJDRSU with a suspicion of CJD, in which this diagnosis was excluded by neuropathological examination or in which there was a definite alterative clinical diagnosis. As a result, there were a limited number of controls that met these criteria over the study period, particularly given the declining rates of post-mortems. Nonetheless, this study achieved neuropathological confirmation in 48% of controls and in 55% of sCJD cases. A methodological limitation of the study was that MRI scans were performed under different conditions on many different MR imaging platforms across the country, which resulted in greater technical heterogeneity of the imaging. In addition to these factors, the MRIs were visually assessed when examining for areas of high signal, rather than the quantitative measures used in other imaging studies. The identification of MRI abnormalities in CJD however, are on the basis of visual assessment as part of the diagnostic process and so this study's analysis is applicable to that encountered in clinical practice.

A total of 8% of sCJD cases had no characteristic signal abnormalities on initial MRI, with the majority of scans (61%) lacking sensitive DWI sequences. These cases were also more likely to have poorer quality MRI scans and the timing of the initial scan, represented as a percentage of illness duration, was statistically more likely to be at an earlier stage of the illness than those with positive imaging. A proportion of those with initial negative imaging had serial studies, 78% of which were positive, predominantly on this basis of DWI sequences. When considering serial scans, only 5% of cases had negative imaging overall. Serial imaging is discussed in more detail in chapter 5. Of note, there were no cases with both negative imaging (including serial scans) and a negative CSF RT-QuIC in this cohort.

In conclusion, the results of this study emphasise the importance of obtaining MRI using DWI sequences in all patients that present with a rapidly progressive dementia. MRI is one of

the most useful investigations in suspected sCJD, as it is readily available, non-invasive and the DWI sequences can be obtained in an agitated patient in a short period of time. In the appropriate clinical context, high signal in the basal ganglia and/or two or more specific cortical regions is strongly suggestive of a diagnosis of sCJD and can elevate the diagnostic classification in life from a possible to a probable case. The presence of these MRI findings on DWI sequences alone has a comparable sensitivity and specificity to CSF RT-QuIC and when both of these tests were used, the sensitivity reached 100%. Although the specificity of DWI sequences was high, it did not reach 100%, and it is important to note that autopsy still remains essential for a definite diagnosis of sCJD.

Unfortunately, CJD remains an untreatable and universally fatal disease. However, the accurate diagnosis in life is vital not only for appropriate clinical care, but also to help safeguard against the potential transmissibility of the condition.

Chapter 4 Evolution of DWI signal abnormalities in sCJD

4.1 Aim

The aim of this chapter was to assess the evolution of signal abnormalities in sCJD on serial diffusion-weighted imaging in order to determine whether changes in scan appearances occur with disease progression.

4.2 Results

4.2.1 Basic demographics

There were 64 cases of sporadic CJD (29 definite and 35 probable) in whom at least 2 serial MRI studies had been undertaken with the inclusion of DWI sequences during each scan. In 9 cases, where there were more than two serial MR studies, the initial and last scans were analysed.

There was a total of 24 anatomical regions that were analysed, in each scan, for abnormal signal changes that included right and left subcortical structures; caudate, putamen, anterolateral thalamus, mediodorsal thalamus, pulvinar thalamus, hippocampus and six cortical areas; right and left cingulate gyrus, insular, frontal, temporal, parietal and occipital regions.

The median age at disease onset for cases was 68 (range 49-82). 34 cases were female. The median disease duration was 4 months (range 1.03-62.03). The median time to first MR scan from disease onset was 2.65 months (range 0.35-23.45) with the median time expressed as a percentage of disease duration of 49% (range 0.3-92%). The median time to the last available MR scan was 3.21 months (range 0.35-61.03) with median time as a percentage of disease

duration of 72% (0.21-99%). The median time of initial to serial scan was 0.84 months (0.06-37.58).

4.2.2 Initial MRI scans

The median number of regions demonstrating signal abnormality on initial scan was 7 of 24 (range 1-20).

4.2.2.1 Cortex

Cortical high signal was the most commonly observed abnormality, with 92% (59 of 64) of cases demonstrating at least one area of cortical hyperintensity (median cortical areas 5 (range 1-12)). The cingulate gyrus was the most commonly involved cortical area, with 73% (47 of 64) involvement (70% bilateral and 30% asymmetric), followed by frontal (62%) and parietal regions (62%). The hippocampus was the area least likely to show signal abnormality (9%).

4.2.2.2 Basal ganglia

Basal ganglia signal abnormalities were observed in 58% of cases (37 of 64) on initial MR. Of these cases, all had caudate head hyperintensity (60% symmetric and 40% asymmetric) and 54% (20 of 64) had associated involvement of the putamen (55% bilateral and 45% asymmetric).

4.2.3 Serial MRI scans

The median number of anatomical regions demonstrating signal abnormalities increased between serial scans, from 7 to 9 respectively. Figure 29 shows the distribution of signal abnormalities between initial and serial imaging.

The number of regions involved correlated positively with disease duration in both initial ($r = 0.29$, $p = 0.02$) and serial scans ($r = 0.25$, $p = 0.04$) (figure 30), but not with age at disease onset.

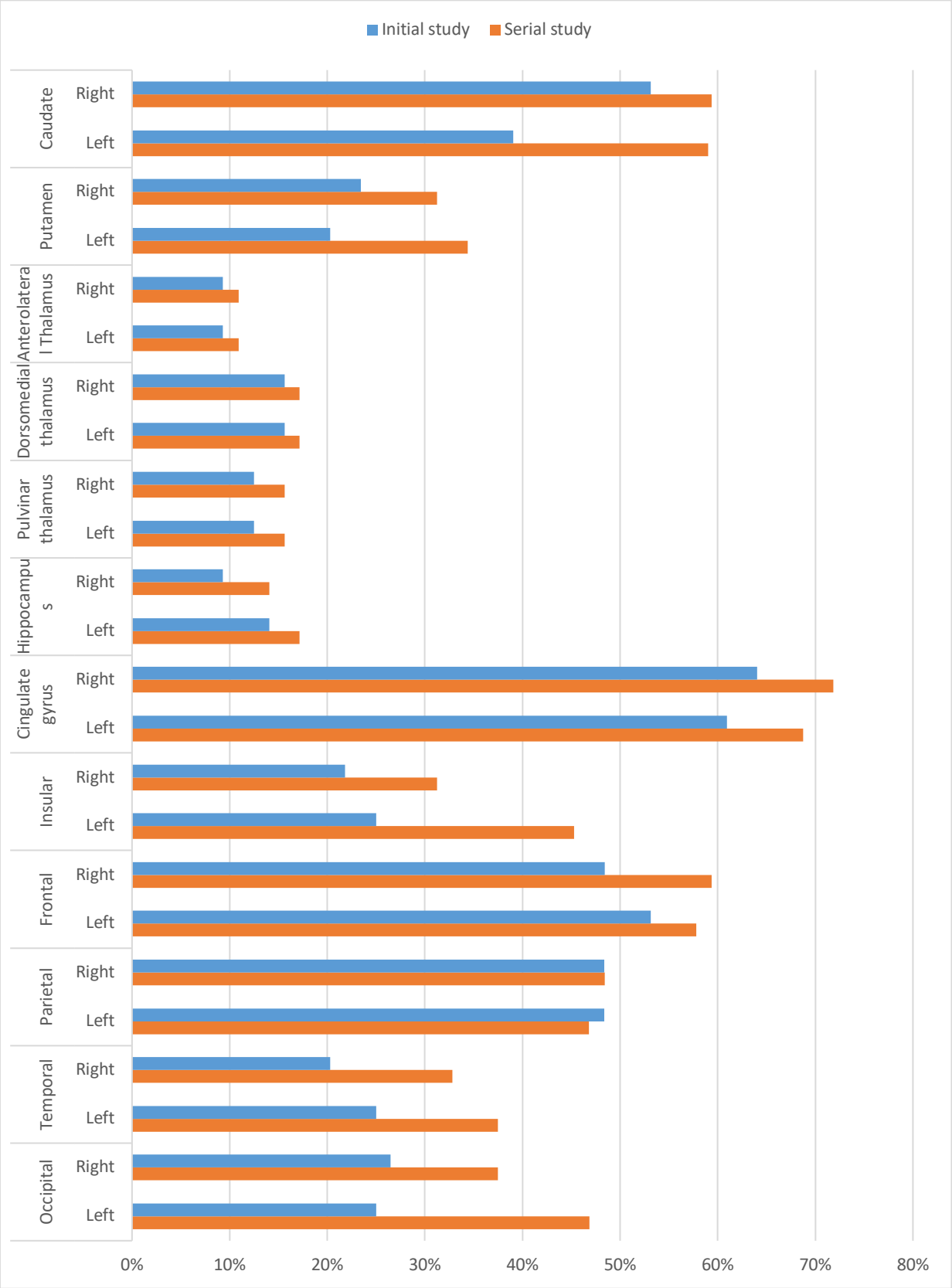


Figure 29: Distribution of signal abnormalities on DWI (%) at initial and last available MRI brain scan in cases with serial imaging (n=64)

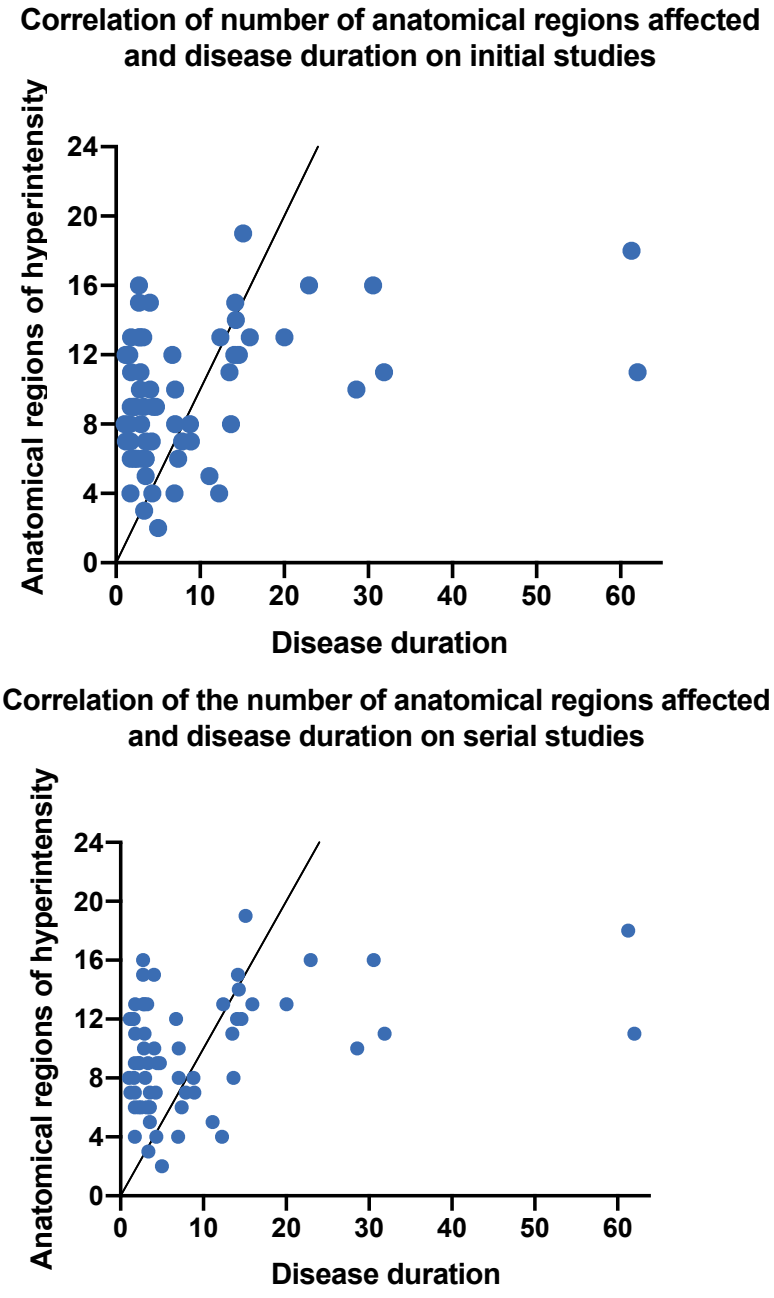


Figure 30: Scatterplots showing positive correlation between number of anatomical regions involved on both initial and serial MRI scans and disease duration (months). Spearman R = 0.29, two-tailed p-value = 0.02

4.2.3.1 Cortex

There was an increase in the overall number of cases demonstrating cortical signal abnormalities on serial imaging when compared to baseline study, with 63 of 64 cases having at least one region of cortical involvement (98% vs 92% respectively). The median number of cortical regions involved increased from 5 to 6 (range 1-12). The cingulate gyrus (77%), frontal (77%) and parietal regions (64%) remained the most commonly affected regions. There was also an increase in the extent of cortical signal abnormalities in serial imaging in 59% of cases (38 of 64) (figure 31). 19% of cases (12 of 64) showed no change in the number of cortical regions involved and 14% (9 of 64) demonstrated fewer areas of cortical involvement on serial imaging.

Coinciding with the cases with decreased cortical involvement on serial study, an increase in the percentage with basal ganglia signal abnormality was observed (44% on initial and 67% on serial study). There was no significant difference observed in age (p=0.69), disease duration (p=0.84), timing of initial (p=0.99) or serial scan (p=0.37) between those with increased and those with decreased cortical involvement on serial imaging.

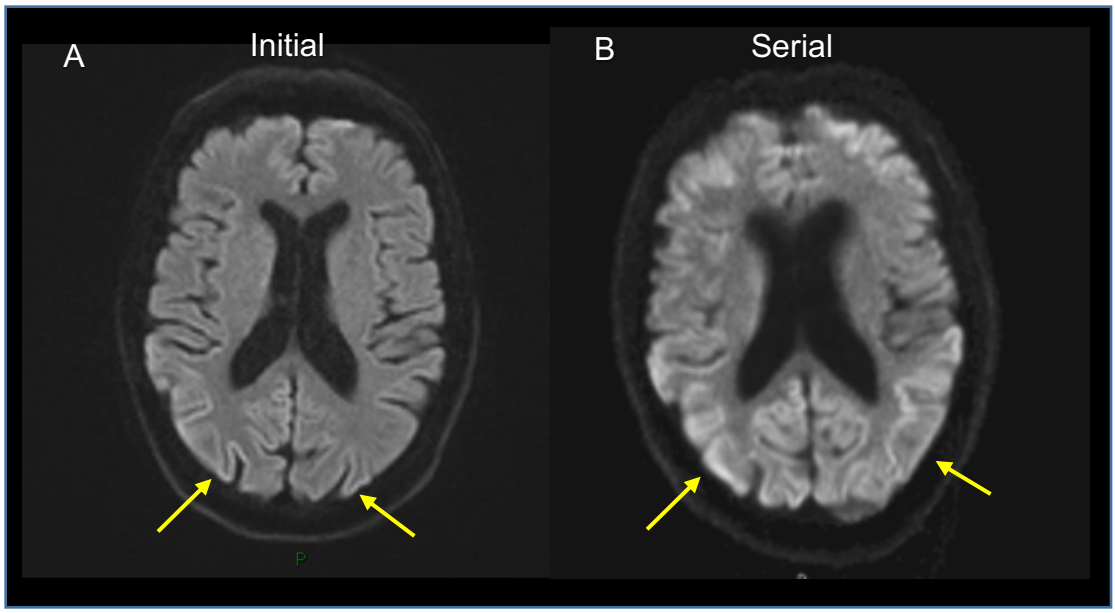


Figure 31: Evolution of DWI signal abnormalities in a 67-year-old with sCJD. On the initial scan (A) there is cortical signal abnormalities predominantly affecting the right parietal region (arrow) and, to a lesser extent, the left parietal lobe (arrow). The subsequent scan (B) 28 days later demonstrates more extensive cortical signal abnormalities involving bilateral parietal regions (arrows) as well as bilateral frontal regions

4.2.3.2 Basal ganglia

The percentage of cases with basal ganglia signal abnormalities increased to 72% (46 of 64) on serial imaging, associated with a progression from asymmetric to symmetric involvement (65% symmetric and 35% asymmetric). All of these cases demonstrated caudate involvement and 59% (27 of 64) had associated involvement of the putamen (56% bilateral and 44% asymmetric). Of those with isolated caudate head basal ganglia signal abnormalities on initial MR (18), 56% (10) developed associated signal abnormalities in the putamen on serial imaging (see figure 32). All cases with signal abnormalities in the putamen on initial scan (20) demonstrated persistent putamen hyperintensity on serial imaging.

Two cases showed apparent disappearance of basal ganglia high signal on serial scan. In both these cases, the basal ganglia signal abnormalities were deemed to be subtle on the initial scan. One case with asymmetric caudate head and cortical signal abnormalities on initial scan showed disappearance of the caudate changes on the serial imaging performed 1.16 months after the first study. The cortical abnormalities persisted however and were more widespread and confluent on serial study (figure 33).

A further case with asymmetric caudate head signal abnormalities on initial study showed disappearance of this signal change on serial study. The quality of the study deteriorated on serial imaging, which may have contributed to the less evident signal abnormality (see figure 34).

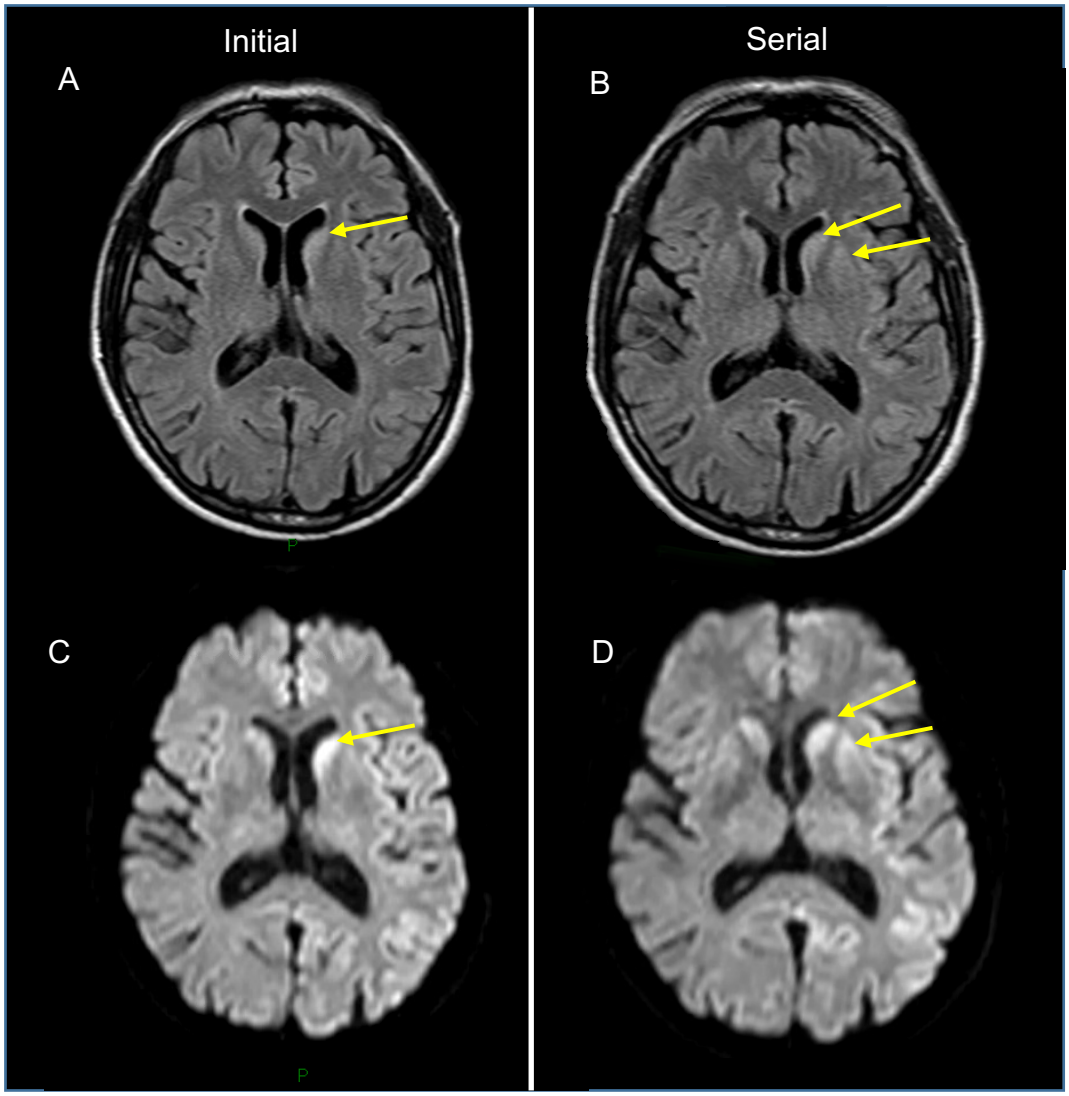


Figure 32: Evolution of basal ganglia signal abnormalities in a 71-year-old with sporadic CJD. The initial scan (A + C) demonstrates left caudate head signal abnormality on FLAIR (A) and DWI (C) (arrows) with associated cortical high signal involving left frontal, insular and parietal regions appreciable on DWI. There is also subtle right caudate head signal abnormality, again, more conspicuous on DWI. The serial scan (B and D), performed 16 days after the first scan, shows progression of the basal ganglia signal abnormality with involvement of the left anterior putamen more conspicuous on DWI (D) than FLAIR (B). The extent of the cortical signal abnormalities is unchanged between the scans.

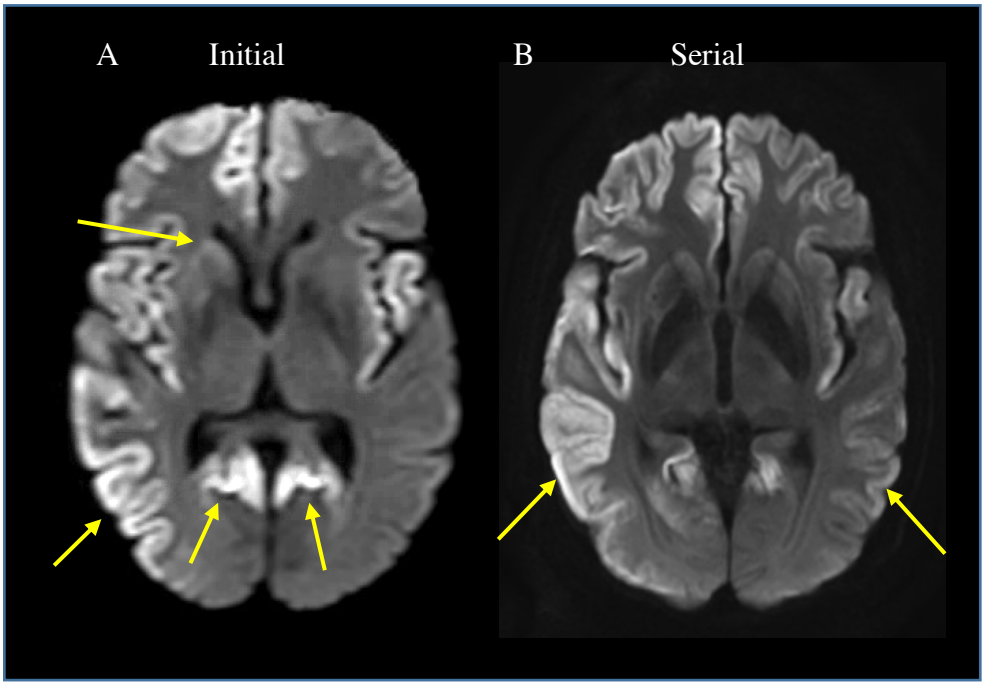


Figure 33: Disappearance of basal ganglia signal abnormality on serial imaging in a 72-year-old with sporadic CJD. The initial study (A) shows cortical signal abnormalities involving right parietal, temporal and frontal regions as well as bilateral insular and hippocampal areas (arrows). There is subtle high signal in the right caudate head (arrow). The cortical signal abnormalities progressed on the serial scan performed 35 days later (B), becoming more conspicuous and spreading to involve left frontal and parietal regions (arrows). The caudate head high signal seen on initial scan was not evident on the serial scan.

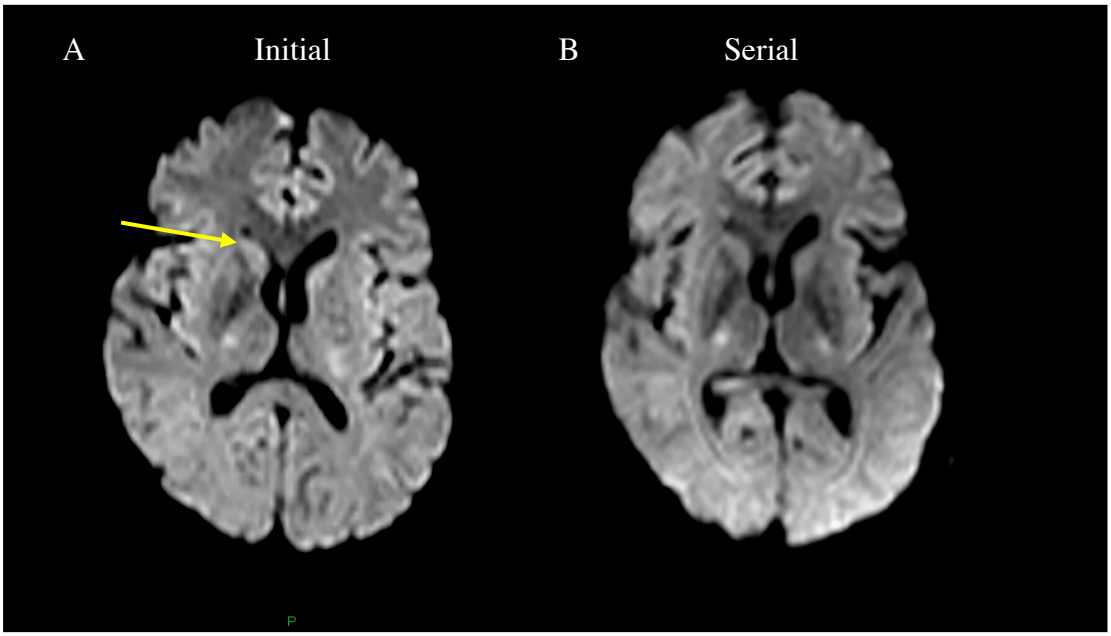


Figure 34: Disappearance of basal ganglia and cortical signal abnormalities on serial imaging in a 53-year-old with MM2 sCJD. Disease duration was prolonged at 27 months. The initial study (A), performed 20 months after symptom onset, shows subtle signal hyperintensity in the right caudate head (arrow) and bilateral insular cortices. The caudate head and insular changes are not evident on the serial scan performed 3 months later. The quality of the serial study deteriorated as a result of movement artefact.

4.3 Discussion

This chapter describes the evolution of MRI signal abnormalities in sCJD on serial diffusion-weighted imaging. The results suggest that disease progression is unsurprisingly associated with an overall increase in the extent of signal abnormalities, in both cortical and subcortical regions, as observed on DWI. Cortical high signal was the most commonly observed abnormality, particularly affecting frontal, cingulate and parietal regions. In the majority of cases there was an increase in the number of cortical regions affected with disease progression; a finding that has been previously reported upon in sCJD, although in smaller cohorts (127, 139, 141, 166, 167).

There was a small minority of cases (9 of 64) where a decrease in the extent of cortical involvement was observed, acting as a potential confusing factor in diagnosis. There were no

significant differences in age, overall disease duration or timing of scan between those with increased and those with decreased cortical involvement on serial scan.

High signal affecting the basal ganglia was the second most commonly observed abnormality on imaging. All cases with basal ganglia high signal had caudate head involvement. There was an overall increase in the number of cases with basal ganglia high signal as well as a general evolution from asymmetric to symmetric basal ganglia involvement with disease progression. A number of cases with isolated caudate head signal change showed posterior progression with involvement of the putamen, a finding that has been previously reported (168). There were two cases where subtle caudate head signal abnormality was not apparent on serial imaging, albeit with progression of cortical abnormalities in one of these cases.

The exact pathological substrate responsible for the DWI signal abnormalities observed in sCJD is yet to be determined. A number of studies have reported increased vacuolation (spongiform changes) in anatomical regions corresponding to DWI signal abnormalities (139, 169, 170) whilst another study described a correlation with both PrP^{Sc} accumulation and, to a lesser extent, vacuolation (171).

Assuming vacuolation does relate to the high signal observed on DWI, it is possible that the overall increase in DWI abnormalities with disease progression is reflective of increased spongiform degeneration in both cortical and subcortical grey matter structures. Disappearance in DWI signal abnormalities was observed in 16% of cases in this study, predominantly affecting the cortical regions. This phenomenon has been reported as occurring in the later stages of the disease in the literature (141, 172, 173). The exact mechanism for this is unknown, but it thought to be due to progressive neuronal death and gliosis in the later stages of the disease, leading to the decrease of abnormal vacuoles and thus resulting in a loss of DWI signal abnormalities prior to the appearance of frank atrophy (132, 139). There were no significant differences however, in the timings of these scans in this study, either as a percentage of disease duration or length of time to serial scan, between those with increased and those with decreased signal abnormalities. Evolution of atrophy was not formally assessed within this study and so it is not possible to comment on whether this was a factor associated with those with reduced signal abnormalities on serial imaging. It may be of interest to formally access this in a similar cohort of cases in the future. There are, of course, a number of methodological problems in a reliable comparative assessment of

cerebral atrophy on MR scans, especially when scans are obtained on different machines with different protocols.

In conclusion, patients with sCJD show an overall increase in the extent of cortical and subcortical signal abnormalities that correlates with disease duration. The evolution of these signal abnormalities is likely a manifestation of progressive spongiform degeneration. Disappearance of signal abnormalities with disease progression can occur, particularly that affecting the cortex, and this should be taken into consideration when interpreting imaging in the diagnosis of CJD.

Chapter 5 Timing of MRI and the role of serial imaging in the diagnosis of sCJD

5.1 Aim

The purpose of this chapter was to assess whether the MRI scan in sCJD is more likely to show characteristic features at different stages of the clinical illness and to determine the role of serial imaging in the diagnosis of the condition.

5.2 Results

5.2.1 Basic demographics

Of the 462 definite and probable cases, 91 (20%) had serial imaging performed with either FLAIR / DWI sequences or both. 75 cases had two scans, 15 had three and one had four. There were no significant differences between cases with serial imaging and those without, in age (median 67 years and 68 respectively, p=0.39) or in illness duration (median 4.42 and 4.08, p=0.14). In the discussion, a 'positive' scan is one that had abnormalities characteristic for sCJD and 'negative' is one without such abnormalities.

5.2.2 Initial MRI negative cases

There were 18 cases with serial scans that were negative on initial imaging. 14 of these cases (78%) had positive scans on subsequent imaging (see figure 35). DWI was available in 55% of initial scans compared to 88% on serial imaging. In 6 of these 14 cases that had a later

positive scan, the signal abnormalities were identified on DWI sequences alone when the initial scan had lacked these sequences.

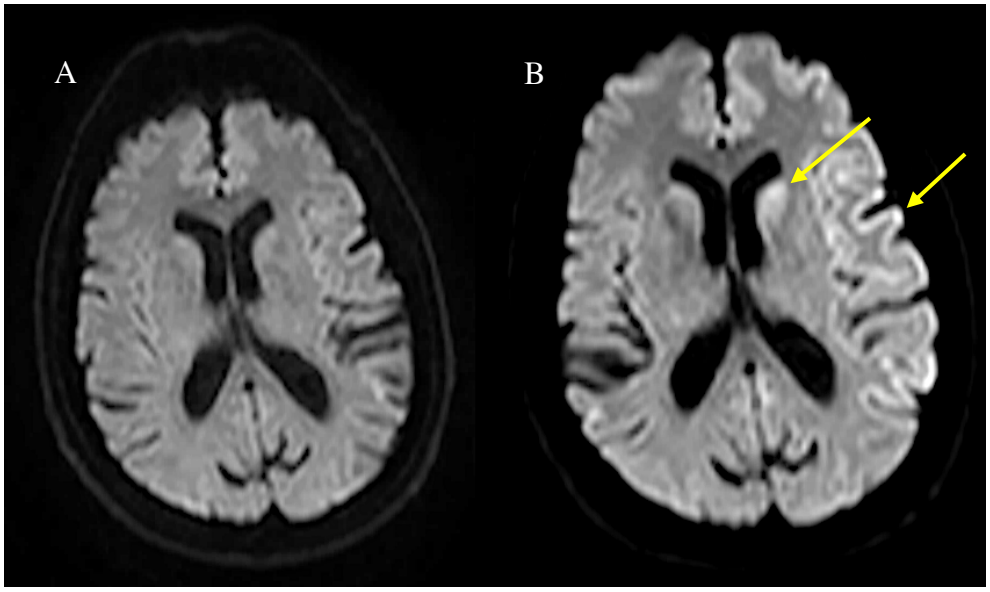


Figure 35: Serial imaging of a definite sporadic CJD case. (A) Initial DWI with no signal abnormalities suggestive of CJD. (B) Repeat DWI performed 1 month later now demonstrating high signal in left caudate head and associated left sided cortical high signal

The median timing between scans that were positive on subsequent imaging was 4.6 months (range 0.55-16.90). Four cases remained negative on serial scan. One case that was positive on the second scan, became negative on the third (see table 15).

Table 15: Serial imaging in cases with negative first scans (* % of illness duration)

Case	Age	Disease duration (months)	1st MR impression (+/-)	DWI (yes/no)	Time to 1st MR (months)	Timing of 1st MR (%*)	2nd MR impression (+/-)	DWI (yes/no)	Time to 2nd MR (months)	Timing of 2nd MR (%*)	3rd MR impression (+/-)	DWI (yes/no)	Time to 3rd MR (months)	Timing of 3rd MR (%*)
1	66	23.06	-	Yes	1.87	8%	-	No	2.74	12%				
2	66	2.84	-	No	0.94	33%	+	Yes	1.35	48%				
3	66	4.42	-	Yes	3.71	84%	-	No	4.03	91%				
4	58	5.16	-	No	2.10	41%	+	Yes	3.58	69%				
5	80	2.97	-	Yes	1.13	38%	-	Yes	1.61	54%				
6	54	2.65	-	No	1.39	52%	+	Yes	2.00	76%				
7	65	2.13	-	No	1.23	58%	+	Yes	1.52	71%				
8	51	17.74	-	No	9.23	52%	+	Yes	9.81	55%	-	Yes	14.29	81%
9	67	24.90	-	Yes	13.55	54%	+	Yes	16.90	68%	+	Yes	18.81	76%
10	64	12.52	-	Yes	3.23	26%	+	Yes	4.23	34%	+	Yes	5.13	41%
11	73	7.48	-	No	5.19	69%	+	Yes	6.71	90%				
12	62	7.52	-	Yes	6.35	85%	+	Yes	6.39	85%	+	Yes	7.00	93%
13	80	8.29	-	Yes	2.03	25%	-	Yes	6.00	72%				
14	67	15.23	-	Yes	0.77	5%	+	Yes	12.61	83%				
15	75	8.90	-	Yes	5.23	59%	+	Yes	6.55	74%				
16	68	1.48	-	Yes	0.10	7%	+	Yes	0.55	37%	+	Yes	1.03	70%
17	76	5.68	-	No	3.19	56%	+	Yes	5.00	88%				
18	67	3.32	-	No	2.13	64%	+	Yes	3.06	92%				

5.2.3 Initial MRI positive cases

Of the 73 cases with positive first MRI scans; all remained positive on subsequent imaging.

5.2.4 Timing of MRI in relation to illness duration

Here, the timing of positive and negative scans using either FLAIR and/or DWI sequences, (including serial studies) was compared. Timing of scans was represented as a percentage of disease duration. The disease duration was calculated using date of onset to date of death inclusive. The onset was defined as the earliest date when symptoms that were deemed to be related to CJD were noted by the patient's family or local clinician. It is recognised that a certain amount of imprecision will exist regarding the date of onset of symptoms, but because information about the illness was obtained via direct interview of the families, using standard methodology, this is likely to be as accurate as is possible. Of course, the onset of symptoms may well not reflect the point at which the disease process starts, but there is no way of retrospectively ascertaining this. Likewise, the date of death may not represent the same disease stage in different individuals, as it may be influenced by other intercurrent factors. Regardless of these considerations, the fundamental point is there are no other means of accurately measuring disease duration in this study.

Figure 36 shows timing of positive and negative MRI scans in relation to illness duration, with negative scan occurring at all stages. The shortest period from symptom onset to the appearance of CJD associated MRI signal abnormalities was 10 days (see figure 37).

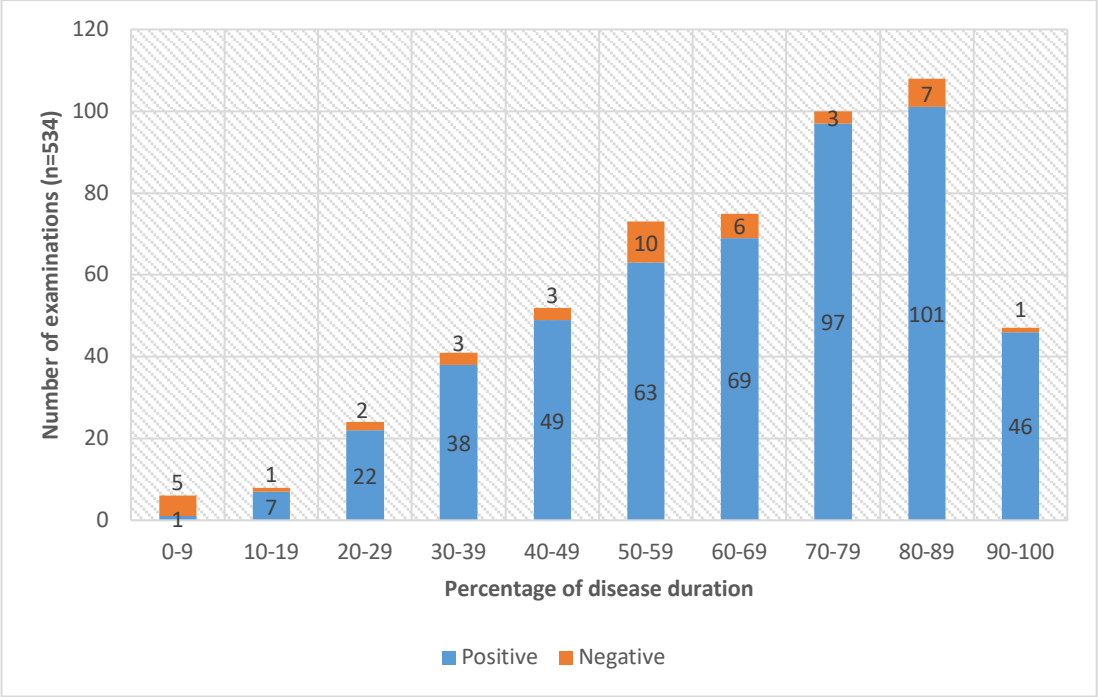


Figure 36: Timing of MR in relation to disease duration (% of total duration) including cases with serial imaging (n=534) with FLAIR and/or DWI

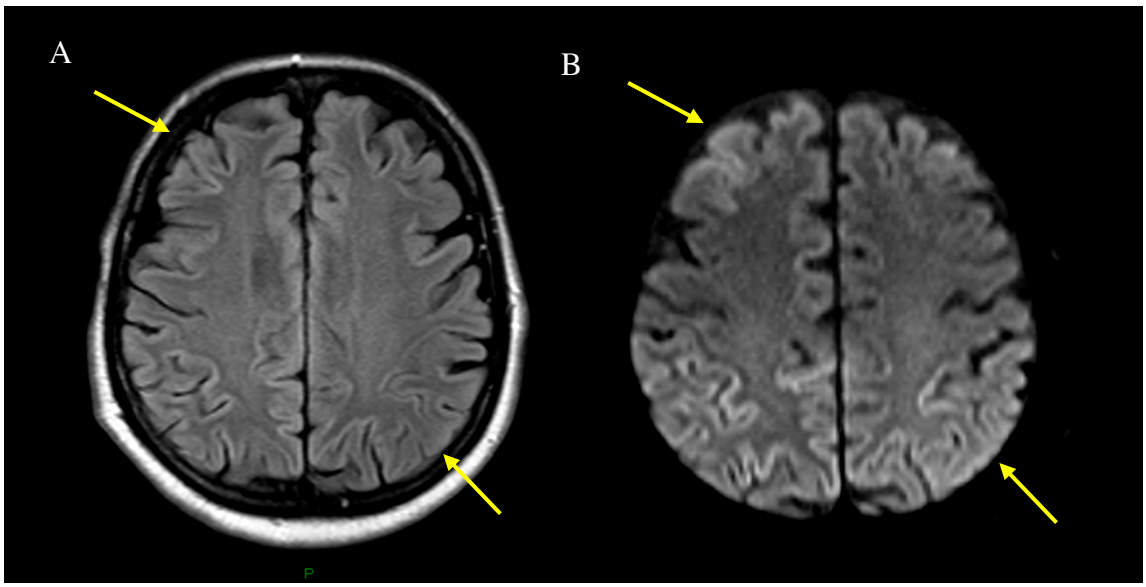


Figure 37: MRI scan 10 days after reported symptom onset with visuospatial disturbance in a 71-year-old with sCJD (MM). Both FLAIR (A) and DWI (B) demonstrate symmetrical cortical high signal of the parietal and frontal regions. There was no clear high signal in the basal ganglia

5.2.5 Timing of FLAIR and DWI sequences in relation to disease duration

There was a statistically significant difference between the median timing of positive and negative imaging (positive = 68%, negative = 56%, p=0.007) when looking at scans on the basis of FLAIR sequences only (n=261). However, no significant difference was observed in the timing of positive and negative imaging on the basis of DWI sequences (n=495) (positive = 68% and negative = 60%, p=0.178).

5.2.6 Timing of signal abnormalities in relation to disease duration

Studies with isolated cortical high signal, including on serial imaging, were statistically more likely to occur earlier on in the disease process (median = 65%) compared to those with basal ganglia signal abnormalities (median = 72%) (p=0.036). This analysis did not take account of rate of disease progression in cases, and it is likely that imaging would be undertaken at different stages of the illness depending on faster or slower rates of progression.

5.3 Discussion

This is the largest review of serial imaging in the diagnosis of sCJD and the results are confirmatory of the findings in smaller case series in the literature (132, 139, 172, 173). The study benefits, as described in previous chapters, from consisting of consecutive and unselected sCJD cases referred to a single specialist centre over the study time period. The findings demonstrate that, in the appropriate clinical circumstances, serial imaging with DWI sequences has a clear role in the diagnosis of sCJD when the initial scans are negative. The majority of these cases went on to develop characteristic CJD signal abnormalities on serial imaging, predominantly identified on DWI sequences.

However, negative imaging occurred at all stages of the disease illness and, when analysing studies on the basis of DWI sequences, there was no statistically significant difference in the timing of MRI (represented as % of illness duration) compared to those with positive imaging. In contrast to this, FLAIR sequences were more likely to be negative in the early stages of the illness. The shortest period from symptom onset to appearance of CJD associated MRI changes was 10 days, which parallels time periods reported in the literature (174) (134).

Only a small percentage of cases had imaging performed in the terminal stages of illness, which may be a reflection of the poor clinical state of the patient, deterring local clinicians from carrying out further investigations including MRI. As well as this, symptoms of agitation and myoclonus, which are often worse in the later stages of illness, may have prevented an MRI from being undertaken or, if one was performed, it may have been too heavily degraded by movement artefact and so not included in this analysis.

Isolated cortical high signal was more likely to occur in the early stages of the illness compared to scans with basal ganglia high signal, which were more often found in the later stages of the disease. These findings are similar to those described in the literature (131, 132, 141, 173, 175, 176).

One definite case that was positive on the second scan, became negative on the third (see table 15). This was a VV2 case with a prolonged disease duration of 17 months and a negative CSF RT-QuIC. The second scan demonstrated extensive cortical high signal on DWI involving most regions as well as high signal of the right caudate head. The third scan showed no clear signal abnormalities. The interval between the second and third scan was 4.5 months. As mentioned in the previous chapter, the disappearance of cortical high signal has been reported in the later stages of the disease in the literature (139, 141, 172), which is thought to be related to the progressive neuronal loss and atrophy with disease progression.

In conclusion, when applied in the appropriate clinical circumstances, serial imaging with the inclusion of DWI sequences, has a clear role in elevating the diagnostic classification of CJD when initial imaging is negative. Furthermore, DWI is shown

to be more sensitive than FLAIR at identifying CJD associated signal abnormalities throughout all the different stages of the illness.

Chapter 6 MRI lesion profiles in sCJD subtypes

6.1 Aim

To determine whether there is variation in the utility of MRI in the diagnosis of sCJD according to codon 129 genotype and prion protein isotype.

6.2 Results

There were 189 definite sCJD cases with detailed MRI (FLAIR, DWI or both) and molecular subtype data available for analysis (excluding cases with mixed subtypes). The patient characteristics and timing of MRI studies are shown in Table 16. 89% of studies had DWI, 83% had FLAIR and 73% had both sequences.

Table 16: Patient characteristics in the six molecular subtypes of sCJD

Disease subtype (n)*	Sex (M:F)	Median age (range)	Median duration (months) (range)	Median Time to 1st MR (months)	Time of MR (% disease duration) (range)
MM1 (111)	40:71	71 (44-88)	2.90 (0.87-25)	1.81 (0.10-8.74)	72% (7-96%)
MM2 (16)	7:6	66 (50-80)	8.90 (0.29-31.87)	6.85 (1.06-12.94)	67% (35-96%)
MV1 (10)	5:5	65.5 (53-75)	5.29 (1.68-19.29)	3.94 (0.87-9.32)	58% (25-87%)
MV2 cortical (20)	10:10	69.5 (54-79)	13.28 (8.03-61.29)	7.16 (3.23-23.45)	52% (20-85%)
MV2 thalamic (0)	-	-	-	-	-
VV1 (3)	2:1	51 (25-76)	17.74 (7.03-17.97)	8.35 (5.06-9.23)	52% (46-72%)
VV2 (32)	15:17	68.5 (52-84)	6.39 (2.19-22.94)	4.60 (0.74-20.39)	70% (5-89%)

6.2.1 Detailed analysis of subtypes

6.2.1.1 MM1

Basal ganglia high signal was frequently observed in the MM1 subtype (70%) (see figure 38) and often associated with widespread cortical high signal (defined as more than 3 cortical regions) (86%) with cingulate, frontal, parietal, temporal and occipital regions being frequently involved. Hippocampal and insular high signal change was less commonly seen compared with MM2 subjects. Thalamic high signal was rare (3%).

6.2.1.2 MM2

Widespread cortical high signal was frequently observed in this subtype (92%), particularly in the temporal, parietal and occipital regions as well as the hippocampus. Basal ganglia involvement was less frequent when compared to MM1 subjects. No thalamic high signal change was observed in any.

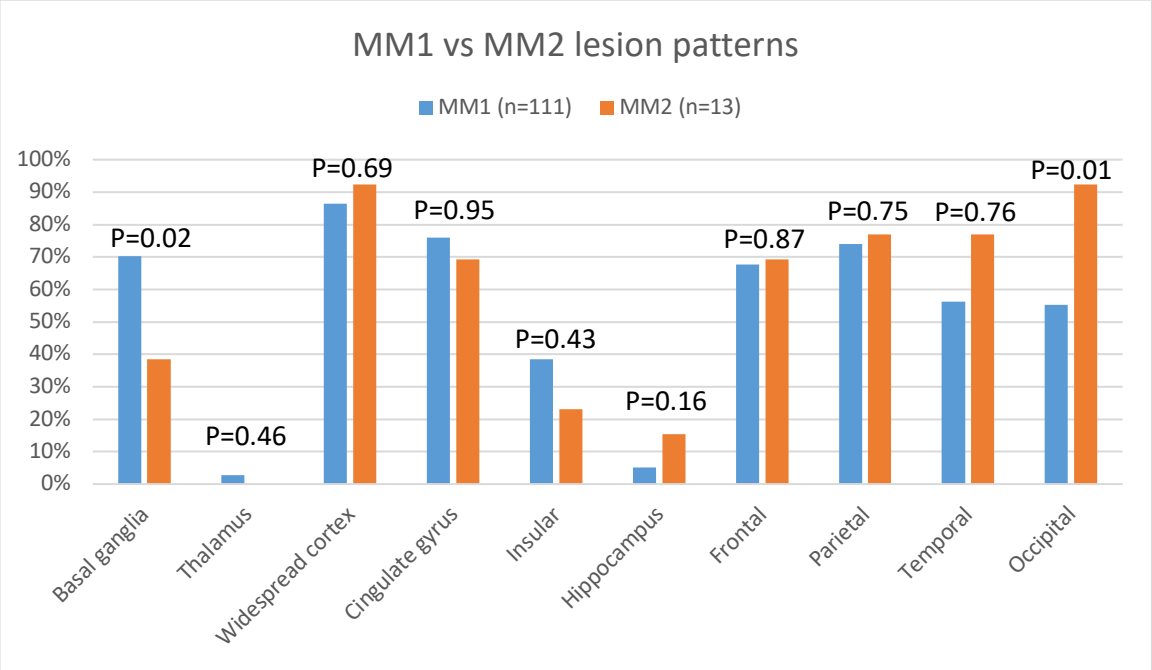


Figure 38: Distribution of MRI signal abnormalities in MM1 and MM2 subtypes

6.2.1.3 MV1

Basal ganglia high signal change was infrequent in the MV1 subtype (20%) (see figure 39), particularly compared to the MV2 group. Widespread cortical high signal was frequently observed (100%), particularly in insular, temporal and occipital regions. No thalamic signal abnormalities were observed in any.

6.2.1.4 MV2

High signal within the basal ganglia (75%) and thalamus (45%) was frequently observed in the MV2 subgroup. Thalamic high signal predominantly affected bilateral dorsomedial (67%) and pulvinar regions (56%). The pulvinar sign was identified in one case with symmetrical basal ganglia signal change and no associated cortical involvement (see previous figure 15). Widespread cortical high signal infrequent and when it was observed it often included the cingulate gyrus and frontal regions.

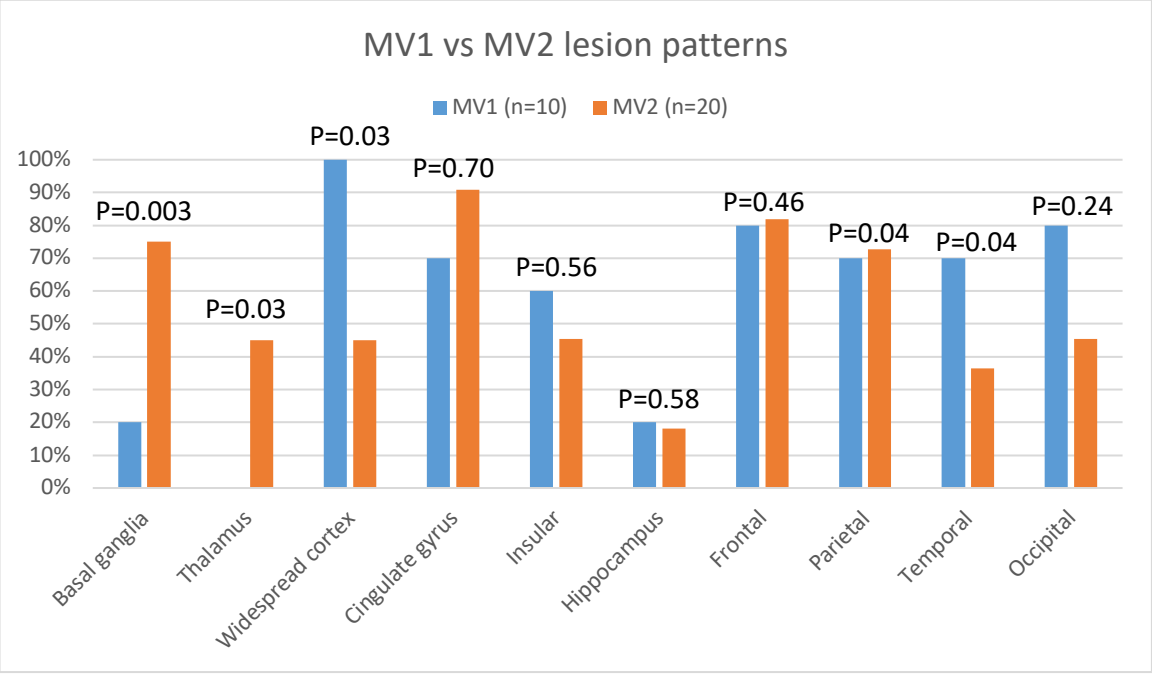


Figure 39: Distribution of MRI signal abnormalities in MV1 and MV2 subtypes

6.2.1.5 VV1

Widespread cortical high signal was often observed in this subtype with basal ganglia high signal being less commonly seen and no thalamic involvement (see figure 40). The cingulate, parietal and temporal regions were frequently involved alongside hippocampal high signal (67%).

6.2.1.6 VV2

Basal ganglia and thalamic signal abnormalities were frequently observed in this subtype (66%) compared to VV1 cases. Thalamic high signal was most frequently seen in the pulvinar region (83%), followed by the mediodorsal (67%) and anterolateral thalami (33%). Cortical high signal was limited to less than 3 regions when compared to other subtypes and was most frequently found in the cingulate gyrus (91%).

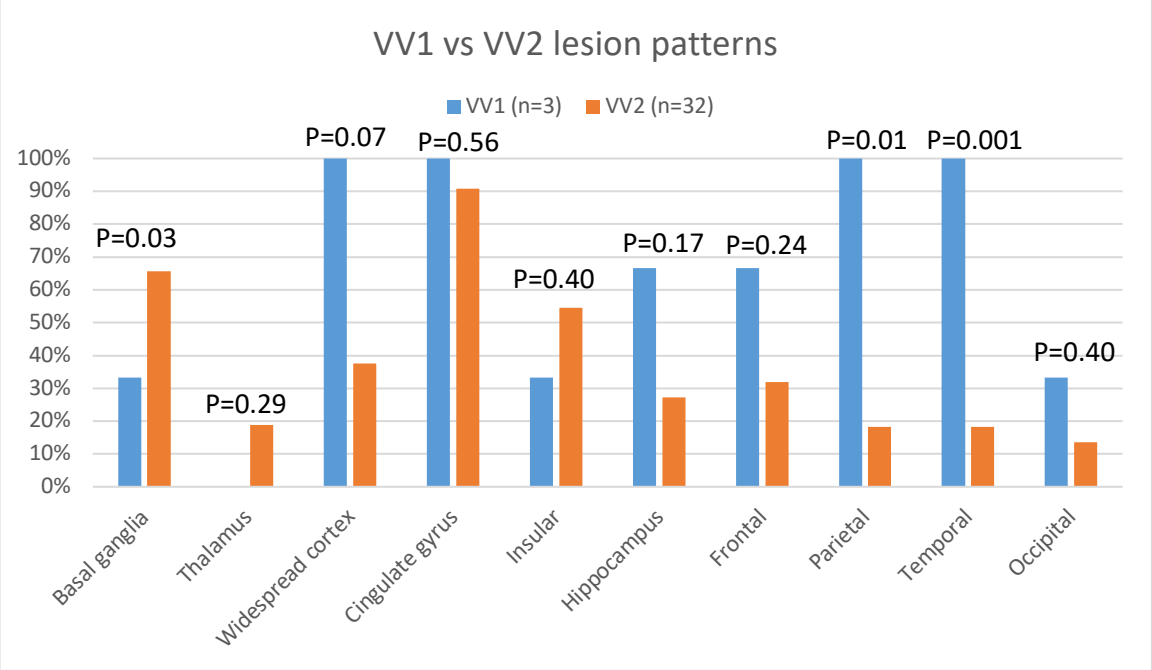


Figure 40: Distribution of MRI signal abnormalities in VV1 and VV2 subtypes

6.2.2 Radiological predictors of sCJD subtype

A univariate analysis was performed to show the increase or decrease in odds of the subtype diagnosis based on the presence of specific regions of signal abnormality on FLAIR and/or DWI. The results are shown in table 17.

Table 17: Radiological predictors of sCJD subtypes (CI – confidence intervals, OR – odds ratio)

	Subtype											
	MM1		**MM2**		**MV1**		**MV2**		**VV1**		**VV2**	
Predictor	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**	**OR (95% CI)**	**P Value**
WIDEPSREAD CORTEX (>3 regions)	2.662 (1.398-5.066)	0.002	5.311 (0.674-41.882)	0.078	0.693 (0.628-0.764)	0.037	0.288 (0.112-0.741)	0.007	0.704 (0.642-0.733)	0.263	0.172 (0.077-0.386)	0.000
Cingulate	1.137 (0.622-2.080)	0.677	1.254 (0.371-4.238)	0.715	1.299 (0.325-5.196)	0.711	0.0509 (0.200-1.293)	0.150	0.640 (0.574-0.713)	0.196	0.899 (0.409-1.975)	0.790
Insular	1.000 (0.541-1.848)	1.000	0.580 (0.154-2.187)	0.416	3.211 (0.872-11.823)	0.066	0.638 (0.221-1.843)	0.403	1.000 (0.089-11.243)	1.000	1.058 (0.474-2.358)	0.891
Hippocampus	0.216 (0.074-0.627)	0.002	1.701 (0.348-8.318)	0.508	2.382 (0.468-12.136)	0.282	0.993 (0.212-4.655)	0.993	19.822 (1.713-230.798)	0.001	2.556 (0.891-7.331)	0.073
Frontal	1.736 (0.968-3.115)	0.063	2.102 (0.624-7.078)	0.222	3.783 (0.781-18.309)	0.078	0.701 (0.276-1.780)	0.454	1.796 (0.160-20.149)	0.630	0.193 (0.079-0.472)	0.000
Parietal	2.691 (1.482-4.886)	0.001	3.043 (0.810-11.435)	0.085	2.063 (0.517-8.234)	0.296	0.419 (0.159-1.104)	0.072	0.532 (0.465-0.609)	0.107	0.086 (0.029-0.257)	0.000
Temporal	1.692 (0.934-3.063)	0.082	4.815 (1.280-18.109)	0.011	3.236 (0.810-12.922)	0.081	0.292 (0.094-0.909)	0.026	0.425 (0.359-0.502)	0.046	0.145 (0.048-0.432)	0.000
Occipital	1.544 (0.855-2.789)	0.149	18.171 (2.311-142.888	0.000	5.676 (1.172-27.494)	0.016	0.398 (0.138-1.145)	0.079	0.648 (0.058-7.274)	0.723	0.102 (0.030-0.349)	0.000
BASAL GANGLIA	2.174 (1.169-4.044)	0.013	0.379 (0.122-1.182)	0.085	0.105 (0.022-0.512)	0.001	1.487 (0.514-4.298)	0.462	0.232 (0.021-2.613)	0.078	0.8922 (0.400-1.991)	0.074
THALAMUS	0.0117 (0.0033-0.419)	0.000	–-	–-	0.899 (0.856-0.945)	0.292	14.545 (4.805-44.033)	0.000	0.903 (0.862-0.947)	0.471	2.788 (0.961-8.090)	0.051
Pulvinar	0.125 (0.027-0.587)	0.02	–-	–-	0.933 (0.897-0.970)	0.933	7.714 (2.180-27.296)	0.000	0.935 (0.901-0.971)	0.649	3.968 (1.173-13.422)	0.018

6.2.3 Timing of MRI in relation to disease duration

6.2.3.1 MM1

A total of 18/111 cases had serial imaging: 15 with 2 scans and 3 with 3. In this section, a 'positive' scan is one that had abnormalities characteristic for sCJD and a 'negative' is one without such abnormalities. 5 cases with initial negative MR examinations became positive (high signal of basal ganglia +/- cortex or >2 regions of cortex) on subsequent imaging. Two of these cases lacked DWI on initial imaging but this sequence was performed on subsequent examinations. Figure 41 demonstrates all positive and negative MR examinations, including serial scans, in relation to disease duration as defined by the presence or absence of basal ganglia signal change +/- >2 areas of cortical ribboning respectively.

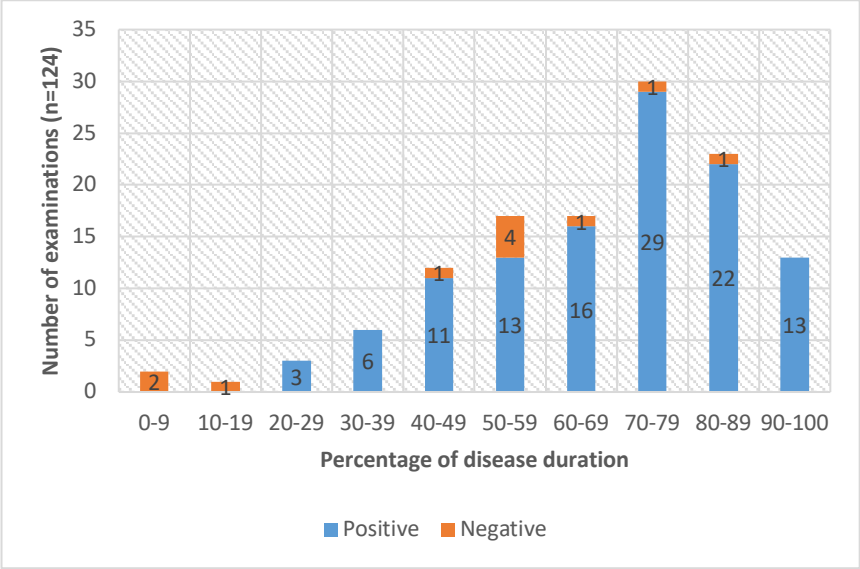


Figure 41: Timing of MR in relation to disease duration in MM1 subtype

6.2.3.2 MM2

3 cases had serial imaging; 2 with 2 images and 1 with 3. All cases were positive on initial and subsequent imaging. One case had multiple areas of cortical high signal on initial imaging with subsequent imaging demonstrating basal ganglia involvement

alongside the cortical high signal. Figure 42 demonstrates the timing of positive MR examinations in relation to disease duration.

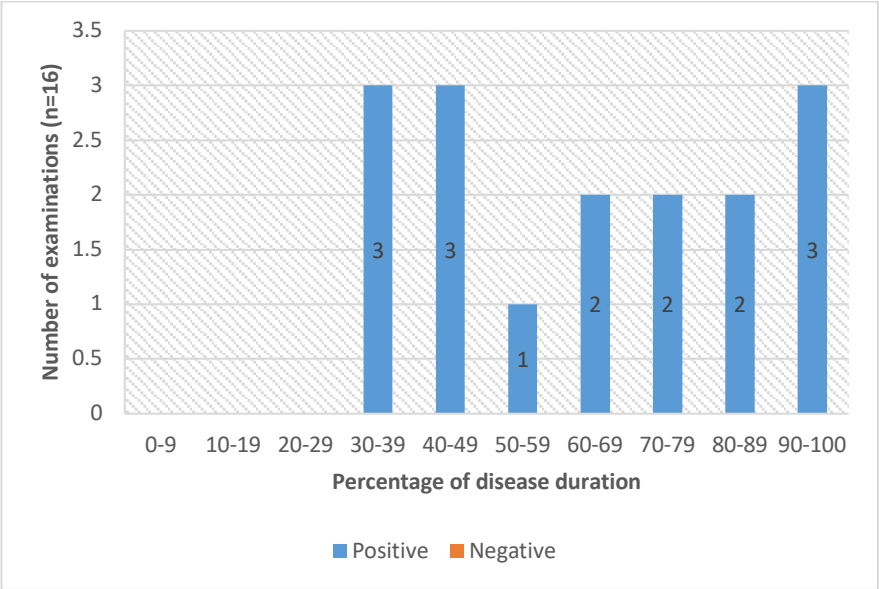


Figure 42: Timing of MR in relation to disease duration in MM2 subtype

6.2.3.3 MV1

2 case had serial imaging; one with 2 and 1 with 3 images. Both cases had cortical high signal in isolation on initial imaging with subsequent imaging demonstrating basal ganglia involvement in both (see figure 43).

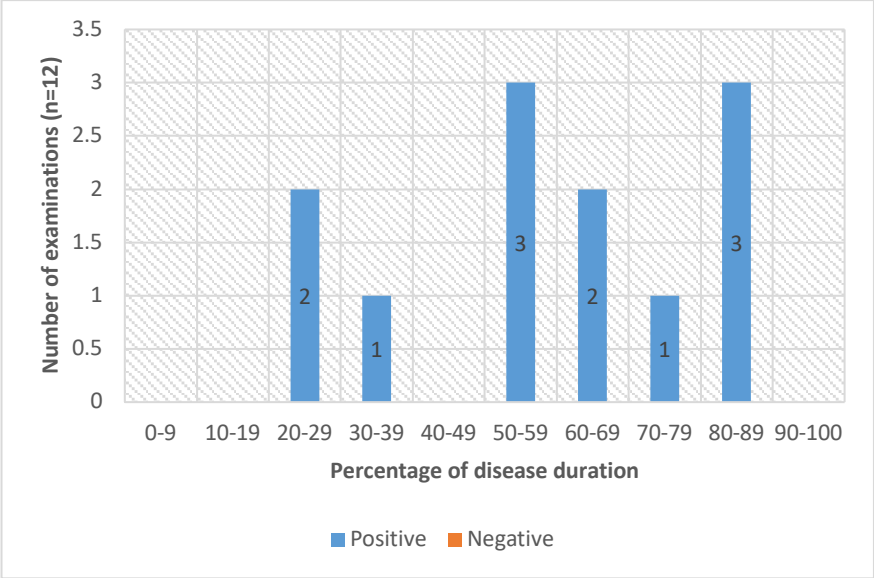


Figure 43: Timing of MR in relation to disease duration in MV1 subtype (1 case lacked date of MRI scan)

6.2.3.4 MV2

6 cases had serial imaging; 4 with 2 and 2 with 3 images. 2 of these cases that were negative initially became positive on subsequent imaging (see figure 44)

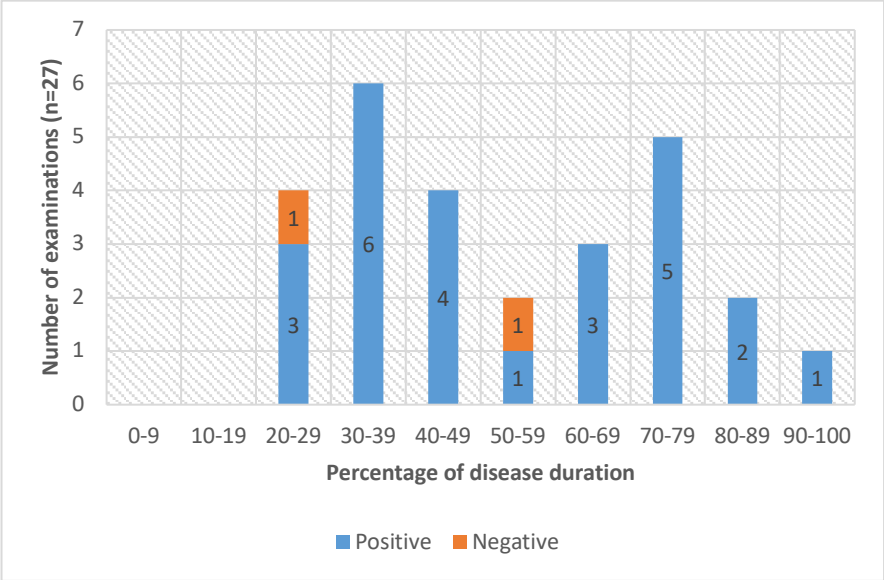


Figure 44: Timing of MR in relation to disease duration in MV2 subtype

6.2.3.5 VV1

2 of the VV1 subtype had serial images; 1 with 2 and 1 with 3. The case with 3 images was negative on initial scan, positive on the 2nd and then negative on the third (see figure 45).

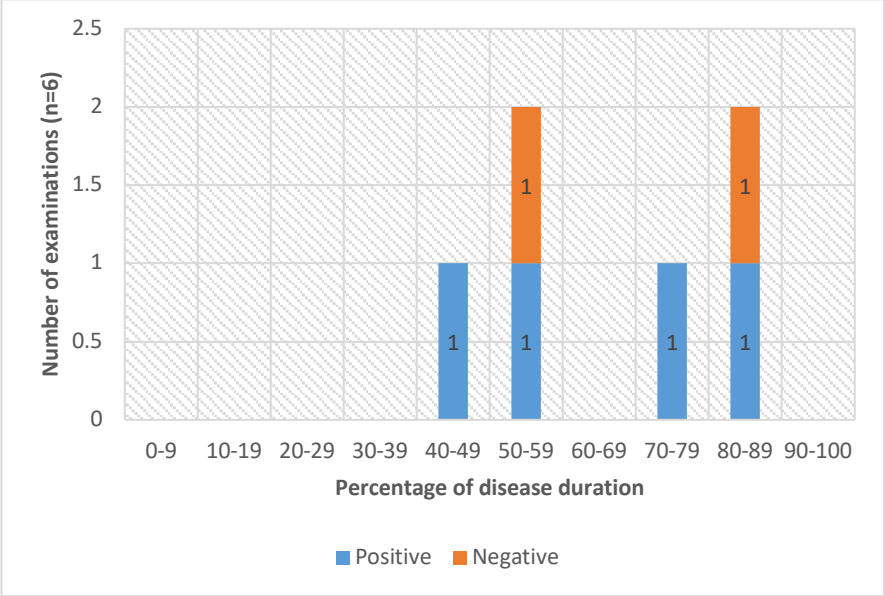


Figure 45: Timing of MR in relation to disease duration in VV1 subtype

6.2.3.6 VV2

7 had serial imaging; 5 with 2 and 2 with 3 images. 4 images were positive on initial imaging and remained positive with subsequent imaging. 3 cases that were negative initially became positive on repeat imaging (see figure 46)

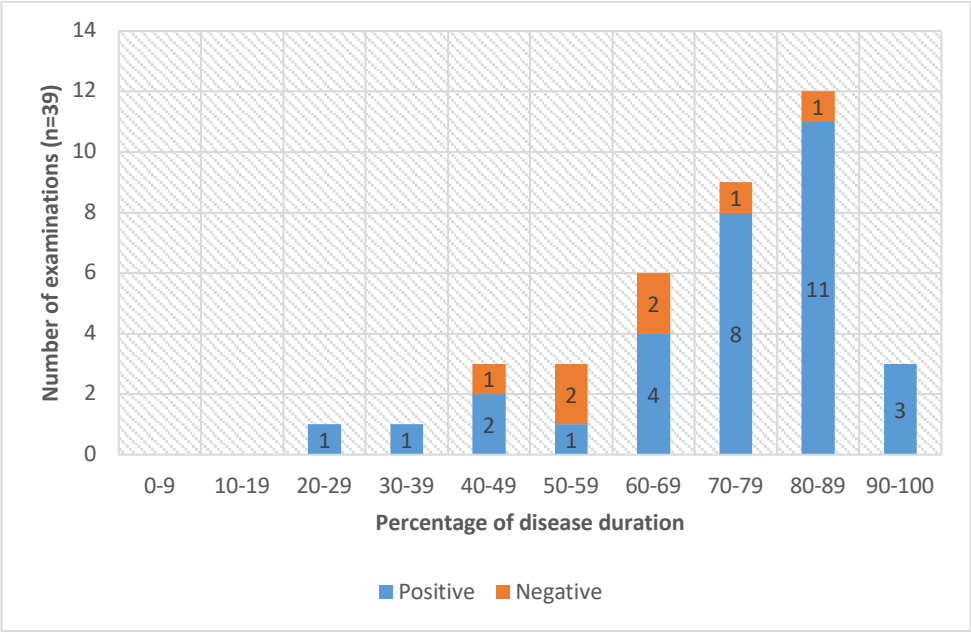


Figure 46: Timing of MR in relation to disease duration in VV2 subtype (2 cases lacked date of MR examination)

6.2.4 Comparative sensitivities of MRI and RT-QuIC for sCJD by subtypes

221 of 260 definite sCJD cases had codon 129 genotyping data available for analysis, including those reviewed by the NCJDSU Neuroradiologist (n=189) and those by the research registrar (n=32). Table 18 details the comparative sensitivities of initial MRI and CSF RT-QuIC in disease subtype.

Table 18: Comparative sensitivities of MRI and CSF RT-QuIC for the diagnosis of sCJD by subtypes

Disease subtype	Basal ganglia high signal	Basal ganglia or >2 cortical regions *	Positive RT-QuIC
MM1	76/127 (60%)	100/127 (79%)	28/39 (72%)
MM2-cortical	7/16 (44%)	16/16 (100%)	6/9 (67%)
MM2-thalamic	-	-	-
MV1	3/11 (27%)	11/11 (100%)	4/5 (80%)
MV2	16/23 (70%)	20/23 (87%)	12/14 (85%)
VV1	3/5 (60%)	4/5 (80%)	1/4 (25%)
VV2	24/39 (62%)	28/39 (72%)	18/20 (90%)

(*parietal, occipital or temporal regions)

6.3 Discussion

This chapter describes the MRI appearances in relation to the six molecular subtypes of sCJD in a large series of cases and confirms the findings of similar studies in the literature (129, 133). The largest of these studies was carried out some years ago by Meissner et al, as an international collaboration, and reported MRI lesion profiles for the different subtypes similar to those found here (133). This was not, however, a systematic study based on contiguous cases and there were multiple radiologists involved in reporting the MRI findings. Furthermore, the number of cases with DWI sequences was relatively low in the study (54%). Given that this is the most sensitive MRI sequence for detecting high signal in both cortical and sub-cortical regions, it

may have led to under-recognition of these signal abnormalities within the study. This chapter expands on the previous literature by analysing MRI appearances in consecutive CJD cases within a single centre over a set period of time. MRIs were reviewed by one specialist neuroradiologist with extensive CJD experience, therefore avoiding inter-observer variability. There was also a high percentage of cases with DWI sequences in this particular chapter (83%) which improves the detection of cortical and subcortical regions of high signal, thereby more accurately depicting signal profiles amongst the molecular subtypes.

In this chapter's analysis, basal ganglia signal abnormalities were a frequent finding across the subtypes, except in VV1 and MV1. Cortical signal changes were a consistent finding, but the extent and regions affected varied between the subtypes, as did thalamic and hippocampal signal changes. The MM1 and MM2-cortical subtypes demonstrated a degree of overlap in the pattern of signal abnormalities, although basal ganglia involvement was more likely to occur in the MM1 group overall.

The MM1 and MV1 subtypes and are traditionally grouped together due to their overlapping histopathological phenotypes (20). In this analysis, MM1 and MV1 subgroups had different MRI lesion profiles, with basal ganglia signal abnormalities being more frequently observed in the MM1 group. The reason for this observation is not clear. It may reflect that the signal abnormalities are influenced by more than the underlying molecular subtype and that other potential genetic factors have an affect on the MRI lesion profile. The observed differences in MRI findings may also be related to differences in disease duration between these subtypes and thus the point at which the MRI was performed.

In agreement with previous literature, both MV2 and VV2 subtypes showed predominant basal ganglia and thalamic signal abnormalities with less frequent cortical involvement, distinguishing these subtypes from the others (133). The VV1 subtype has been previously described as demonstrating isolated widespread cortical involvement with less frequent sub-cortical changes. This analysis found similar results, with all VV1 cases demonstrating widespread cortical signal changes and an

absence of thalamic involvement. This subgroup was also more likely to have hippocampal involvement compared with the others.

The MV2 group were more likely to have thalamic involvement compared to any of the other subtypes and were the only subtype to demonstrate a positive pulvinar sign. This, together with relatively limited cortical involvement, make this subtype more likely to be mistaken for variant CJD on the basis of MRI, as mentioned in previous case reports (177-179). This unique signal profile should therefore be taken into consideration in the diagnosis of sCJD, given its main differential diagnosis includes that of vCJD. Indeed, Kaski et al reported a 'possible' case of MV vCJD with a pulvinar sign on FLAIR (180). The DWI in this case displayed basal ganglia and thalamic involvement as well as cortical high signal involving bilateral cingulate, frontal, parietal and occipital regions, similar to that observed in MV2 cases in this analysis. This is discussed further in chapter 9.

MRI scans with no CJD associated signal abnormalities (i.e. negative imaging) occurred at varying points of the clinical illness in all subtypes apart from MV1 and MM2-cortical groups. Aside from in the MM1 subtype, imaging was rarely undertaken early on in the clinical illness, which may be a reflection of the more atypical phenotypes (longer disease duration and younger age at onset) encountered amongst these other subtypes leading to a delay in undertaking investigations like MRI. Serial imaging was useful in some of the subtypes, with initial negative scans becoming positive later on in the clinical process.

The sensitivity of MRI for sCJD subtype, based on the criteria of the presence of basal ganglia high signal, varied amongst the different subtypes, with MM2-cortical and MV1 groups having relatively low sensitivities since basal ganglia high signal was uncommon in these two subtypes. The current international diagnostic criteria for MRI, which allows for basal ganglia high signal and/or more than two regions of cortical high signal, improves recognition of these rarer subtypes resulting in a higher sensitivity of MRI for sCJD overall.

Indeed, the sensitivity of MRI was superior to that of CSF RT-QuIC for all subtypes apart from in the VV2 group. MRI had an overall sensitivity of 81% compared to

76% with CSF RT-QuIC. However, this comparison was limited by the number of cases with CSF RT-QuIC results. In a large, combined retrospective and prospective study of sCJD CSF samples, CSF RT-QuIC had an overall sensitivity of 94% for sCJD (123). This study also demonstrated that the sensitivity of CSF RT-QuIC was dependent on sCJD subtype, being lower for rarer subtypes such as VV1 and MM2-cortical (75% and 78%, respectively) (123), similar to the findings here.

In summary, basal ganglia and cortical signal abnormalities were the most frequent MRI findings in sCJD and together were most characteristic of the MM1 subtype, albeit with a degree of overlap with the MM2-cortical subtype. VV1 and MV1 were less likely to have basal ganglia signal changes, and MV2 and VV2 subtypes were typified by predominant sub-cortical involvement. Overall, MRI brain had a high sensitivity across the subtypes ranging from 72% in the VV2 subtype to 100% in the MM2-cortical and MV1 subtypes.

Chapter 7 Local reporting of MRI

7.1 Aim

The aim of this chapter was to assess the accuracy of local reporting of the MRI findings in CJD and whether this changed with time.

7.2 Methods

Local (clinical diagnostic service) MRI reports from cases of CJD (both definite and probable) were reviewed for inclusion of comments on the presence/absences of abnormalities associated with CJD and compared to those observed by the Neuroradiologist at the NCJDRSU. The types of signal abnormalities accepted as 'CJD associated abnormalities' included those observed within the basal ganglia (caudate, putamen or both), the thalamus (dorsomedial, anterolateral and pulvinar) and those involving the cortex.

7.3 Results

7.3.1 Basic demographics

Data from 485 MRI scans from 412 cases of sCJD (226 definite and 179 probable) were compared. 189 males, 223 females. Median age 68 (range 25-87). Median disease duration 4.21 months (range 0.87-62.03).

7.3.2 Reported signal abnormalities

The NCJDRSU found characteristic MRI signal abnormalities in 445 of 485 scans (overall sensitivity of 92%). CJD associated abnormalities were not found in 40 cases, 24 of whom lacked DWI sequences. The local MRI reports described CJD associated abnormalities in 215 of 485 examinations (overall sensitivity of 44%). Inter-observer agreement between local reporters as a group and the NCJDRSU neuroradiologist was poor with 43.7% concordance (kappa 0.121, p=0.000) (see figure 47).

Two scans judged by the NCJDRSU to have no CJD associated changes had been reported as having cortical high signal by the local centre. In both of these cases, the cortical high signal was identified in the frontal regions by local reporting and interpreted as ischaemia/stroke in both. The NCJDSU interpreted the frontal high signal as likely artefact. One of these cases had a positive RT-QuIC and the other had serial imaging which had CJD-associated signal abnormalities identified by the NCJDRSU.

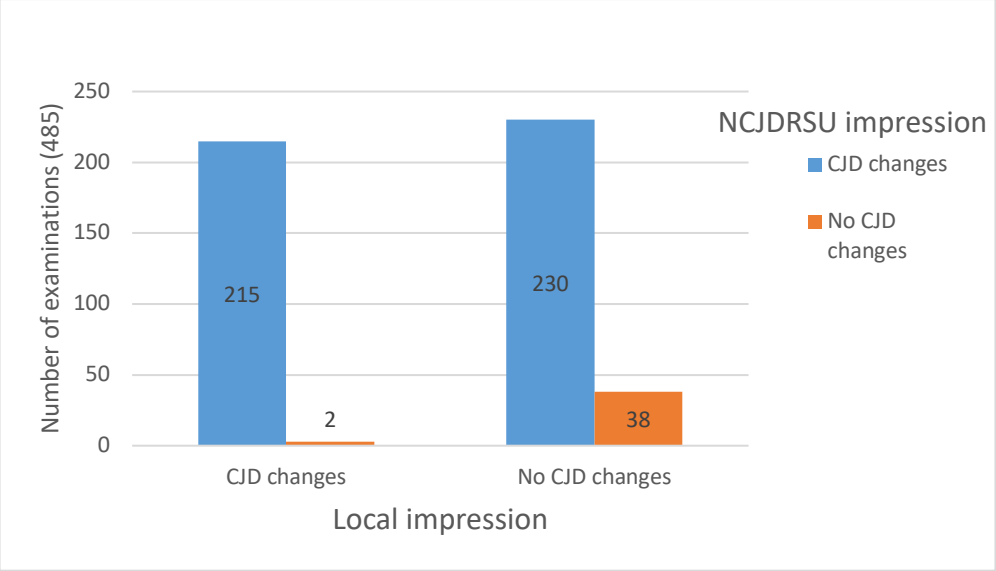


Figure 47: Accuracy of local reporting of MRI associated findings in CJD compared to NCJRSU review

CJD associated abnormalities were frequently observed in the cortex (84.3%) followed by the basal ganglia (74%). The thalamus was less often involved (12.57%) (see figure 48). The most common region of high signal not documented on local reporting was the cortex (observed in 22%), although there were statistically significant discrepancies in all affected areas.

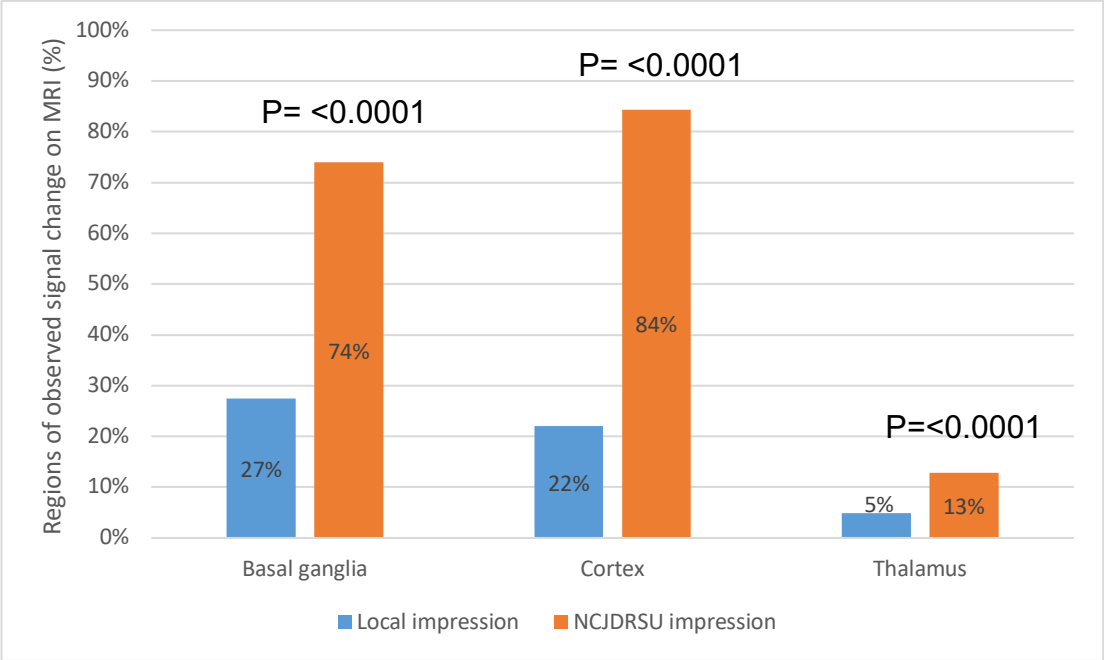


Figure 48: Comparison of observed signal changes on MRI examinations between local reporting and NCJDRSU

7.3.3 Local reporting diagnosis

Of the 215 examinations reported locally as demonstrating CJD associated changes, 162 (75%) were said to be in keeping with CJD. Of these, 52 were said to be sporadic CJD, 6 variant CJD (4 of whom had thalamic high signal, one of which with a positive pulvinar sign and 2 with cortical changes only) and the others (104) unspecified CJD. The remaining 53 examinations mentioned alternative diagnoses

for the observed MRI changes, predominately: stroke (n=32), autoimmune/infective/toxic encephalitis (n=10), posterior reversible encephalopathy syndrome (PRES) (n=3), malignancy (n=2), Wernicke's encephalopathy (n=2), artefactual (n=2), seizure (n=1) and 'unknown' (n=1).

7.3.4 Accuracy and interpretation of local reporting over time

The accuracy of local reporting, both in the identification of signal abnormalities (sensitivity) and interpretation/impression of these findings (specificity) varied over the 5-year study period. There was no significant overall change observed (see figure 49).

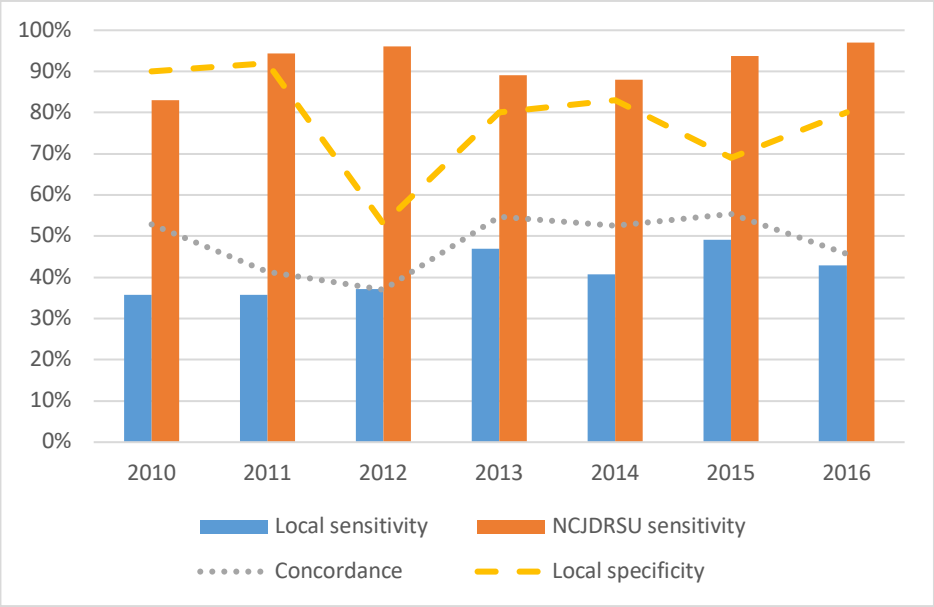


Figure 49: Accuracy of local reporting (sensitivity and specificity) compared to that of the NCJDRSU over the 5-year study period

7.4 Discussion

This is the largest review of local MRI reporting in sCJD cases and expands on similar studies (181-183) by assessing not only the accuracy of identification of signal abnormalities, but also their interpretation and how this has varied over time.

This study shows that the MRI findings associated with CJD are often not identified by local centres. Signal abnormalities occurred in 92% of MRI scans overall, commonly involving the basal ganglia and cortex, but local centres reported upon these findings in less than half of cases. Cortical abnormalities were the most common region not to be documented on local reporting, followed by thalamic and basal ganglia signal abnormalities. The accuracy of local reporting of MRI in CJD varied over the study time period but overall, there was no significant change.

There are a number of reasons to explain why the MRI findings in CJD are often overlooked by local centres. Firstly, the abnormalities can be subtle, particularly those limited to the cortex, and so can easily be overlooked. Furthermore, CJD may have not been considered as a potential diagnosis at the time of arranging the MRI scan and so was not included in the MRI request details to guide the radiologist towards these changes. The local radiologist would therefore be required to consider a broader range of differentials as opposed to the specialist radiologist at NCJDRSU, who would have a higher level of consideration for CJD abnormalities. It is also likely that some MRIs were reviewed at local radiology meetings where signal abnormalities were identified, but no addendum of these findings added to the original report.

Lastly, MRI studies with isolated cortical signal abnormalities may have been observed but attributed to artefact and so not mentioned within the report. Indeed, there were a few incidences where the report mentioned cortical high signal but said that these changes were due to artefact. Of note, relatively high signal intensities within the cingulate, frontal and temporal cortices, are frequently seen in normal individuals and many radiologists will interpret abnormalities in these regions with caution. This is probably further compounded by the MRI being degraded by motion artefact, a common occurrence in patients with CJD due to confusion and/or myoclonus, with any perceived signal abnormalities being attributed to this. In these circumstances, it is more useful to interpret the DWI sequences, which is acquired in a relatively short period of time compared to other sequences, and so is less prone to image degradation from movement of the patient. As well as this, cortical high signal

which is asymmetric and/or involvement of the basal ganglia, can add further support to signal changes being genuine rather than artefactual.

When MRI signal abnormalities were recognised, the majority of reports (75%) mentioned CJD as a potential cause for these findings followed by alternative conditions including stroke, encephalitides and PRES. These conditions can be associated with MRI signal abnormalities resembling CJD, but the clinical features are often distinct. It is unlikely however, that the local radiologist reporting the scan has the full clinical details to hand. CJD is a rare disease, and it is likely that many radiologists have not encountered the condition and so will be inexperienced with the characteristic MRI changes. Conditions like stroke on the other hand, are more common and also occur in a similar age demographic to CJD. Nonetheless, it is important to note that the signal abnormalities identified on DWI in CJD do not conform to specific vascular territories, like that observed in stroke (168) and this may be one way to distinguish these conditions on the basis of radiological appearance.

In conclusion, signal abnormalities characteristic of sCJD are present in the majority of cases but are often not identified in clinical practice. This observation highlights the importance for clinicians, both radiologists and neurologists, to familiarise themselves with the pattern of signal abnormalities typical of CJD in order to make an accurate diagnosis. Therefore, in situations where CJD is suspected clinically, even when the MRI has been reported as normal, it is recommenced the imaging is reviewed at a local radiology meeting or by an experienced Neuroradiologist such as that within the NCJDRSU. This may ultimately lead to an earlier diagnosis, which can be helpful in guiding the patient's management as well as avoiding repeating tests unnecessary.

Chapter 8 Diffusion-weighted MRI findings and clinical presentation

8.1 Aim

The aim of this chapter was to evaluate for any potential relationship between diffusion-weighted MRI appearances and the mode of clinical presentation in sCJD.

8.2 Methods

For the purpose of this analysis, the mode of clinical presentation was classified as either 'Typical' (a rapidly progressive encephalopathy with cognitive decline as a prominent feature, along with other neurological features) or 'Atypical' (a mixed group, characterised by features as further detailed below (also see Appendix 3).

The 'Atypical' group is characterised by one of several characteristics: an isolated progressive neurological deficit (eg cerebellar ataxia); a relatively slowly progressive cognitive impairment without other initial neurological features; a psychiatric presentation; a sudden-onset, stroke-like presentation; some miscellaneous less common presentations (such as alien limb syndrome) (see figure 50).

It should be emphasised that 'Typical' cases may well have cerebellar, visual or other abnormalities either initially or very shortly after onset; they are generally characterised by a rapidly progressive encephalopathic illness that may have a number of features. The 'Atypical' forms that are characterised as 'Cerebellar' or 'Visual' are different in that they present with purely cerebellar or visual features, respectively, as progressive isolated abnormalities (though progress on to a more global picture in time).

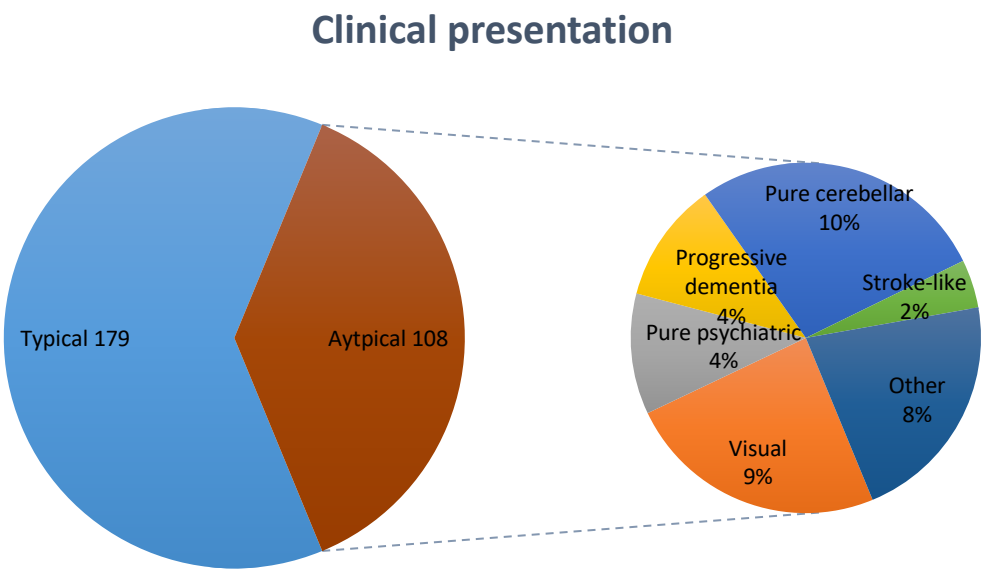


Figure 50: Distribution of mode of clinical presentation in sporadic CJD. 'Typical' presentation defined as those with a rapid cognitive decline at onset (n=179) against 'Atypical' presentations (n=108): pure visual, pure psychiatric, pure cerebellar, progressive dementia, stroke-like and 'other' (consisting of a variety of presentations including movement disorder, sensory disturbance, isolated language impairment, sleep disorder and alien limb syndrome).

Cases with DWI were used for analysis, given that this sequence is recognised as the most sensitive for detecting signal abnormalities in CJD. The MRI scans were classified according to appearance as follows:

- Basal ganglia signal abnormalities and the extent of these (none/unilateral/bilateral)
- Cortex signal abnormalities and the extent of these (which cortical regions, number of cortical regions and whether asymmetry present (defined as >2 cortical regions on either left or right hemisphere when compared to contralateral side).

A basal ganglia index score was created to reflect the extent of observed basal ganglia signal abnormalities as follows: 1=no basal ganglia involvement, 2= asymmetric involvement (either right or left caudate head +/- putamen), 3= symmetrical involvement (bilateral caudate head +/- putamen). A two-way analysis

of variance (ANOVA) was conducted to look for an association between the above MRI variables and mode of clinical presentation, followed by a post hoc t-test analysis. Values are presented as mean ± SEM.

8.3 Results

There were 303 cases of sCJD (56% definite) with mode of clinical presentation and DWI sequences available for analysis. 131 male: 172 female. High signal in the basal ganglia had a statistically significant association with mode of clinical presentation (F=4.33, p-value = 0.0382) (see table 19). No significant associations were observed for degree or asymmetry of cortical signal abnormalities on mode of clinical presentation.

Table 19: Two-way ANOVA

Source	Sum of squares	Degrees of Freedom	Mean Square	F	Significance
Basal ganglia	1.6904	1	1.69042	7.21	0.0076
Cortex	0.1281	1	0.12813	0.55	0.4603
Error	70.3263	300	0.23442	-	-
Total	73.602	303	-	-	-

8.3.1 Association of basal ganglia high signal on clinical presentation

Post hoc t-test analysis indicated the mean degree of basal ganglia signal abnormalities, using the index scoring system above, was significantly different between the 'Typical' presentation group (M=2.93, SEM = 0.0652) and the

'Atypical' group (M=2.63, SEM= 0.07) (t-value = 2.15, p-value = 0.03) (see figure 51). The results indicate that those with a 'typical' clinical presentation tended to have more symmetrical basal ganglia signal abnormalities, whereas those with an 'atypical' presentation were more likely to have limited, asymmetric basal ganglia involvement.

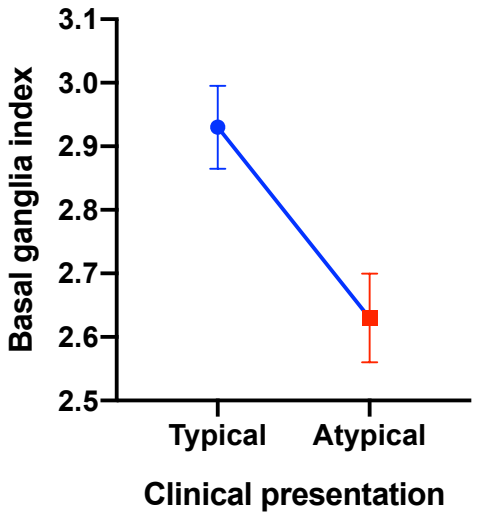


Figure 51: Basal ganglia index score (indicating extent of basal ganglia signal abnormalities) between 'Typical' and 'Atypical' clinical presentations in sCJD

8.4 Discussion

The majority of cases of sCJD follow an overall rather uniform clinical course, typically characterised by a rapidly progressive cognitive syndrome, ataxia, some other neurological features, and myoclonus, terminating in akinetic mutism. However, there is considerable variability in the clinical presentation of sCJD, which can make the diagnosis challenging in the initial stages. The main variations well described in the literature are cases that present with isolated neurological deficits and those with an overall atypical phenotype. Of those with isolated deficits at onset,

the two most defined profiles are those with a pure cerebellar ataxia (known as the "Brownell-Oppenheimer" variant) (81) and those with pure visual disturbance, typically progressing to cortical blindness (known as the "Heidenhain" variant) (82). In total, six clinical phenotypes are defined for the purposes of this study (see appendix 3). Both the clinical phenotypic and pathological heterogeneity in sCJD largely correlate with variations in the *PRNP* codon 129 genotype and the PrP^{sc} protein type deposited within the brain, as used in the Parchi molecular classification system (20).

The aim of this study was to determine whether the clinical presentation of sCJD correlated with patterns of signal abnormality on MRI. Previous studies have examined correlations with signal abnormalities and clinical findings in sCJD, but none have systemically examined for a correlation with specific clinical presentations (184-186). In 2004, Meissner et al analysed 219 patients with sCJD to determine whether clinical features, prion protein codon 129 and molecular subtype correlated with MRI basal ganglia signal abnormalities (129). They reported that those with basal ganglia signal abnormalities had a shorter illness duration and were more likely to have cognitive impairment at an earlier stage of the disease. The study was limited by a number of factors, however. Firstly, the identification of basal ganglia signal abnormalities was made on T2-weighted sequences only, as an insufficient number of scans had the more sensitive sequences of FLAIR or DWI. As a result, the study did not include the assessment of cortical high signal. Furthermore, their data on clinical features were collected retrospectively by reviewing the medical notes of identified cases. This has recognised limitations in that symptoms and signs may not be recorded accurately in the medical notes, if at all, leading to inconsistencies in the quality of this data. Gao et al found similar results in their study, in that CJD patients with basal ganglia signal abnormalities on DWI had a shorter disease duration and higher incidence of myoclonus (92 versus 44%) than those without basal ganglia involvement (184).

Other studies have looked at particular clinical presentations with an assessment of investigations, including that of MRI, but these were small numbered and were not systematic studies assessing the MRI findings against a range of clearly defined

clinical presentations. For instance, Bairardi et al analysed 18 patients with sCJD that had isolated visual symptoms at onset (the Heidenhain variant) and found that the majority of these cases had cortical high signal affecting the occipital regions on MRI (187). There have also been several case reports that have correlated MRI abnormalities with clinico-pathological findings. Geschwind et al reported a case of sCJD where clinical findings of severe aphasia and asymmetric, focal neurological deficit of the right limbs, correlated with a predominance of DWI signal abnormalities of the left cortex and basal ganglia (171). The neuropathological changes in this case were quantitatively worse in the left hemisphere.

In comparison to the above studies, the analysis within this chapter of the thesis examines specific patterns of signal abnormalities identified on DWI sequences, comparing these to the mode of clinical presentation in a large and unselected sCJD cohort. This study has produced novel results, indicating through the use of a unique basal ganglia scoring index an association between 'Typical' and 'Atypical' presenting features and the extent of basal ganglia signal abnormalities on DWI sequences. Patients with a 'typical' clinical presentation, with rapid cognitive decline at onset, were statistically more likely to have more extensive and symmetrical basal ganglia signal abnormalities, at the time of MRI, compared to those presenting with 'atypical' clinical features. This latter group tended to have more limited, asymmetric basal ganglia signal abnormalities at the time the MRI was performed.

The study benefits from consistent and continuous data collection due to the national referral system to the NCJDRSU, where attempts are made to review every suspected case of CJD in person by a clinician from this unit. Where possible the next of kin is interviewed in order to obtain a detailed history of the clinical presentation and supplement other reviewed sources including medical and GP records. Despite these measures, there are general limitations to note in this study. Firstly, as with many neurodegenerative disorders, there is often difficulty with the identification of the presence or absence of clinical features and the accurate time of illness onset in a condition, especially one that affects cognition. The reliability of accurate reporting of symptoms may be variable and the data provided by relatives prone to recall bias, with the potential for over- or under- reporting a clinical feature. Another

consideration relates to the definition of the clinical presentation. As mentioned above, the presenting symptoms have been classified using specified criteria. For instance, to fulfil the criteria for the Cerebellar/Brownell-Oppenheimer variant, as it is defined, a case needs to present with isolated cerebellar ataxia for a period of two weeks or more, without any other significant clinical features within this time period. The strict application of defined criteria might lead to under-ascertainment of certain clinical presentations in this study.

In conclusion, the results of this study suggest a correlation between the extent of basal ganglia signal abnormalities and the clinical presentation type in sCJD. Those with a 'typical' clinical presentation were more likely to have the characteristic CJD-associated MRI findings of symmetrical basal ganglia high signal. Whereas the 'atypical' presenting group were more likely to have limited, asymmetric basal ganglia signal abnormalities. Knowledge of this relationship may aid the diagnosis of sCJD, with the particular consideration that 'atypical' clinical presentations tend to have less characteristic MRI appearances.

Chapter 9 Diffusion-Weighted Imaging in variant Creutzfeldt–Jakob disease

9.1 Aim

The aim of this chapter was to investigate the image appearances and sensitivity of DWI in the radiological diagnosis of vCJD including its role in identifying cases of MV vCJD.

9.2 Methods

Using the NCJDRSU database, MR images from patients with both probable and definite vCJD with DWI sequences were identified for analysis. If serial MRIs were available, the first examination was used for analysis. Where studies were incomplete or had not been retained, copies of MR images were requested from the original referring hospital.

The scans were reviewed by the NCJDRSU Neuroradiologist, experienced in the assessment of MRI in CJD, for the presence or absence of features associated with these diseases. The Neuroradiologist was aware of the suspicion of CJD but was blinded to the specific diagnosis (i.e. sporadic, variant etc). The images were also reviewed within a larger mixed cohort of CJD studies including cases of sCJD.

As the majority of probable cases of vCJD were classified as such on the presence of a supportive MR examination (positive pulvinar sign), it was decided to analyse these cases separately to those confirmed neuropathologically so as not to confound results.

9.3 Results

9.3.1 Basic demographics

There have been 178 cases of definite or probable variant CJD identified in the United Kingdom to date (123 definite and 55 probable cases who did not undergo post-mortem). MR imaging was performed in 176 cases, of which 27 were identified as having DWI sequences. Two cases were excluded from analysis as their DWI sequences were of too poor image quality. Of the 25 remaining, 15 were neuropathologically confirmed and 10 classified as probable. The latter group were predefined on the basis of an appropriate clinical history together with positive MR imaging or positive tonsil biopsy or a combination of both (table 20).

Table 20: Clinical characteristics of probable vCJD cases including supportive diagnostic investigation

Case no	Age	Disease duration (months)	Positive Pulvinar sign	Positive tonsil biopsy
1	25	13	Yes	N/A
2	33	15	Yes	N/A
3	34	11	Yes	N/A
4	29	12	Yes	N/A
5	23	13	No	Yes
6	20	6	Yes	N/A
7	47	45	No	Yes
8	39	16	Yes	Yes
9	22	22	Yes	N/A
10	46	12	Yes	N/A

The date of MRI scans ranged between November 1999 and August 2015. The majority of images were Digital Imaging and Communications in Medicine (DICOM) data, with 8 images on plain film. A total of 34 MR examinations were available from the 25 cases; 7 with serial images (6 had 2 images and 1 had 4). The

median disease duration was 13 months (range 6 - 45) with median age of disease onset 33 (range 15 - 74).

The majority of images were of sufficient diagnostic quality; with minimal motion artefact. Of the 35 studies, 29 were graded as good or average and 4 as sufficient. One case was graded insufficient based on a combination of movement artefact and lack of FLAIR sequences.

9.3.2 Probable cases of vCJD

9.3.2.1 MRI signal abnormalities

Of the 10 probable cases of vCJD, 8 were positive for the pulvinar sign on FLAIR images and 2 were negative (figure 52). The latter two cases had positive tonsil biopsies. In comparison to FLAIR, only 5 cases had pulvinar signs identified on DWI (all of which were positive on FLAIR). There were no cases where the DWI was positive when the corresponding FLAIR was negative. 6 cases demonstrated basal ganglia hyperintensity on FLAIR compared to 8 on DWI. Cortical high signal was only identified in 3 cases and only on DWI.

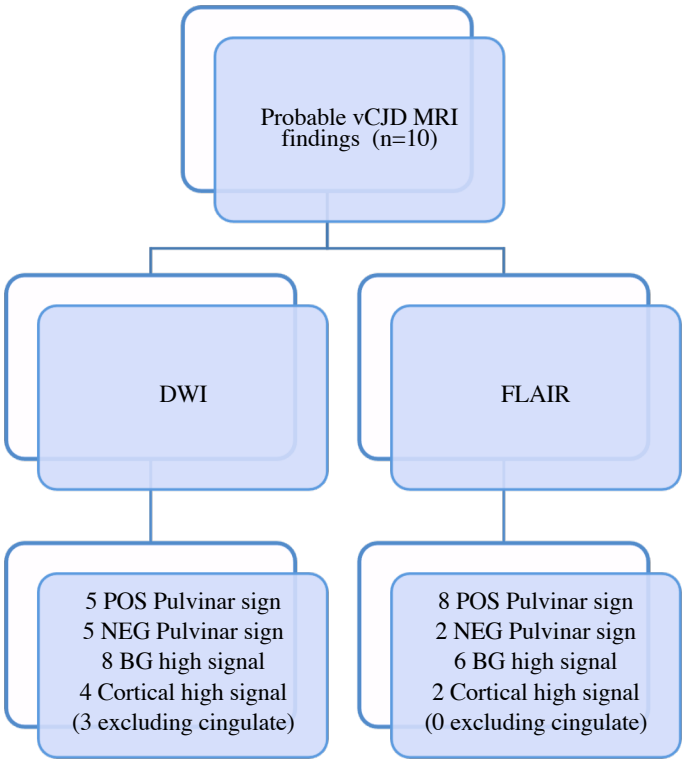


Figure 52: MRI abnormalities observed in probable cases of vCJD

9.3.2.2 MRI diagnosis

On FLAIR, 8 scans were classified radiologically as variant CJD, based on the presence of the pulvinar sign, and 2 as sporadic CJD due to basal ganglia hyperintensity and absent pulvinar signs (figure 53). In the DWI, 5 scans were classified as vCJD due to positive pulvinar signs, all of which were FLAIR positive.

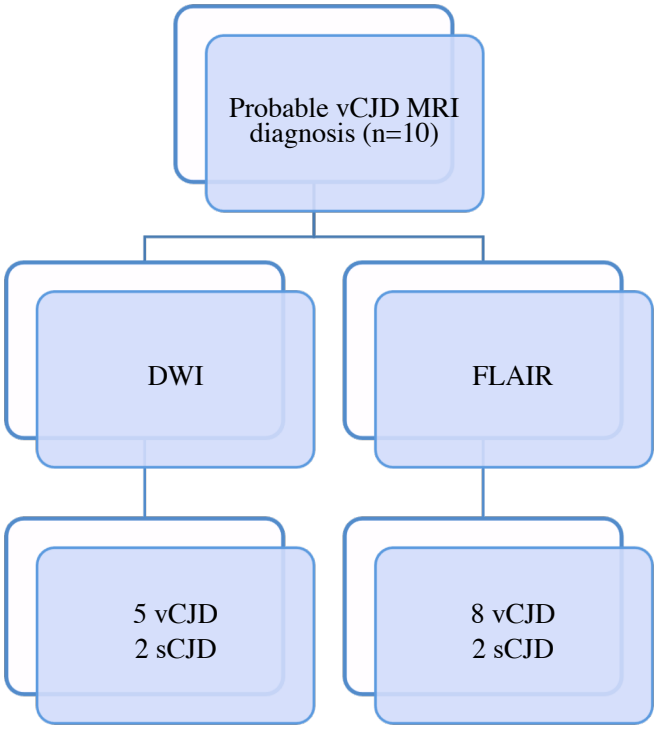


Figure 53: Radiological diagnosis in probable cases of vCJD

9.3.3 Definite cases of vCJD

9.3.3.1 MRI signal abnormalities

Of the 15 neuropathologically confirmed cases of vCJD, 12 had a combination of both FLAIR and DWI and 3 had DWI alone (figure 54). Of the 12 cases, 8 were positive for the presence of the pulvinar sign on FLAIR and 4 were negative. In the corresponding DWI sequences, 4 were positive and 8 were negative (see figure 55). There were no incidences where the DWI was positive when the FLAIR was negative.

Basal ganglia high signal was present in 8 cases on FLAIR compared to 10 on DWI. Cortical ribboning was not evident on any of the FLAIR sequences but was present in 3 on DWI. Of the cases with only DWI available (n=3), all had basal ganglia and thalamic high signal, one had associated cortical ribboning, and all were negative for the pulvinar sign.

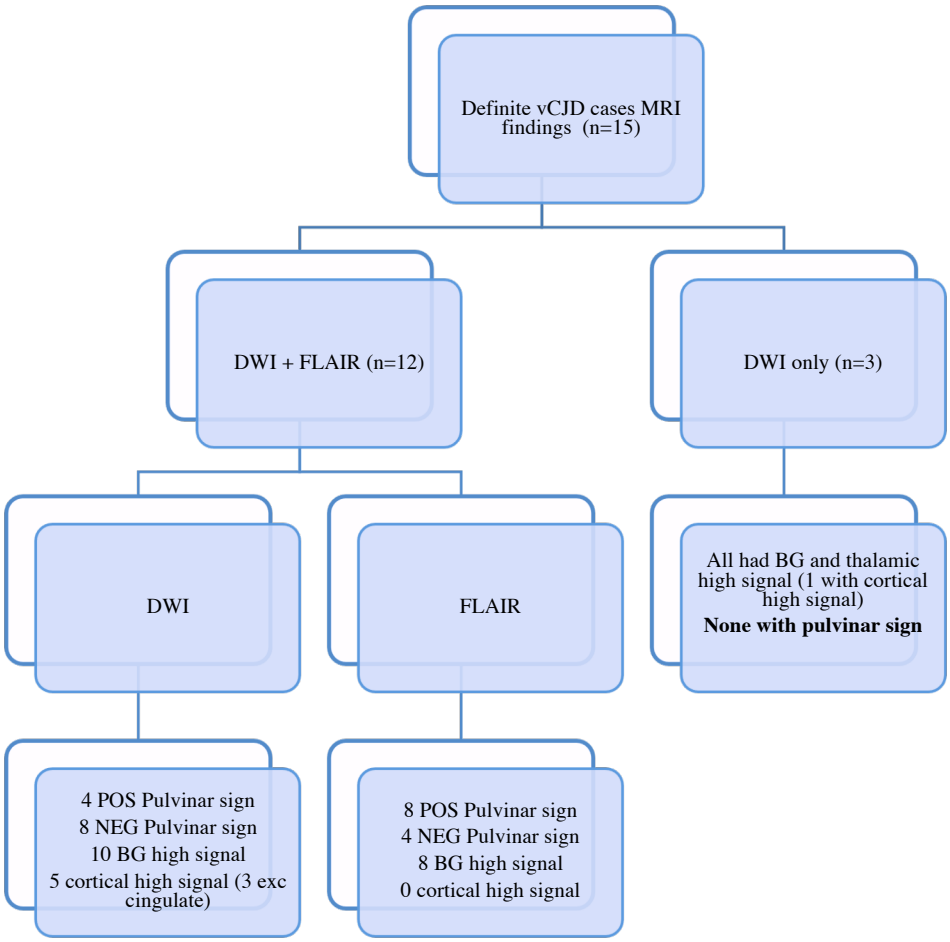


Figure 54: MRI abnormalities observed in the definite cases of vCJD

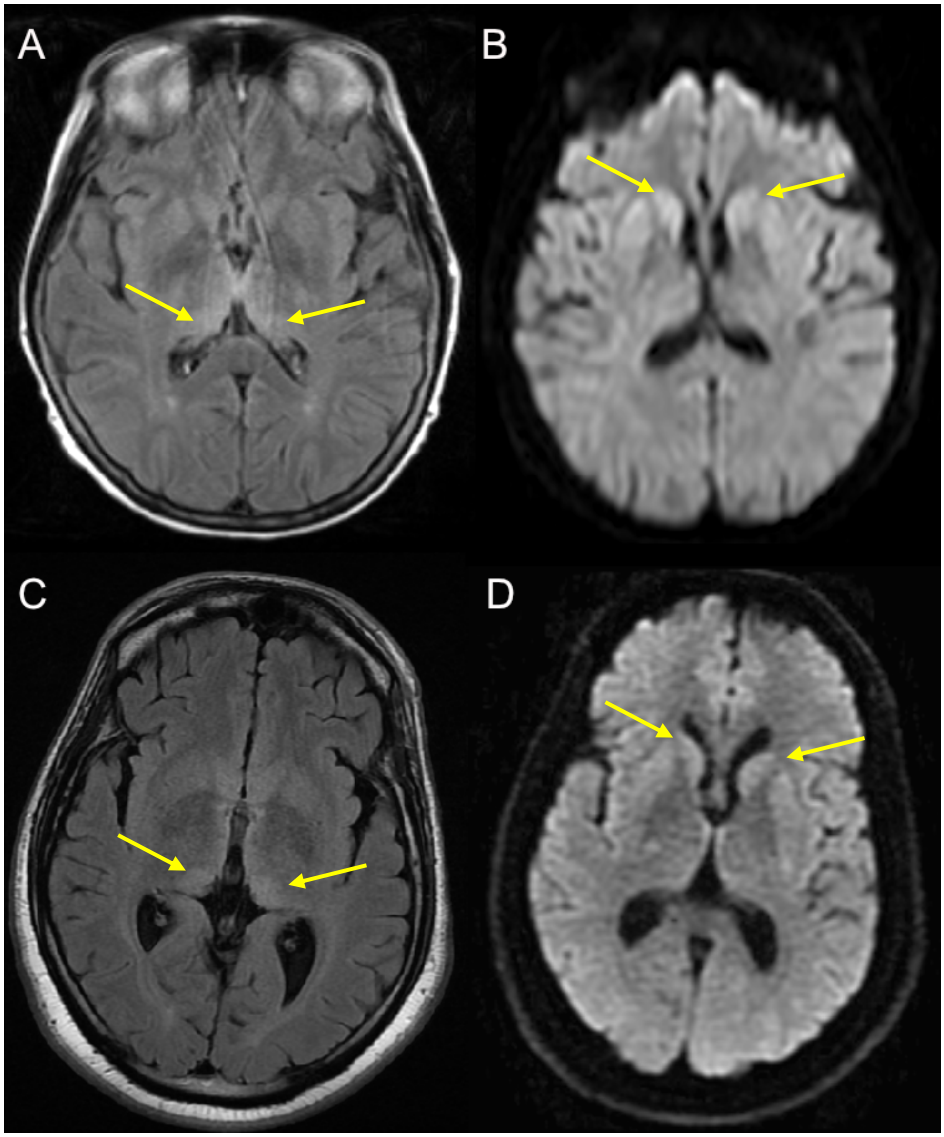


Figure 55: MRI findings in two neuropathologically confirmed cases of vCJD (A+B) and (C+D): Axial FLAIR images in both cases (A and C) demonstrating positive pulvinar signs with the corresponding DWI (B and D respectively) showing more conspicuous high signal in the basal ganglia in the absence of clear pulvinar signal abnormality

9.3.3.2 MRI diagnosis

In the 12 cases with FLAIR and DWI, 7 scans were classified radiologically as vCJD on FLAIR, 4 as sCJD and 1 non-suggestive (figure 56). On the corresponding DWI, 4 had appearances of vCJD, 4 sCJD (see figure 57) and one non-suggestive. Of the 3 with DWI only, 2 were classified as CJD of uncertain subtype and one suggestive but not diagnostic.

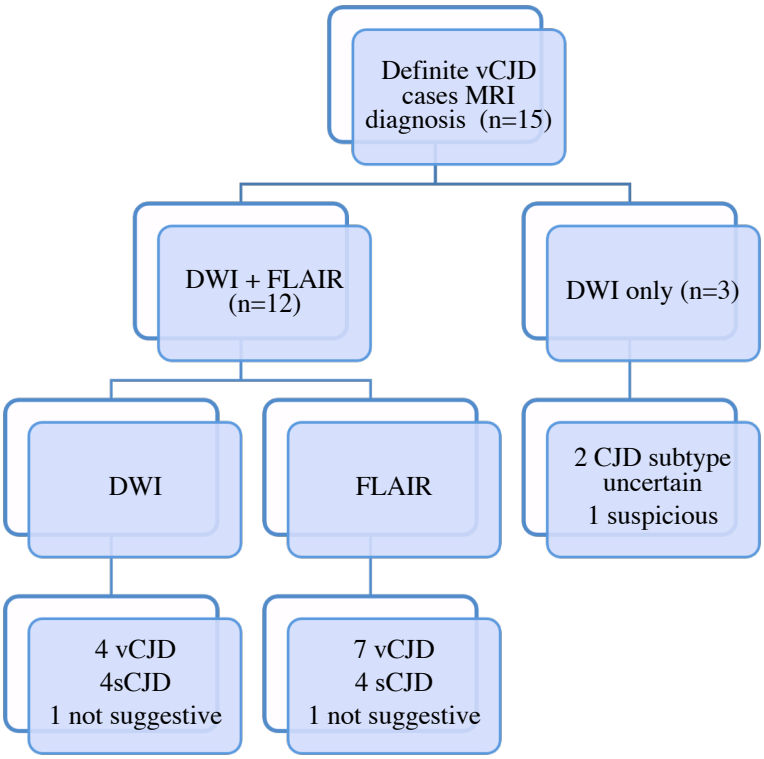


Figure 56: Radiological diagnosis in definite cases of vCJD

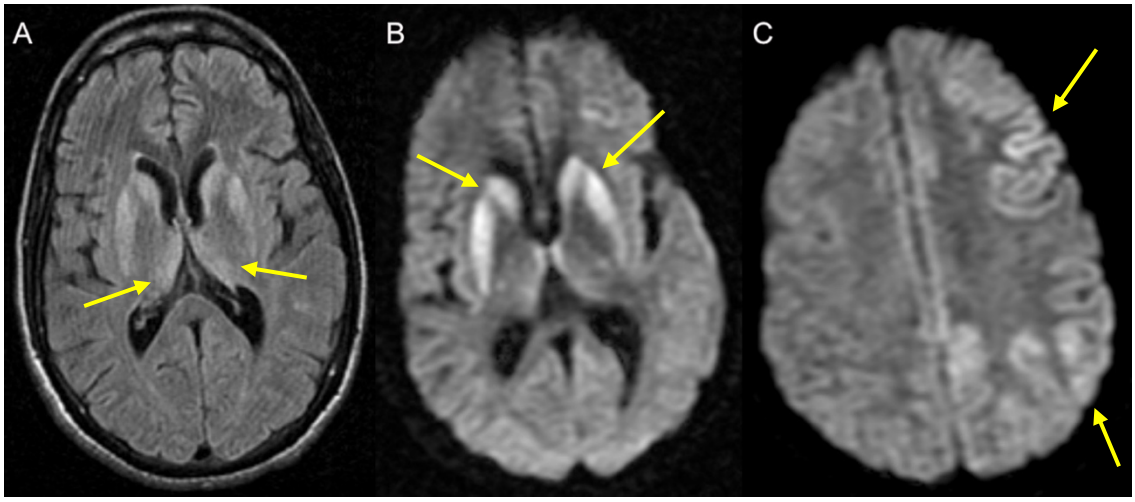


Figure 57: MRI in a confirmed case of vCJD with appearances more characteristic of sporadic CJD (A) Axial FLAIR showing mediodorsal and pulvinar high signal (arrows) of equal intensity to basal ganglia. (B) DWI showing more conspicuous high signal in basal ganglia (arrows) with less marked dorsomedial thalamic abnormalities with (C) associated left parietal and frontal cortical hyperintensity (arrows)

9.3.4 Cases with multiple MRI scans

9.3.4.1 Probable cases

A total of 4 probable cases had serial imaging; 3 with 2 images and one with 4. Two of the 4 probable cases were positive for the pulvinar sign on initial images (based on FLAIR, with one having corresponding pulvinar sign on DWI). The second examinations from these cases had identical appearance. The 2 other cases with serial scans were both negative for the pulvinar sign on initial imaging. One of these cases had a positive pulvinar sign detected on subsequent imaging (FLAIR only). The other case had 4 serial examinations that were all negative for the pulvinar sign.

9.3.4.2 Definite cases

There were 3 neuropathologically confirmed cases with two MRI scans. Two of these were positive for the pulvinar sign on FLAIR images (one having a corresponding pulvinar sign on DWI) on both the initial and subsequent MR examinations. Of these 2 with pulvinar signs, one was classified as variant CJD based on image appearance alone and the other as sporadic; due to the presence of cortical ribboning on DWI (bilateral cingulate and insular). The latter case changed classification on subsequent MR examination to variant CJD, due to a positive pulvinar sign in the absence of clear cortical ribboning.

The remaining scan that was negative for the pulvinar sign was classified as non-suggestive of CJD on initial and subsequent examination. This was a blood transfusion related case and the initial scan showed bilateral cingulate high signal, with the subsequent scan showing bilateral cingulate and unliteral insular high signal.

9.3.5 Diagnostic utility of FLAIR and DWI sequences in vCJD

When combing definite and probable cases of vCJD, the pulvinar sign was identified on 16 (72.7%) of FLAIR sequences and 9 (36%) of DWI sequences.

9.3.6 Cortical hyperintensity on DWI

Of the 10 probable cases, 4 were observed to have cortical hyperintensities (3 when excluding the cingulate gyrus). The median areas of cortex involved was 2, ranging between 1 - 4. In decreasing frequency of those areas involved; parietal 29% (n=2), cingulate 29% (n=2), frontal 14% (n=1), insular 14% (n=1) and occipital (14%) n=1.

Of the 15 definite cases, 6 were observed to have cortical hyperintensities (3 when excluding cingulate gyrus). The median areas of cortex involved was 2, again ranging between 1 - 4. In decreasing frequency; Cingulate 56% (n=5), insular 22% (n=2) frontal 11% (n=1) and parietal 11% (n=1).

9.3.7 Timing of MR in relation to disease duration

Negative MR imaging occurred at most stages in the disease process on both FLAIR and DWI (see figure 58-59).

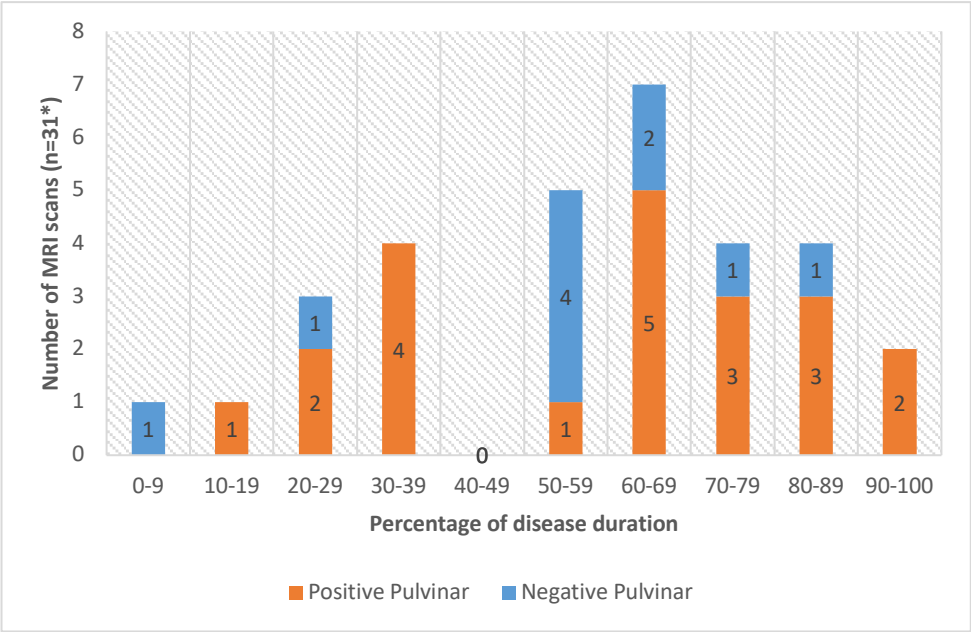


Figure 58: Graph showing timing of FLAIR imaging in relation to disease duration in both probable and definite cases (*including serial scans)

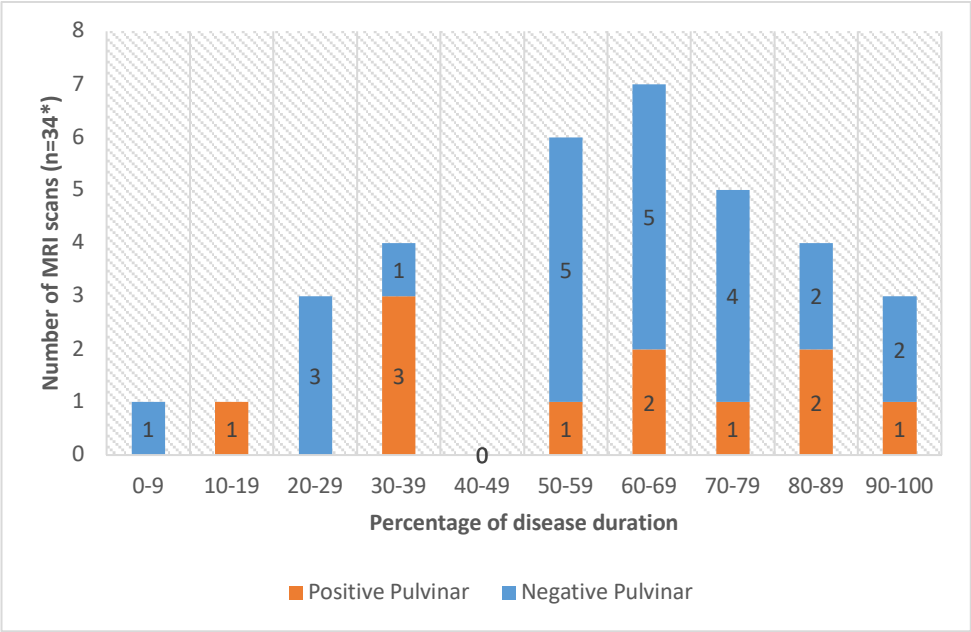


Figure 59: Graph showing timing of DWI in relation to disease duration in both probable and definite cases (including serial scans)

9.4 Discussion

To our knowledge, this is the largest study to date evaluating DWI appearances in vCJD. Prior to this analysis, it was expected that DWI would be more useful than FLAIR imaging in vCJD, but this appears not to be the case. Instead, pulvinar hyperintensity was often less conspicuous on DWI than on FLAIR images. Conversely, basal ganglia and cortical hyperintensities were more conspicuous on DWI, leading to appearances associated more frequently with sCJD. Overall, DWI appears to be a less useful sequence compared to FLAIR in identifying the pulvinar sign (sensitivities of 36% and 72.7% respectively). Furthermore, cortical hyperintensity was an infrequent finding in this study and, when present, was limited to only a few cortical areas. This differs to the widespread cortical involvement often observed in sCJD.

The neuropathologically confirmed heterozygous case of vCJD was reported to have an MRI more compatible with sCJD. Based on this study, the DWI appearances were not too dissimilar to that observed in the homozygous cases; with marked basal ganglia hyperintensities and minimal cortical involvement in the absence of clear pulvinar hyperintensity.

As mentioned previously in this thesis, Kaski et al reported a suspected diagnosis of vCJD in an MV genotype case in 2009 who died without an autopsy (180). The MRI in this case demonstrated the pulvinar sign on FLAIR imaging but not on DWI. However, in contrast to the neuropathologically confirmed MV case and other MM cases, the DWI displayed widespread cortical hyperintensity (bilateral frontal, cingulate, parietal and occipital) together with basal ganglia and thalamic involvement (see figure 60).

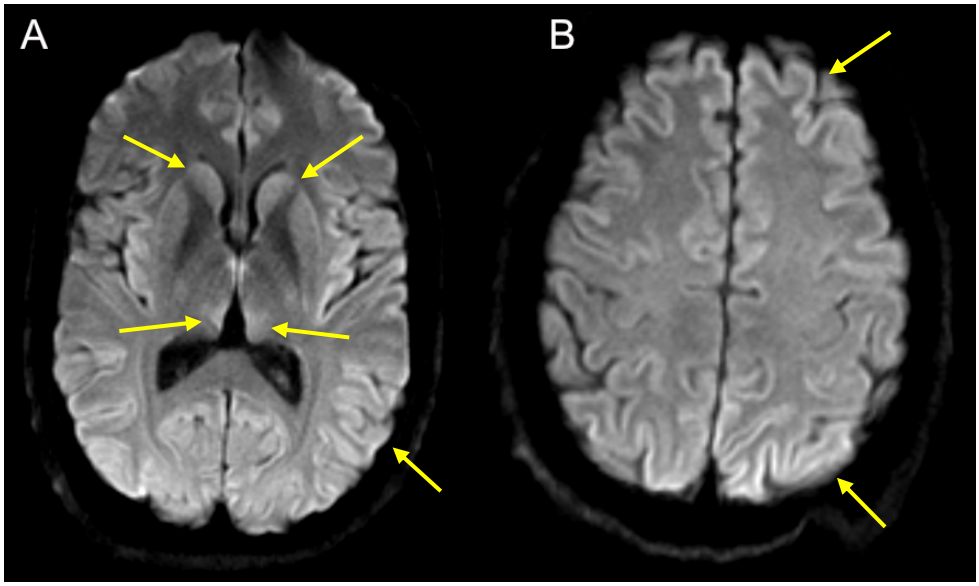


Figure 60: Axial DWI sequences in 'possible' MV vCJD case showing (A) basal ganglia and pulvinar high signal (arrows) with associated widespread cortical ribboning (A and B)

It is clear that there is substantial overlap in the appearances of grey matter structures on MRI in different forms of CJD, particularly so in sCJD. Meissner et al assessed MRI signal profiles in sCJD based on protein subtype. In their study, MV1 cases often showed cerebral cortex and basal ganglia involvement. In the MV2 cases, the basal ganglia and thalamus were characteristically affected, with the thalamic high signal observed frequently within the pulvinar area and the pulvinar sign being present in three patients (133).

Of course, in clinical practice a diagnosis of CJD is not based on the MRI brain findings in isolation. Rather, the MRI requires to be interpreted alongside the appropriate clinical context such as age of onset, clinical features and disease duration. Iatrogenic CJD can often be distinguished based on previous exposure (e.g. human pituitary-derived growth hormone/human dura mater grafts) and familial CJD on the basis of genetic analysis. The difficulty arises when clinical features overlap, particularly so in sporadic and variant CJD. It was originally felt that in these situations MRI would be a reliable means of distinguishing between variant and sporadic CJD, but it appears not to be the final arbitrator in all cases. Although CSF

RT-QuIC has emerged as a highly sensitive and specific test for sCJD, the assay in its current form does not amplify the PrP^{Sc} in vCJD (188). CSF protein misfolding cyclic amplification (PMCA) on the other hand, appears to be a promising ante-mortem test for vCJD and may differentiate patients with heterozygous vCJD from those with sCJD.

This study is open to a number of criticisms. Similar to previous chapters, the MRI scans in this study were performed under different conditions in different scanners across the country, which resulted in reduced comparability of the scans. It is also limited by the small number of cases with DWI as well as being generally limited by the quality of DWI sequences during the study time period. Of the cases that had DWI, a proportion had only hard copy images available which introduces a number of uncontrolled limitations in image quality compared to digital data, such as the inability to change window settings.

In conclusion, DWI appears to be a less sensitive sequence in detecting the pulvinar sign compared to FLAIR imaging. Both basal ganglia and cortical hyperintensities are more conspicuous on DWI, leading to radiological appearances more typical of sCJD. Therefore, the DWI should be interpreted with caution when considering a diagnosis of vCJD. It is therefore a recommendation from this study, that the presence or absence of the pulvinar sign, according to the current diagnostic criteria, be recorded as ‘present ‘or ‘absent’ on the basis of axial FLAIR imaging.

Chapter 10 Thesis conclusion

This thesis demonstrates that MR imaging of the brain, particularly with the inclusion of DWI sequences, has a high sensitivity for sporadic CJD, used in the context of an established set of internationally accepted clinical diagnostic criteria. In this context, when other clinical criteria are met, the presence of characteristic MRI abnormalities allows an elevation of diagnosis from possible to probable sCJD. These criteria require either defined MRI or CSF abnormalities, and the presence of high signal in the basal ganglia and/or more than two specific cortical regions on DWI sequences alone had a comparable sensitivity and specificity to a positive CSF RT-QuIC test and when both tests were utilised, the sensitivity reached 100%. The MRI abnormalities vary with the molecular subtype and clinical presentation, with 'atypical' clinical phenotypes being more likely to have atypical MRI findings. Understanding this variation is important in clinical diagnosis.

In contradistinction to sCJD, DWI appears to be less a sensitive sequence compared to FLAIR in the radiological diagnosis of variant CJD. This is important to consider given the potential for further cases of vCJD in codon 129MV individuals, where clinical features might overlap with sCJD. It is a recommendation of this work that DWI be interpreted alongside FLAIR imaging, and not in isolation, when considering a diagnosis of variant CJD.

In conclusion, it is clear that the presence of characteristic abnormalities on MRI are extremely useful in the diagnosis of CJD in the appropriate clinical context. It is important to note that the MRI findings are not unique to CJD and can occur in other conditions. As such, MRI findings should not be interpreted in isolation in the diagnosis of CJD and instead should only be applied when other clinical criteria are met, particularly with the exclusion of alternative, potentially treatable diseases.

Unfortunately, all forms of CJD remain untreatable and universally fatal disease. However, accurate diagnosis in life is vital not only for appropriate clinical care, but

also to help safeguard against the potential transmissibility of the condition. Earlier, more reliable clinical diagnosis would become more important if treatments were developed.

10.1 Potential Future research

This thesis provides a detailed assessment of the role of MRI brain in the clinical diagnosis of CJD and its unlikely that this work can be further expanded upon using routinely performed diagnostic MRI sequences out with research settings.

On the basis of the data in this thesis, brain MRI in clinical settings is unlikely to be a reliable biomarker of disease progression in CJD given that a number of cases demonstrated regression of signal abnormalities over the course of their illness. However, this study was not designed to address this question and it is possible that MRI could have some biomarker progression role.

There has been recent interest in using MRI with Diffusion Tensor Imaging (DTI) as a quantitative imaging biomarker in a number of neurodegenerative conditions including amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease (189, 190). DTI is a relatively new MRI technique that can be used to evaluate microstructural changes in the brain by measuring the motility of water molecules in tissue (189). As a result, the indices measured in DTI are established biomarkers of brain tissue microstructural change (188). There are a limited number of studies accessing DTI in CJD (191-194) and it would be of interest to undertake further work in this field to establish whether DTI could be used a reliable marker for disease activity in the condition, which could be utilised in potential future treatment trials.

Recent developments in Positron emission tomography (PET) imaging have allowed the detection of β-amyloid in vivo in AD (195, 196). Although the amyloid deposits associated with CJD are different to those found in AD, they share similar physical-chemical properties (197). A limited number of studies have attempted to modify

PET imaging in order to detect CJD associated amyloid deposits, with the potential to utilise this as a diagnostic tool in the condition(198-200). So far, these studies have reported mixed results, although it remains a potential area for future research. One factor to take into consideration is that PET imaging is not widely available in clinical settings and this may act as major limitation to its use.

When CJD was first described nearly 100 years ago, pneumoencephalography was the most sophisticated brain imaging technique that existed. By 1982, when Stanley Prusiner coined the term 'prion' for the transmissible agent in CJD, the clinical use of MRI was all but in its infancy. Since this time, there has been considerable advancements in MRI technology, including the advent of new sequences, which have underpinned MRI as a crucial component of the diagnostic criteria for CJD. In the future, there is the potential for additional imaging techniques to aid the diagnosis of CJD, but these are unlikely to replace MRI as a diagnostic tool in routine clinical practice due to its wide availability and its capabilities of excluding alternative conditions.

Appendix 1: Current list of publications:

- ***'Mackenzie G, Will R. Creutzfeldt-Jakob disease: recent developments'.*** *F1000Res. 2017 Nov 27;6:2053. Review. PubMed PMID:29225787*

- - ***'Abu-Rumeileh S, Redaelli V, Baiardi S, Mackenzie G, et al. Sporadic Fatal Insomnia in Europe: Phenotypic Features and Diagnostic Challenges'.*** *Ann Neurol. 2018 Sep;84(3):347-360. Original Research. PubMed PMID:30048013*

- ***'Knight R, Mackenzie G. Clinical Aspects of Human Prion Diseases', in Sakudo A, & Onodera T. (Eds.) (2019).*** *Prions: Current Progress in Advanced Research (Second Edition).* Norfolk: Caister Academic Press

Appendix 2: MRI Proforma

Patient name: _____

NCJDSU number:_____

MRI date:_____

Format: CD / Films/IEP Films loaded: Yes / No
MRI sequences: T1/T2 ☐ C / S / A ; Flair ☐ C / S / A ; DWI ☐ C / S / A
Quality of scan (grade 1-6): _____
(1=excellent, 2=good, 3= average, 4= sufficient, 5= insufficient, 6= poor)

		Right		Left	
		FLAIR	DWI	FLAIR	DWI
Basal ganglia	Caudate				
	Putamen				
	Globus pallidus				
Thalamus	Anterolateral thalamus				
	Mediodorsal thalamus				
	Pulvinar				
Cortex	Cingulate gyrus				
	Insular region				
	Hippocampi				
	Frontal				
	Parietal				
	Temporal				
	Occipital				

Summary:
Pulvinar sign: Yes/No
Basal ganglia high signal: Yes/No
Number of areas of cortical high signal: _

Overall impression: Inadequate scan ☐
Not suggestive ☐
Suspicious but not diagnostic ☐
Positive scan ☐
Suggestive of CJD subtype: variant/sporadic/non-specific

Local report:
Normal MRI brain ☐
MRI differential mentioned CJD ☐
Changes documented: BG ☐ cortex ☐ thalamus ☐

Appendix 3: European classification of mode of clinical onset in Sporadic CJD

The following is an attempt to classify the different modes of clinical presentation in sCJD (Mrs Terri Lindsay, NCJDRSU)

RAPIDLY PROGRESSIVE DEMENTIA

The precise presenting symptom will vary from case to case, but the picture is an encephalopathic illness with dementia (and other neurological features), progressing rapidly over weeks to a few months, with no individual cognitive or physical deficit being present alone for more than two weeks

SLOWLY PROGRESSIVE DEMENTIA

These cases present with a slowly progressive dementia, developing over months to years, without any other significant neurological features for the first six months.

CORTICAL BLINDNESS (HEIDENHAIN)

These cases present with impairment of visual acuity and/or field, progressing onto cortical blindness, without other significant clinical deficit for the first two weeks of illness. The visual symptoms might include visual loss, visual inattention, visual illusions and visual hallucinations. It is essential that the symptoms progress to cortical blindness. Cases with other onsets that progress to include cortical blindness are NOT included in this category.

PSYCHIATRIC ONSET

These cases present with psychiatric symptoms such as depression, anxiety, paranoia, and delusions, without the presence of other features for a period of at least four weeks. Non-specific malaise or apathy do not count unless accompanied by some of

the above symptoms. Visual or auditory hallucinations alone do not count but may accompany the above features. It may be difficult to distinguish between the early features of dementia and a more specifically psychiatric onset. Behavioural change straightforwardly due to a developing dementia is not included in this category. The essential characteristic of this presentation is that the patients present with a disturbance that suggests a psychiatric disturbance rather than an obvious dementia and that specifically neurological features are absent.

CEREBELLAR ONSET

The presentation is with a progressive cerebellar syndrome without other significant features, for at least two weeks.

EXTRAPYRAMIDAL ONSET

The presentation is with an extrapyramidal syndrome involving Parkinsonian features with or without chorea, athetosis or dystonia, but without other significant features for at least two weeks.

STROKE-LIKE ONSET

The PRESENTATION is abrupt enough for a diagnosis of stroke to be entertained in the initial stages.

OTHER

None of the above-described presentations is applicable.

Appendix 4: NCJDRSU Surveillance Questionnaire

CLINICAL AND EPIDEMIOLOGICAL REVIEW

SECTION 1

GENERAL INFORMATION

1.

IDENTIFICATION INFORMATION

ID Number:

1.1 What is *subject's* name? First name: _____

Surname: _____

Other name(s): _____

(if subject is female and is/has been married)

record *subject's* maiden name or previous married names if different from current surname

_____

1.2 Sex of *subject?* ☐ 1=male 2=female

1.3 When was *subject* born? ____/____/_____ (dd/mm/yyyy)

1.4 ☐ 1=Caucasian 4=Afro-Caribbean

What do you consider to be *subject's* ethnic origin?

2=North African 5=Asian

3=Other African 6=Other

1.5 What is *subject's* marital status? ☐

1=single 4=divorced

2=married 5=separated

3=widowed 6=cohabitating

1.6 What is *subject's* present home address?

(*if subject is deceased)* What was *subject's* last home address before s(he) became ill?

House and street: _____

Town: _____

County: _____

Postcode: _____

1.7 Name of *subject's* consultant: _____

1.8 Hospital address

Name of hospital: _____

Street: _____

Town: _____

Postcode: _____

Tel. number: _____

1.9 *Subject's* hospital record number: _____

1.10 *Subject's* NHS number: _____

1.11 Name of *subject's* GP Name/Practice: _____

Street: _____

Town: _____

Postcode: _____

Tel. number: _____

1.12 Name of *subject's* dentist Name/Practice: _____

Street: _____

Town: _____

Postcode: _____

Tel No: _____

1.13 Date of interview/examination: ____/____/_____ (dd/mm/yyyy)

1.14 Interview/examination performed by: _____

_____

1.15 What is your (*respondent's)* name?

First name _____

Surname _____

Other names(s) _____

1.16 What is your *(respondent's)* relationship to subject? ☐

If other, *specify:*_____

1=partner
2=son/daughter
3=nephew/niece
4=sibling
5=cousin
6=father/mother
7=subject themselves
8=other

1.17 How long have you know *subject*? ☐☐☐☐

(record year since which *subject* known)

1.18 What is your *(respondent's)* address?

House and street _____

Town _____

County _____

Postcode _____

Telephone _____

1.19 Place of interview: ☐

1=hospital
2=home
3=other

SECTION 2

CLINICAL SURVEILLANCE AND EPIDEMIOLOGICAL REVIEW

BACKGROUND HISTORY

2.1 Surgical History

Surgical history questions refer to *subject's* history **BOTH BEFORE AND AFTER ONSET OF ILLNESS**

Has *subject* ever had any operations, including eye operations or stitching of wounds? ☐ 1= yes

2= not to respondent's knowledge

(If yes), record the year, hospital and type of operation:

1.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐
2.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐
3.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____

	Operation: _____ Group Category _____	☐
4.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐ _____	
5.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐ _____	
6.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐ _____	

7.	Year: _____ Accuracy code (filled in at data entry stage) Name of Hospital: _____ Operation: _____ Group Category	☐ ☐
8.	Year: _____ Accuracy code (filled in at data entry stage) Name of Hospital: _____ Operation: _____ Group Category	☐ ☐
9.	Year: _____ Accuracy code (filled in at data entry stage) Name of Hospital: _____ Operation: _____ Group Category	☐ ☐
10.	Year: _____ Accuracy code (filled in at data entry stage) Name of Hospital: _____ Operation: _____ Group Category	☐ ☐

11.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐
12.	Year: _____ Accuracy code (filled in at data entry stage) ☐ Name of Hospital: _____ Operation: _____ Group Category ☐

Total no.of fracture operations (88=N/A 99=N/K) ☐☐

Record the total number of operations ☐☐ 88=not applicable

2.2 Organ or Tissue Transplant

Has *subject* ever received an organ or tissue transplant, including corneal or bone marrow transplant?

(If yes), record year hospital and organ/tissue(s) received: ☐

1=yes

2=not to respondent's knowledge

Hospital _____ Organ: _____

Hospital _____ Organ: _____

1=yes

2=not to respondent's knowledge

If yes, record year

If no, record 8888

Cornea	☐	☐☐☐☐
Bone Marrow	☐	☐☐☐☐
Kidney	☐	☐☐☐☐
Liver	☐	☐☐☐☐
Other	☐	☐☐☐☐

2.3 Previous Medical History

All further questions in this section refer to *subject's* history **PRIOR TO THE ONSET** of the current illness

Does the *subject* have a record of previous hospital admissions EXCLUDING surgery AND unrelated to the present illness? ☐

1= yes

2= not to respondent's knowledge

(If yes,) on how many occasions has the *subject* been admitted to hospital (excluding surgery)? ☐☐

88=not applicable

(If yes), record the hospital name, the date(s) of admission and the reason(s) for admission:

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

Hospital: _____

Date of Admission: _____

Reason: _____

☐☐ Total no.of fracture admissions

(non-surgical) 88=N/A 99=N/K)

2.4 Dental History

Since the beginning of 1980 has *subject* had dental treatment? ☐

1=yes

2=not to respondent's knowledge

6=yes, fillings only

(If yes), record a description of the treatment(s) with date(s):

Treatment:	Date:
Treatment:	Date:
Treatment:	Date:
Treatment:	Date:
Treatment:	Date:
Treatment:	Date:
Treatment:	Date:

2.5 **Blood transfusion**

Has *subject* ever received a blood transfusion (blood components or plasma products)?

For suspect cases do not include any transfusions related to current illness

If yes, give year, hospital and reason: ☐ 1=yes

2=not to respondent's knowledge

Year:

Hospital:

Reason

2.5 **Blood transfusion (cont'd)**

Year:

Hospital:

Reason

Year:

Hospital:

Reason

Has *subject* ever received a transfusion of albumin or immunoglobulin?

For suspect cases do not include any transfusions related to current illness

If yes, give year, hospital and reason: ☐ 1=yes

2=not to respondent's knowledge

Year:

Hospital:

Reason

Year:

Hospital:

Reason

Year:

Hospital:

Reason

2.6 **Blood Donation**

Has *subject* ever been a blood donor? ☐ 1=yes

2=not to respondent's knowledge

If yes, date(s) and place(s):

2.7 Has *subject* ever been diagnosed with diabetes or bowel disease? ☐ 1=yes

(If yes, please give details) 2=not to respondent's knowledge

☐

1=inflammatory bowel disease; 2=insulin-dependant diabetes;

3=other; 4=other bowel disease; 8=not applicable

2.8 Has *subject* ever been to see a psychiatrist?

For suspect cases (and hospital controls) do not include consultations relating to current illness

(If yes, record year, name of psychiatrist, reason and treatment) ☐ 1=yes

2=not to respondent's knowledge

Year:

Psychiatrist:

Reason:

Treatment:

Year:

Psychiatrist:

Reason:

Treatment:

Year:

Psychiatrist:

Reason:

Treatment:

2.9 **Course of Injections**

Has *subject* ever received a treatment involving a course of injections, for example, human growth hormone, human gonadotrophin, insulin, fertility treatment?

For suspect cases and controls do not include treatments related to current illness

(If yes), record year, name of therapy, frequency and reason): ☐ 1=yes

2=not to respondent's knowledge

Year:

Therapy:

Frequency:

Reason:

2.9 Course of Injections (continued)

Year:

Therapy:

Frequency:

Reason:

Year:

Therapy:

Frequency:

Reason:

Year:

Therapy:

Frequency:

Reason:

2.10 Vaccinations

☐ 1=yes

Since beginning of 1980, has *subject* been vaccinated? 2=not to respondent's knowledge

Year:

Vaccine:

Route:

Year:

Vaccine:

Route:

Year:

Vaccine:

Route:

Year:

Vaccine:

Route:

Year:

Vaccine:

Route:

2.11

Procedures involving skin puncture

Has *subject* ever been tattooed?

☐ 1=yes

☐ 2=not to respondent's knowledge

Has *subject* ever undergone ear or body piercing?

2.12 Family history

Pedigree (indicating years of birth and death)

Subject's **grandparents**

(including names and dates of birth)

Subject's **parents** and **parents' siblings**

Subject and **siblings**

(including names and dates of birth)

2.13 **Family History**

Have any of the blood relatives of the *subject* included in the pedigree above died with dementia (or remain alive with dementia)?

☐ 1=yes

2=not to respondent's knowledge

3=respondent unsure

Have any of these individuals been diagnosed as having Creutzfeldt-Jakob disease?

☐ 1=yes

2=not to respondent's knowledge

3=respondent unsure

(If yes), record the person's name and the approximate date of illness:

Name: _____

Date of illness: _____

Confirmation of family history of CJD from surveillance database

☐ 1=definite case 4=unable to confirm

2=probable case 5=not a case

3=possible case 8=not applicable

2.14

Social Contact

Has *subject* had social contact, through family, friends or work, with someone else who developed CJD?

☐ 1=yes

2=not to respondent's knowledge

3=respondent unsure

(If yes), record the person's name and the approximate date of illness:

Name: _____

Date of illness: _____

Confirmation of social contact with case of CJD from surveillance database

☐ 1=definite case 4=unable to confirm

2=probable case 5=not a case

3=possible case 8=not applicable

2.15

Dietary History 1=yes; 2=not to respondent's knowledge

Has *subject* ever been a vegetarian for a period of one year or more? ☐

(If yes), during what period(s) was *subject* vegetarian, and did s(he) eat any meat or fish at all during this time?

_____

2.17 RESIDENTIAL HISTORY (begin with the most recent and work backwards)

	From (dd/mm/yyyy)	To (dd/mm/yyyy)	Street Town County	Postcode	OS grid reference
1	/ /	/ /			E N
2	/ /	/ /			E N
3	/ /	/ /			E N
4	/ /	/ /			E N
5	/ /	/ /			E N
6	/ /	/ /			E

											N							
7	/ /	/ /									E							
											N							
8	/ /	/ /									E							
											N							
9	/ /	/ /									E							
											N							
10	/ /	/ /									E							
											N							

2.17 RESIDENTIAL HISTORY (continued)

	From (dd/mm/yyyy)	To (dd/mm/yyyy)	Street Town County	Postcode	OS grid reference
11	/ /	/ /			E
					N
12	/ /	/ /			E
					N
13	/ /	/ /			E
					N
14	/ /	/ /			E
					N
15	/ /	/ /			E
					N
16	/ /	/ /			E

											N							
17	/ /	/ /									E							
											N							
18	/ /	/ /									E							
											N							
19	/ /	/ /									E							
											N							
20	/ /	/ /									E							
											N							

2.18 OCCUPATIONAL HISTORY OF SUBJECT (begin with the most recent occupation and work backwards)

	From (dd/mm/yyyy)	To (dd/mm/yyyy)	Name of employer	Town	Description of work
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

2.19	**Summary coding of *subject's* occupational data**	1=ever 2=never	Number of years elapsed from last work to onset (88=not applicable)
	medical/paramedical/nursing/dentistry	☐	☐☐
	animal laboratories	☐	☐☐
	pharmaceutical laboratories	☐	☐☐
	other research laboratories	☐	☐☐
	animal farming/veterinary medicine	☐	☐☐
	meat industry (butcher's/abbattoirs/rendering plants etc)	☐	☐☐

catering industry

other occupational involving animal products (eg leather worker)

2.20 Exposure to animals

Since the beginning of 1980, has *subject* worked or lived or stayed for more than one week on a farm?

1=lived or worked

2=stayed

3=not to respondent's knowledge

(If lived, worked or stayed), which type of farm was it?

1=arable

2=livestock

3=mixed arable and livestock

4=other

8=not applicable

Duration of stay? _____

2.21 **Education**

How many years of full time education did *subject* complete? | | |

(including school, college and university)

2.22 EDUCATIONAL HISTORY OF SUBJECT

(full time education for *subject's* aged less than 50; begin with most recent university/college/school and work backwards

	From (dd/mm/yyyy)	To (dd/mm/yyyy)	Name of Institution Town County	Postcode							OS grid reference						
1	/ /	/ /									E						
											N						
2	/ /	/ /									E						
											N						
3	/ /	/ /									E						
											N						
4	/ /	/ /									E						
											N						
5	/ /	/ /									E						
											N						
6	/ /	/ /									E						

											N							
7	/ /	/ /									E							
											N							
8	/ /	/ /									E							
											N							
9	/ /	/ /									E							
											N							
10	/ /	/ /									E							
											N							

SECTION 3

EXAMINATION REVIEW

	Examination from notes State of *subject* at admission/first examination by a neurologist
3.1	General Appearance:
3.2	Mental state/speech function:
3.3	Cranial nerves:
3.4	Motor system: Involuntary movements:

3.5	Sensory system:
3.6	Reflexes: primitive: tendon: plantar:
3.7	Cerebellar function/co-ordination
3.8	**NCJDSU Clinician – Examination of the *subject*** General Appearance: bed bound NG/PEG catheterised akinetic mute posture myoclonus startle

	other involuntary movements
3.9	Mental state/speech functions: best motor response best verbal response eye opening
3.10	Cranial nerves: fields/response to menace pupils EOMs/Doll's eyes
3.11	Motor system: tone power wasting
3.12	Sensory system

3.13	Reflexes primitive: grasp palmomental pout rooting tendon (incl. jaw jerk): plantar:
3.14	Cerebellar function/co-ordination:
3.15	General examination:

3.16 Detailed history of present illness:

CLINICAL HISTORY

3.17 Note the source of information eg hospital notes, relatives, other.

hospital notes ☐

relatives ☐

other ☐

1=yes
2=no
9=not known

If other, specify: _____

3.18 What were the first symptoms of illness noted by the *subject* or their family?

_____

3.19 When did these symptoms first occur? (dd/mm/yyyy)

_____

3.20 When did the *subject* seek medical attention for the illness? (dd/mm/yyyy)

_____

When was the *subject* first referred to a neurologist? (dd/mm/yyyy)

_____

3.21

3.22 When was the *subject* seen by a neurologist? (dd/mm/yyyy)

3.23 When was the *subject* first admitted for the current illness? (dd/mm/yyyy)

3.24 Since the start of the illness, has the *subject* been seen by a psychiatrist? ☐ 1=yes 2=no

3.25 If yes, record date of the first consultation: (dd/mm/yyyy)

3.26 SPECIFIC ILLNESS DETAILS Since the start of the illness, until the current time, has the *subject* exhibited the following neurological symptoms/signs:

Symptoms:		**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**	**Signs:**	**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**
Forgetfulness/ memory impairment				Cognitive impairment		
Other higher function impairment				Agnosia		
				Apraxia		
				Visuospatial impairment		
Language disturbance				Dysphasia		
				Dyslexia		
				Dysgraphia		
Psychiatric symptoms				Grasp reflex		
Depression						
Anxiety						
Behavioural disturbance						
Apathy/withdrawal						
Delusions						
Hallucinations	Visual					
	Auditory					
Other psychiatric symptoms						
Disturbance of gait				Cerebellar gait ataxia		

				Spastic		
				Lower motor neurone		
				Other, specify		
Bedbound				Akinetic mutism		
Speech disturbance				Dysarthria		
Visual symptoms	Diplopia			Ocular motor palsy		
	Visual impairment			Hemianopia		
				Cortical blindness		
Weakness or clumsiness of limbs				Pyramidal weakness		
				Extrapyramidal signs		
				Cerebellar signs/nystagmus		
				Lower motor neurone		

Symptoms:	**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**	**Signs:**	**1=Yes 2=No 8=NK 9=Missing**	**Date appeared (dd/mm/yyyy)**
Increased limb tone			Rigidity		
			Spasticity		
			Gegenhalten		
			Hyperreflexia		
			Extensor plantar responses		
			Muscle wasting		
			Fasciculation		

				Hypo- or Areflexia		
Involuntary movements				Myoclonus		
				Chorea		
				Dystonia		
				Other, specify		
Sensory symptoms	Numbness/tingling/ paraesthesia				**1=yes 2=no 3=central origin 4=peripheral origin 9=uncertain**	**Date appeared dd/mm/yyyy**
	Pain /burning / discomfort			Sensory signs		
Seizures						

3.27 TREATMENT		0=No treatment
Specify drug therapy for TSE	☐	1=Quinacrine 2=Oral PPS 3=Intra-ventricular PPS 4=Tetracycline (doxycycline) 5=Other, specify: _____
Complications of surgery	☐	1=yes

Complications of intra-ventricular catheter

2=no

9=missing

☐

Complications of pump

Significant drug side effects

☐

1=yes, withdrawal of drugs

2=yes, decrease of therapeutic dose

3=yes, but no change of the therapeutic dose

4=no

9=missing

Significant evidence of benefit

☐

1=clinical improvement

2=EEG improvement

3=MRI improvement

4=14-3-3 improvement

5=other improvement

6=no

8=Other, specify _____

9=missing

SECTION 4

REVIEW OF INVESTIGATIONS

INVESTIGATIONS

4.1 EEG

Has the *subject* undergone an EEG?	☐ 1=yes; 2=no; 9=not known
(If yes), on how many occasions?	☐☐
(If yes), record date of most recent EEG	____/____/_____(dd/mm/yyyy)
Are EEG records/copies available in the Unit	☐ 1=yes 2=yes,some
Have the EEGs been examined by a Unit staff member?	☐ 3=no 8=not applicable
Has the patient recorded an EEG characteristic of CJD (generalised triphasic periodic complexes with frequency about 1/s)	☐ 1=yes, confirmed by Unit staff 2=yes, reported by local staff, EEG not available for confirmation by Unit staff 3=no 8=no EEG performed

(If yes), record the date on which the first characteristic EEG was recorded

____/____/_____ (dd/mm/yyyy)

4.2 **CT Scan**

Has the *subject* ever had a CT scan? ☐

1=yes; 2=no; 9=not known

Has the patient ever had an abnormal CT scan? ☐

1=yes, confirmed by Unit staff

2=yes, reported by local staff, scan not available for confirmation by Unit staff

3=no

8=no scan performed

(If yes), record the date on which the first abnormal scan was performed?

____/____/_____ (dd/mm/yyyy)

(If yes), specify what abnormalities have been observed

_____

_____

_____

4.3 MRI Scan

Has the *subject* ever had an MRI scan? ☐

1=yes; 2=no; 9=not known

(If yes), on how many occasions? ☐☐

(If yes), record date of most recent scan _____/_____/_____(dd/mm/yyyy)

Are MRI scans available in the Unit ☐

1=yes

2=yes,some

3=no

Have the MRI scans been examined by a Unit staff member? ☐

8=not applicable

Has the patient ever had an abnormal MRI scan? ☐

1=yes, confirmed by Unit staff

2=yes, reported by local staff, scan not available for confirmation by Unit staff

3=no

8=no scan performed

(If yes), record the date on which the first abnormal scan was performed? ____/____/_____ (dd/mm/yyyy)

(If yes), specify what abnormalities have been observed

_____

_____

_____

(If an abnormal MRI scan has been reported by someone outside the unit) who reported the abnormal scan?

Name: _____

Address _____

_____

_____

4.4 **CSF findings** (fill coding boxes with 8s if test results are not available)

Date of first CSF collection

____/____/_____ (dd/mm/yyyy)

Results:

protein ☐☐.☐☐ g/l

CSF glucose

serum glucose ☐☐.☐ mmol/l

WBC

RCC ☐☐☐☐ count/mm^3

14-3-3 ☐

1=negative

2=equivocal

3=positive

	NSE	☐☐☐.☐ ng/ml
	S100b	☐☐.☐☐ ng/ml
	tau	☐☐☐☐☐ pg/ml
Ig oligoclonal bands in:	CSF	☐ 1=positive, 2=negative
	blood	☐ 1=positive, 2=negative

Date of second CSF collection

____/____/_____ (dd/mm/yyyy)

Results:	protein	☐☐.☐☐ g/l
	CSF glucose serum glucose	☐☐/☐☐ . ☐/☐ mmol/l
	WBC RCC	☐☐☐☐ / ☐☐☐☐ count/mm^3
	14-3-3	☐ 1=negative 2=equivocal 3=positive
	NSE	☐☐☐.☐ ng/ml
	S100b	☐☐.☐☐ ng/ml
	tau	☐☐☐☐☐ pg/ml

	Ig Oligoclonal bands in:	CSF	☐ 1=positive, 2=negative
		blood	☐ 1=positive, 2=negative

4.5 Has the *subject* had neurophysiology studies done? ☐

1=yes

2=no

9=not known

4.6 Has the *subject* had any abnormalities on other routine biological/haematological investigations during this illness? ☐

1=yes

2=no

9=not known

(If yes), describe the investigation(s) and the abnormalities:

_____

_____

_____

_____

_____

4.7 Has the *subject* undergone neuropsychological assessment? ☐ 1=yes 2=no 9=not known

Type of examination? ☐ 1=MMSE 2=Other, specify_____ 3=Formal Neuropsychometry 4= Multiple

Result Score for MMSE _____

Score from other test (score)

_____

(please specify other test)

Is neuropsychology report available? ☐ 1=yes 2=no 9=not known

4.8 Has the *subject* undergone a brain biopsy? ☐ 1=yes 2=no 9=not known

(If yes), were there any complications? ☐ 1=yes 2=no 9=not known

Result _____

4.9 Has the *subject* undergone a tonsil biopsy? ☐ 1=yes 2=no 9=not known

(If yes), were there any complications? ☐ 1=yes 2=no 9=not known

Result _____

4.10 Specimens Collected

	1=yes 2=no	Quantity (mls)
Blood: Frozen for general use	☐	☐☐
Separated and frozen	☐	☐☐
Urine	☐	☐☐
CSF	☐	☐☐

4.11 Post Mortem

Date of death: ____/____/_____

Was a post mortem performed? ☐ 1=yes 2=no

9=not known

(If yes), is neuropathological material available? ☐

1=yes

2=no

(If material is available) is material available in Edinburgh? ☐

8=not applicable

9=not known

Are post mortem results available? ☐

1=yes

2=no

9=not known

4.12 PrP Genotype

Are PrP genotype data available? ☐

1=yes

2=no

9=not known

(If yes), what was the *subject's* codon 129 genotype ☐

1=MM

2=MV

3=VV

(If yes), did the subject carry a mutation? ☐

1=yes

2=no

8=not done

9=missing

(If mutation), specify: _____

4.13 **Subject Classification**

On the basis of the available information, what is the classification of the *subject*? ☐☐

1.0=definite CJD
2.0=probable CJD
. 3.0=possible CJD
4.1=diagnosis unclear
4.2=CJD thought unlikely
4.3=definitely not CJD
5.0=GSS
0.0=unclassified

(If subject is classified as at least possible CJD or GSS) which category of disease is suspected? ☐

S=sporadic CJD
N=variant CJD
F=familial CJD
I=iatrogenic CJD
G=GSS

Presenting symptoms ☐

1=rapidly progressive dementia
2=Heidenhain
3=Pure psychiatric onset
4=progressive dementia

5=pure cerebellar onset

6=stroke-like onset

8=other, specify: _____

9=missing

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