The development of imaging biomarkers for the diagnosis of human prion diseases

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University College London (UCL)
DECLARATION STATEMENT

I, Marie-Claire Porter, confirm that the work I presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

______________________
Marie-Claire Anne Porter
Abstract

Future therapeutic trials in human prion disease will require the use of biomarkers of disease activity such as MRI in order to assess efficacy of treatment. Whilst the development of biomarkers is of importance it is also necessary to be able to understand and interpret what imaging findings characterise at post-mortem and furthermore how they correlate with clinical symptoms. In this thesis I investigate whether both conventional and quantitative imaging parameters can act as biomarkers to predict disease progression in symptomatic patients. I also assess what conventional MRI findings represent on a microstructural level and how imaging findings correlate with clinical symptoms of prion disease such as sleep disturbance.

This work is detailed in four projects, the first of which I investigate if abnormalities found on conventional MRI brain scans, PRNP genotype and prion strain can act as predictors of disease progression in patients with the sporadic form of prion disease. I was unable to show that conventional MR brain imaging helps to predict disease progression in this patient group, but I was able to show that codon 129 remains the main predictor of disease progression and strain subtype has an additional effect.

In the second project I test the hypothesis that MTR, a quantitative imaging parameter can predict disease progression in symptomatic patients. I found that both on cross-sectional and longitudinal analysis there were significant differences in symptomatic patients and that there was a strong correlation with the MRC Scale score, clinical outcome measure, and MTR value in patients with symptomatic disease which could be used as a clinical biomarker in combination to predict response to therapeutics in future clinical trials.

In the third project I focus on investigating the prevalence of sleep disturbance and its association with other features of disease and imaging findings. I found that sleep disturbance was highly prevalent in all forms of prion disease. I also found that there was a significant association found between thalamic signal
change seen on MRI scan and sleep symptomatology. In order to capture more data on the diversity of sleep symptoms in this population I constructed the Prion Disease Sleep Questionnaire a bedside screening tool that can be used to both record and monitor the incidence and severity of sleep disturbance.

In the final project I assess if specific histopathological findings on post-mortem correlate with cortical imaging abnormalities seen on DWI in patients diagnosed with sporadic CJD. I found that there were significant difference between patients with and without cortical ribboning present on their MRI brain scans those with DWI signal change had more frontal cortex spongiosis than those that didn’t. There was also a modest correlation identified between the 3 histopathological parameters: PrP\textsuperscript{Sc} deposition, gliosis and spongiosis.
Impact statement

Prion diseases are self-propagating, progressive and uniformly fatal neurodegenerative disorders. At present there is no current disease modifying treatment, but research is ongoing in the hope of finding one. Prior to commencing clinical trials there is first a need characterise the disease and develop biomarkers of progression that can be used in future clinical trials.

MRI measures have been found to be sensitive diagnostic markers of the presence of microstructural pathology in human prion disease but few studies have examined longitudinal change and correlation with clinical measures of disease progression. Diagnosis of prion disease is based on the recognition of clinical features of the disease accompanied by supportive investigation findings such as those found on magnetic resonance imaging (MRI). MRI has become an important diagnostic tool in prion disease and is included in the World Health Organisation (WHO) criteria for diagnosing both variant and sporadic forms of the disease. The clinical features of prion disease reflect the underlying neuropathological change occurring in the brain and the imaging changes are representative of microstructural change occurring.

Quantitative MRI changes in prion disease are well-described and a number of studies have shown a relationship between quantitative imaging changes and the presence of microstructural pathology in human prion disease, but there have been conflicting results on what that is. There have been a small number of studies that have also demonstrated longitudinal change in quantitative serial MRI parameters. There have however been no studies to date that have shown an association between disease progression and quantitative longitudinal MRI change in all forms of human prion disease.

My research focuses on investigating in what way MRI findings reflect the underlying pathology that occurs in human prion disease, which is essential in furthering our knowledge of the underlying mechanisms of the disease process.
I investigate which MRI parameters may be used as imaging biomarkers, this research therefore has an impact on the measurement of rate of disease progression in human prion diseases, this is of importance, as these parameters may be used as biomarkers in future clinical trials that measure response to disease modifying therapy.

I also investigate the prevalence and phenomenology of sleep related symptoms in prion disease including treatment response, it is important to also concentrate clinical research on patient’s symptoms and assess if current symptomatic treatment is effective. This body of research therefore has an impact on furthering medical research in human prion disease, which I hope will have a positive impact on both patients and the relatives that care for them.
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Abbreviations

ADC  Apparent diffusion coefficient
AC-PC  Anterior commissure-posterior commissure
As  Asymptomatic patients
Au  Arbitrary unit
CI  Confidence interval
CJD  Creutzfeldt Jakob Disease
Co  Control
Coeff  Coefficient
Cons  Constant
CRF  Clinical research form
CSF  Cerebrospinal fluid
DTI  Diffusion tensor imaging
DWI  Diffusion weighted imaging
EEG  Electroencephalogram
EMG  Electromyelogram
ESS  Epworth Sleepiness Scale
fCJD  Familial Creutzfeldt Jakob disease
FDR  False discovery rate
FWE  Family wise error
FFI  Fatal Familial Insomnia
FLAIR  Fluid attenuated imaging
GFAP  Glial fibrillary acidic protein
GH  Growth hormone
<table>
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<td>GM</td>
<td>Grey matter</td>
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<td>GSS</td>
<td>Gerstmann-Straussler-Scheinker syndrome</td>
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<td>H&amp;E</td>
<td>Haemotoxylin and eosin</td>
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<td>IEP</td>
<td>Image exchange portal</td>
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<td>iCJD</td>
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<td>MAPS</td>
<td>Multi-atlas propagation and segmentation</td>
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<td>MM</td>
<td>Methionine homozygous</td>
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<td>MR</td>
<td>Magnetic resonance</td>
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<td>MRC</td>
<td>Medical Research Council</td>
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<td>MRC Scale</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>MS</td>
<td>Multiple sclerosis</td>
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<td>MT</td>
<td>Magnetisation transfer</td>
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<td>MTR</td>
<td>Magnetisation transfer ratio</td>
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<td>MV</td>
<td>Methionine/valine</td>
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<td>NCS</td>
<td>Nerve conduction studies</td>
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<td>NHNN</td>
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<td>OD</td>
<td>Object density</td>
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<td>PACS</td>
<td>Picture Archiving and Communication System</td>
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<td>Parkinson’s Disease Sleep Scale</td>
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<td>Prion Diseases Sleep Questionnaire</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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**PRNP**  Prion protein gene

PrP  Prion protein

PrP<sup>C</sup>  Cellular prion protein

PrP<sup>Sc</sup>  Scrapie prion protein

PSQI  Pittsburg Sleep Quality Index Sleep Quality Scale (SQS)

REM  Rapid eye movement

RF  Radiofrequency

ROI  Region of interest

RT-QuiC  Real time quaking-induced conversion

sCJD  Sporadic Creutzfeldt Jakob disease

SCN  Suprachiasmatic nucleus

SD  Standard deviation

SFI  Sporadic fatal insomnia

SE-EPI  Type spin echo– ultrafast echo planar imaging preparation

SPM  Statistical Parametric Mapping

SQS  Sleep Quality Scale

SWS  Slow wave sleep

Sy  Symptomatic

T  Tesla

T1W  T1 weighted sequences

T2W  T2 weighted sequences

Thal  Thalamus

UK  United Kingdom

UV  Ultraviolet
<table>
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<td>Voxel based analysis</td>
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<td>VBM</td>
<td>Voxel based morphometry</td>
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<td>vCJD</td>
<td>Variant Creutzfeldt Jakob disease</td>
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<td>VPSP</td>
<td>Variably protease-sensitive prionopathy</td>
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<tr>
<td>VV</td>
<td>Valine homozygous</td>
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<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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<td>WM</td>
<td>White matter</td>
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Aims

The aims of this body of research are:

- To ascertain if codon 129 genotype and MRI signal change can act as a combined predictor of rate of disease progression in patients with sporadic CJD and to determine if the addition of if PrP_{Sc} strain type with codon 129 genotype proves to be a better predictor of disease progression in patients with sporadic CJD.

- To test if quantitative MTR parameters can define microstructural change in patients with or at risk of developing prion disease and whether MTR measures can correlate with clinical rating scores of disease severity both on cross-sectional and longitudinal analysis.

- To assess the prevalence of sleep abnormalities in all forms of prion disease, to test if they are primarily caused by thalamic pathology and therefore will correlate with specific thalamic signal change seen on MRI brain scans.

- To determine if diffusion weighted imaging (DWI) signal change identified on MRI brain scans in patients with prion disease correlate with quantitative analysis of histopathology on autopsy and if an association exists between deposition of PrP_{Sc}, gliosis and spongiosis in both patients with and without signal change on DWI.

Hypothesis

My hypothesis is that MRI signal change can act as a predictor of rate of disease decline in patients with prion disease. That imaging changes correlate with clinical features of disease such as sleep disturbance and disease severity and that imaging abnormalities parallel quantitative markers of neuropathology in human prion diseases.
1. **Introduction**

1.1. **Background**

Prion diseases, also known as transmissible spongiform encephalopathies, are a group of neurodegenerative conditions that exist in both humans and animals. Aetiologically they can be divided into sporadic, acquired and inherited forms. The name of the disease is derived from the deposition of abnormal cellular prion protein, PrP$^{Sc}$, in the brain. This accumulation of abnormal prion is also associated with other neuropathological features, these being: neuronal loss, gliosis and spongiform change. Prions (proteinaceous infectious particles) were first identified in 1982 but it was at least 10 years before the field accepted that prions are the causal agents of prion diseases$^{10}$. Since then, progress in research has been swift and we now have a better understanding of the molecular, neuropathological and clinical features of disease as well as the association between these factors. Advances in prion disease research, including knowledge of propagation and transmissibility of disease have inadvertently had a wider impact on research conducted in other neurodegenerative diseases. It is now thought that so-called “prion-like” propagation and transmissibility may be relevant mechanisms for several abnormal proteins, which accumulate in common neurodegenerative diseases. Indeed, this work raises questions regarding whether human transmissibility of neurodegenerative disease might occur in some specific circumstances in other protein misfolding diseases such as Parkinson’s and Alzheimer’s disease$^{11-14}$. If this is the case there will be even more importance in performing research in the prion disease field and any future discoveries made could have a wider impact on the understanding of other neurodegenerative illnesses.

With studies now being aimed at the development of therapeutics and disease modifying treatments, there is a necessity to develop biomarkers that can both predict the onset of disease in at-risk individuals and be used as clinical outcome measures. As the majority of prion diseases are rapidly progressive,
early diagnosis will become increasingly important if disease-modifying
treatment is to be commenced promptly to prevent further neurodegeneration
from occurring. At present diagnosis of sporadic and acquired forms of prion
disease are based on WHO recommendations of certain clinical features being
present plus supportive investigation findings. In clinical practice it is evident
that many patients give a history of prodromal symptoms, similar to that seen in
other neurodegenerative diseases, prior to the onset of definite clinical
symptoms and signs. It is probable that a pathological process is already
occurring at the prodromal stage, therefore the development of sensitive
biomarkers that may be able to indicate this process or be used to predict
conversion to definite disease will be important in future clinical trials. The
development of clinical outcome measures is of equal priority, as understanding
the natural history of prion diseases is not only useful in indicating disease
progression and prognosis but will also be necessary for measuring therapeutic
treatment response. Already developed is the MRC Prion Disease Rating Scale
(MRC Scale), a clinical marker of disease progression, that is both a reliable
and valid predictor of disease progression\(^\text{15}\), however other non-clinical
indicators may be more sensitive in indicating earlier disease onset/progression
as has been seen with amyloid beta and tau load in Alzheimer’s disease\(^\text{16}\), it is
therefore unlikely that in prion disease there will be one sole marker of disease
onset and progression utilised but rather a battery of different indicators that
may be used either independently or together as combined biomarkers. Taking
into account how these markers are likely to be affected by an individual’s
different molecular and genetic phenotype and having a comprehensive
approach that identifies the effect of these factors is likely to be required in the
development of either individual or combined models.

1.2. History and discovery of the prion diseases

The discovery of prion disease, prions and the nature of their transmissibility
really began its journey in veterinary medical research. Scrapie, a prion disease
affecting sheep and goats was first identified in the 1730’s in Spanish merino sheep. Importation of the infected Spanish sheep resulted in an outbreak of the disease in the UK and this in turn had a major negative impact on the wool industry, prompting the investigation of the pathogenesis and transmission of the disease. It was thought that scrapie was infective or transmissible but it wasn’t until the mid-twentieth century that accidental identification of its transmission was discovered. This discovery was made after an outbreak of the illness occurred in sheep that had been inoculated with a brain and spleen derived vaccine, which had been prepared from scrapie-affected sheep. The infectious agent was initially thought to be a slowly growing virus and inter-species transmission was demonstrated in both goats and later in mice. In 1967 J.S Griffith postulated that the scrapie agent may be an aberrant form of protein that could spontaneously be made and act as a template to induce production of more aberrant forms. Ionising radiation experiments conducted by Alper demonstrated that the infectious agent was not affected by UV radiation and therefore it could not be predominantly made from nucleic acid, further supporting the idea of it being a protein rather than a living organism. In 1982, Stanley Prusiner’s group isolated a protease resistant protein that was only present in the brain of animals with scrapie, and named it the prion protein.

In the UK, at around the same time as the isolation of the prion protein, there was an epidemic of what was later to be discovered as another animal form of prion disease, this was bovine spongiform encephalopathy (BSE), (also known as mad cow disease). This notorious disease was documented in over 180,000 cattle in the 1980’s and 1990’s, although it is estimated that 1-3 million animals may have entered the human food chain. This epidemic occurred predominantly in the UK, but BSE has also been identified in several other countries. The source of the disease was recognised as coming from the high source protein (meat and bone meal) supplement given to dairy cattle during the milking cycle. The removal of the hydrocarbon-solvent extraction process used in its production may have resulted in enough infectious prion protein from
either the carcasses of scrapie sheep or of cattle with sporadic prion disease being present in the feed to cause the epidemic.

Interspecies transmission of BSE has also been demonstrated, with it being transmitted to several other mammals including zoo animals, small ruminants and domestic cats. Most notably in the late 1990s the same prion strain was identified as causing the acquired form of human prion disease, variant CJD (vCJD)\textsuperscript{25, 26, 27}.

Other animal forms of prion disease include transmissible mink encephalopathy in farmed mink and chronic wasting disease the latter affecting cervids: mule deer, white-tailed deer, moose and elk.

Of the human prion diseases, sporadic CJD (sCJD) the most common form, it was first described by neurologists Hans Gerhard Creutzfeldt and Alfons Maria Jakob in the 1920s however no links with other types of human or animal prion disease were made until many years later. Human to human transmissibility was first recognised in the 1960s when Australian anthropologists Ronald and Catherine Berndt reported an unusual neurodegenerative illness called kuru affecting the Fore linguistic group and surrounding populations of the Eastern Highlands Province of Papua New Guinea. Further investigation of the nature and pathogenesis of the disease was commenced by chief medical officer Vincent Zigas and American paediatrician Carlton Gajdusek. Initially the disease was thought to be genetic in origin\textsuperscript{28, 29} but this hypothesis was questioned after it was observed that women and children immigrating to the kuru-region from neighbouring linguistic groups were also affected\textsuperscript{30} and therefore it was more likely that a food substance was responsible for the source of infection. The Fore group practiced ritualistic cannibalism, which is now thought to have resulted in oral prion transmission and development of the disease kuru, since the practice of cannibalism has ceased, kuru has died out\textsuperscript{31}. 

25
The link between sheep scrapie and kuru was made when William Hadlow noted the similarity in the neuropathology between the two diseases and thought that both of them must be related or caused by similar viruses\(^{32}\). Kuru was successfully transmitted to chimpanzees in 1966\(^{33}\) and a little later intracerebral transmission of both CJD and later GSS, a familial form of prion disease, were also made to primates\(^{34,35}\) and later oral transmission of this form of prion disease was also achieved\(^{36}\).

Around this time the first cases of iatrogenic transmission of human prion disease were also documented, a small number of patients who had undergone neurosurgery developed what appeared to be the sporadic form of Creutzfeldt Jakob disease within 18-24 months of their operation. It was found that the instruments used in neurosurgery had been previously employed in neurosurgical operations in patients who later died of sCJD and the link was made between the transmission of the disease between these two groups of patients\(^{37}\).

In addition to this corneal transplant surgery was also identified as a source of transmission of prion disease, the first case of this was reported in 1974 in a patient who received an infected cadaveric cornea and then went on to develop the disease themselves\(^{38}\). Another source of transmission identified was stereotactic intracerebral electrodes that were used for electroencephalogram recording\(^{39}\). Human pituitary gonadotropin hormone and blood transfusion from patients with variant CJD have also caused a number of iatrogenic prion disease cases\(^{40-43}\). By far the largest sources identified are cadaveric dural mater grafts and cadaveric human pituitary growth hormone, with over 220 cases of each having been documented worldwide\(^{44,45}\). Incubation periods of disease are highly variable and cases of iatrogenic CJD secondary to human growth hormone exposure are still being reported up to 40 years after exposure\(^{45}\).
1.3. Molecular biology of prions and strain pathophysiology

After Prusiner's team identified an abnormal form of prion protein associated with prion disease, there was still debate as to whether it was truly the cause of disease and its mechanism of action. Further discoveries including the prion protein gene (PRNP) in 1985, and linkage of the first mutations of PRNP to disease occurred a few years later and many forms of genetic prion disease were discovered, this further concreted the hypothesis of the involvement of prions in neurodegeneration. Positive transmission studies in transgenic PrP mice and conversely the demonstration of protection from prion disease in animals in which the PRNP had been removed re-enforced the argument for the role of prions in causing disease. Later, studies demonstrating knock out of the gene after transmission of disease showed halting of the disease process and even reversal these findings solidified the argument further. Over the last 10-15 years additional advances in the field of prion research have been made that support the hypothesis for the role of prions in transmission of disease and neurodegeneration. These important steps include amplification of prions by cycles of sonication and incubation from recombinant material, and generation of infection in wild type animals from recombinant PrP and spontaneous generation of prions in mice after exogenous prion introduction.

Understanding of the pathogenesis and propagation of prion diseases must accommodate the fact that they are all associated with deposits of an abnormal form of the naturally occurring prion protein (PrP). PrPC is the normal cellular isoform of PrP and is encoded on the PrP gene (PRNP). The human gene is located on the short arm (p) of chromosome 20 and is composed of two exons and a single intron. To date there are over 30 pathogenic PRNP mutations known (see figure 1). Additionally there are also a number of non-pathogenic polymorphisms that have been found. The polymorphism of methionine/valine (M/V) at codon 129 is the most recognised of these. This polymorphism has been found to have a strong influence on disease susceptibility, incubation time and clinical phenotype in sporadic, variant and genetic forms of prion disease.


**Figure 1**

*The prion protein gene, demonstrating both the pathogenic mutations (definite, probable and possible), risk factors, and polymorphisms (“poly”), including the polymorphisms which may have protective effects including the methionine/valine (M/V) change at codon 129 with known influence on disease susceptibility, incubation time and clinical phenotype (figure supplied by the MRC Prion Unit)*

The prion protein, PrP<sup>C</sup>, is a membrane bound glycoprotein (see figure 2) that is composed of a GPI membrane anchor, a structured C terminal with disulphide bond and an unstructured 5 octapeptide repeat N terminal that has high affinity copper binding sites. Features of PrP<sup>C</sup> are that it is digested by proteinases including proteinase K and is soluble. The physiological function of normal cellular PrP is unknown, it is possible that it aids synaptic transmission<sup>56,57</sup>, affords protection against oxidative stress<sup>58</sup>, has a role in copper binding, as suggested by its copper binding sites<sup>59</sup> or has a role in transmembrane signaling<sup>60-62</sup>. What is known, and is important in the development of therapeutics which target the removal of PrP, is that transgenic mice who have
had removal of \( PRNP \), both before and after naissance, appear to have, at least overtly, a normal clinical phenotype\(^{50,63,64} \). The abnormal form of the prion protein \( \text{PrP}^{\text{Sc}} \). A seed of \( \text{PrP}^{\text{Sc}} \), the Sc denotes the “scrapie” form may arise via introduction from the external environment, spontaneously from conversion of normal \( \text{PrP}^{\text{C}} \) or in the case of genetic forms of prion diseases because of inherited mutations of \( PRNP \).

Figure 2

*The prion protein, composed of a GPI membrane anchor, a structured C terminal with disulphide bond and an unstructured 5 octapeptide repeat N terminal that has high affinity copper binding sites (figure supplied by the MRC Prion Unit)*

Once a prion seed is present an autocatalytic process is thought to occur whereby normal \( \text{PrP}^{\text{C}} \) binds the prion seed, which then acts as a template to enable further misfolding, this results in the steady accumulation and
aggregation of the abnormal form of the protein. Prions can be identified through a surrogate, PrP\textsuperscript{Sc} that can be recognised because of its distinct biochemical properties; it has a rich \(\beta\) sheet content, unlike PrP\textsuperscript{C} that is predominantly composed of \(\alpha\)-helices, it also has a propensity to aggregate, resistance to protease digestion, particularly proteinase K, and is insoluble to mild detergents. The conversion and accumulation of PrP\textsuperscript{Sc} is typically associated with neuronal loss, scarring gliosis and neurodegeneration, although the exact mechanism of its toxicity remains unknown (see figure 3).

---

**Figure 3**

Prion protein propagation, conversion of PrP\textsuperscript{C} (monomer) by binding to the seed (the abnormal form of PrP\textsuperscript{Sc}, results in an abnormal fibre that undergoes fragmentation resulting in increased numbers of prion seeds and thereby is propagated and increases in the brain increases exponentially. The different shapes of seed in B and C illustrate the different strain type (figure supplied by the MRC Prion Unit)
Prion strains

As there are different clinically identified forms of prion disease, so there have been recognised different strains of prion, and the existence of these distinct strain isolates goes someway to explaining the differing clinical phenotypes of prion disease. These distinctive individual strains of infectious prions have unique influences on the type of lesion and anatomical distribution of prion disease neuropathology, the disease severity and incubation period\textsuperscript{65}. The difference in individual strains not only relies on a stable structural variation of PrP\textsuperscript{Sc} but also conservation of that isolate when propagated\textsuperscript{66}. The identification of different strain types was first made in animal forms of prion disease\textsuperscript{67}, but was later recognised as occurring in human forms of prion disease too\textsuperscript{68,69}. The strain subtypes are identifiable on Western blot analysis secondary to their differing glycosylation pattern and electrophoretic mobility after partial proteinase K digestion\textsuperscript{70,71} (see figure 4).

Figure 4
Western blot of homogenated brain, depicting London classification of strain subtypes. The individual bands seen are the glycosylation pattern formed from the diglycosylated, monoglycosylated and unglycosylated PrP\textsuperscript{Sc} (figure supplied by the MRC Prion Unit)
Strain types 1-3 are seen in sCJD and strain 4 seen in vCJD. There have been a very few cases of type 3 strain occurring in patients with the MM genotype and 1 definite case of type 4 strain being found in a patient heterozygous at codon 129\textsuperscript{72}.

As aforementioned, in human prion diseases the 129 M/V polymorphism is known to influence clinical phenotype and incubation time, the 129 polymorphisms are associated with certain strain types which have been found to result in differing clinical phenotypes of disease. It is probable that the differing amino acid sequence of PrP\textsuperscript{C}, in part dictated by the 129 M/V polymorphism, influences the strain type of PrP\textsuperscript{Sc} that is formed after misfolding of the protein occurs, with each strain subtype having its own individual biochemical and disease characteristics, including the rate and pattern of strain propagation\textsuperscript{27,73}. There are several strain types recognised in human prion diseases and at present two classification systems used to describe them\textsuperscript{68,69}. In the Parchi system, the strain types are 1, 2A and 2B and in the London classification system there are strains 1-4\textsuperscript{26,74} see table 1. Parchi strain type 1 corresponds with London strains 1 and 2, with the London classification system identifying a difference on Western blot between the two strains (see figure 4). Parchi 2a correlates with the London classification strain type 3 and Parchi strain 2b is the same strain as the London classification type 4 seen in patients with variant CJD. London strain type 6 does have a corresponding described Parchi strain in the literature. London classification type 1 has solely been associated with MM homozygosity at codon 129; type 2 is seen in all types of the polymorphism and type 3 predominantly in the MV or VV cases. Strain types 1-3 have all been identified in sporadic and iatrogenic forms of human prion disease. Type 4 is unique in its association with vCJD and until very recently had only been associated with the MM genotype, there has been identification of one definite case of variant CJD in an individual heterozygous at codon 129 and one probable case\textsuperscript{75}.
<table>
<thead>
<tr>
<th>Parchi classification</th>
<th>London classification</th>
<th>Codon 129</th>
<th>Type of prion disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>MM</td>
<td>sCJD, iCJD</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>MM, MV, VV</td>
<td>sCJD, iCJD</td>
</tr>
<tr>
<td>2a</td>
<td>3</td>
<td>MV, VV (few cases of MM)</td>
<td>sCJD, iCJD</td>
</tr>
<tr>
<td>2b</td>
<td>4</td>
<td>MM (1 definite case of MV)</td>
<td>vCJD</td>
</tr>
<tr>
<td>?</td>
<td>6</td>
<td>MM</td>
<td>sCJD</td>
</tr>
</tbody>
</table>

**Table 1**

The two main classification systems used for denoting PrP<sup>Sc</sup> strain subtype. The London classification and the Parchi classification for PrP<sup>Sc</sup> type.

1.4. The human prion diseases

Human prion diseases can be divided into 3 subtypes: sporadic Creutzfeldt-Jakob disease (sCJD), the inherited clinical syndromes, Gerstmann-Straussler-Scheinker syndrome (GSS), familial CJD and fatal familial insomnia (FFI), PrP systemic amyloidosis, and acquired forms such as variant Creutzfeldt-Jakob disease (vCJD), kuru and iatrogenic CJD.

**Sporadic prion disease**

sCJD accounts for around 85% of all cases of prion disease. sCJD occurs in every country, with a surprisingly similar annual incidence of approximately 1-2 cases per million. The disease usually affects people between the ages of 45-75 years with an average age of onset of around 65 years. The clinical course of the disease is typically rapidly progressive, with a median survival time of 4-5 months<sup>76</sup>. The majority of cases are fatal within 6 months; however the clinical course can vary and in some individuals the disease duration can be longer, in a minority over 3 years.
At present the aetiology of sCJD is unclear. The initial trigger for sCJD is also unknown, it may arise from a spontaneous conversion of PrP\textsuperscript{C} to PrP\textsuperscript{Sc}, from a somatic mutation occurring on \textit{PRNP}\textsuperscript{67,77,78} or it is possible that sCJD may also result from an unknown environmental source.

As mentioned, the codon 129 genotype affects both disease susceptibility and phenotype. Patients who are homozygous at codon 129 have an increased susceptibility to disease\textsuperscript{79,80}, as well as a shorter disease course\textsuperscript{48,81}. It is evident that the duration of clinical disease is heavily influenced by both the codon 129 genotype and partially by the accompanying PrP\textsuperscript{Sc} strain\textsuperscript{26,74}.

Patients classically present with symptoms of cognitive decline accompanied by a movement disorder. In many cases of sCJD the onset of disease is insidious, prodromal symptoms such as mood and/or sleep disturbance are often reported several months prior to the onset of more definite symptoms and signs. The impact these prodromal symptoms have on the patient as well as their response to treatment has received less focus than the more dominant clinical features of disease such as myoclonus and ataxia, an evaluation of sleep symptomatology in prion disease is made in chapter 4 to identify the prevalence of sleep symptomatology. A number of clinical subtypes of sCJD have been well described. The Heidenhain variant of sCJD is associated with visual symptoms that typically progress rapidly to complete blindness. The Brownell-Oppenheimer variant presents with prominent cerebellar disturbance\textsuperscript{82}, in addition to these are documented classical and thalamic (or sporadic fatal insomnia disease) phenotypes\textsuperscript{83}. As the disease is not necessarily specific to a particular part of the brain neuroanatomy it can present in a number of different ways, including stroke-like presentation\textsuperscript{84,85}, Alzheimer’s-like and more recently described corticobasal syndrome-like presentation\textsuperscript{86}. Diagnosis therefore rests on the combination of clinical features as well as investigation findings. The World Health Organisation (WHO) has published criteria that aid in the diagnosis of sCJD; it includes clinical signs of disease accompanied by
investigation findings (see table 2). There have been recent studies that have suggested that the accuracy of diagnosis may be improved by the inclusion of other investigation findings, such as CSF tau\(^{87,88}\) and RT-QuIC\(^{89}\).

<table>
<thead>
<tr>
<th>Diagnosis of sCJD</th>
<th>sCJD diagnostic categories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible</td>
<td>Category 1</td>
</tr>
<tr>
<td>The presence of category 1 and two clinical signs from category 2, with a duration of less than two years</td>
<td>Rapidly progressive dementia</td>
</tr>
<tr>
<td>Probable</td>
<td>Category 2</td>
</tr>
<tr>
<td>The presence of two clinical signs from category 1 and at least one positive investigation finding from category 3.</td>
<td>Myoclonus</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite</td>
<td>Category 3</td>
</tr>
<tr>
<td>Neuropathological confirmation</td>
<td>Periodic sharp wave complexes on the EEG</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**

*Diagnostic criteria for sCJD, adapted from the WHO criteria*

**Acquired prion disease**

**Kuru**

Human to human transmissibility was first recognised in the 1960s during the investigation of an epidemic of spongiform encephalopathy in the Fore linguistic group and surrounding populations located in the Eastern Highlands Province of Papua New Guinea.
Kuru resulted through the practice of ritualistic cannibalism. In the Fore population women and children were primarily affected, as they were the ones to consume the brain and internal organs of deceased relatives during cannibalistic feasts. It is thought that the trigger for the kuru epidemic may have come about through the recycling of prions from a member of the population who had sCJD. The disease onset is wide and ranges between 4 and over 60 years. The mean incubation period for kuru is approximately 12 years but there are cases that have occurred after a minimum exposure of 41 years. Between 1957 and 2004 over 2700 cases of kuru were reported. The main features of the disease are cerebellar ataxia, tremor and dementia.

**Iatrogenic CJD**

Iatrogenic transmission of prions has also been identified as a means of acquiring prion disease, with several routes of transmission having been identified.

Cadaveric derived pituitary growth hormone was used as treatment for growth problems in children, it is thought that a number of the pituitary glands harvested were from patients with unknown sCJD. The practice of using cadaveric sourced growth hormone ceased in 1985 when artificially synthesised growth hormone became available. At present there are between 0-6 cases of growth hormone induced iatrogenic CJD (iCJD) diagnosed in the UK each year. The incubation period is variable, the longest period between inoculation and disease onset has been found to be over 40 years. The duration of illness is variable but usually between 8-18 months. Cerebellar symptoms are a prominent feature of the disease followed by late onset dementia, other symptoms reported to occur include lower limb pain and sleep disturbance.

There have been a small number of cases of iCJD occurring in women treated for fertility problems secondary to human derived pituitary gonadotropin, most of these cases occurred in Australia.
Human derived dura was used for dural repair surgery until 1992 when a synthetic form was introduced. Although cases have been reported worldwide, the majority of reported patients with iCJD secondary to infected dural grafts have been in Japan where the surgery was more common, the grafts having originally come from Germany, a minimal number of cases have been reported in the UK. The incubation period again is highly variable, between 14 months and 30 years\textsuperscript{44}.

The presenting clinical symptoms are usually memory and cognitive problems; the majority of cases follow a clinical course similar to that seen in sCJD.

Other routes of transmission include neurosurgical procedures, inadequately sterilised intracerebral electrodes and corneal grafting\textsuperscript{91,92}.

**Variant Creutzfeldt-Jakob disease**

The first cases of vCJD were reported in 1995, 12 years after the identification of bovine spongiform encephalopathy BSE\textsuperscript{93,94}. These cases were initially thought to be an unusual form of sCJD, however as the number of cases grew and both atypical clinical and pathological features were described, the new disease was identified as new variant CJD\textsuperscript{95}. It is well established that vCJD is caused by dietary exposure to the same prion strain which is responsible for BSE\textsuperscript{27}. The number of clinical cases of vCJD is 229 (correct of December 2019, National CJD Research and Surveillance Unit) with the majority of cases having occurred in the UK, a small number in comparison to the large number of people who have been exposed to BSE prions. A study identified that approximately 1 in 2000 individuals have prion present in appendix tissue\textsuperscript{96}. There is therefore considerable uncertainty as to whether the epidemic is over given the potential for extremely long incubation periods in prion diseases.

In contrast to sCJD, vCJD typically affects a younger population, with a median age of onset of 28 years, with a range of 12-74 years, the incidence occurs
equally in males and females. In addition to the younger age of onset, the clinical presentation of vCJD differs substantially from the sporadic form (see Table 3); the vCJD phenotype is a less rapidly progressive disease, with an average duration of illness of 13-14 months. Early reported clinical features are often psychiatric, with mood disturbance, irritability and anxiety being commonly reported symptoms, in addition to this many patients are symptomatic with peripheral limb pain and/or paraesthesia. As the disease progresses the commonest neurological features to develop are cerebellar ataxia, dysarthria, cognitive impairment and movement abnormalities such as chorea, dystonia and myoclonus. Chorea in particular is one of the clinical features that aids in distinguishing the disease from sCJD and is included in the WHO diagnostic criteria (see table 3).
### Diagnosis of vCJD

#### vCJD diagnostic categories

<table>
<thead>
<tr>
<th>Possible:</th>
<th>Category 1</th>
</tr>
</thead>
</table>
| The presence of 1 of category 1 and 4 from category 2, with EEG unsupportive of sCJD | Progressive neuropsychiatric disorder  
Duration of illness >6 months  
Routine investigations do not suggest an alternative diagnosis  
No history of potential iatrogenic exposure  
No evidence of a familial form of TSE |

<table>
<thead>
<tr>
<th>Probable:</th>
<th>Category 2</th>
</tr>
</thead>
</table>
| The presence of 1 of category 1 and 4 from category 2, with EEG unsupportive of sCJD (although PSWC’s are rarely seen at end stage of disease) and MRI brain scan showing bilateral symmetrical pulvinar high signal | Early psychiatric symptoms  
Persistent painful sensory symptoms  
Ataxia  
Myoclonus or chorea or dystonia  
Dementia |
| Or | |
| The presence of features of category 1 and positive tonsil biopsy (category 4) | |

<table>
<thead>
<tr>
<th>Definite: The presence of features of category 1 and neuropathological confirmation</th>
<th>Category 3</th>
</tr>
</thead>
</table>
| EEG does not show the typical appearance of sCJD (or no EEG)  
MRI brain scan shows bilateral symmetrical pulvinar high signal | |

<table>
<thead>
<tr>
<th>Category 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A positive tonsil biopsy</td>
</tr>
</tbody>
</table>

**Table 3**  
*Diagnostic criteria for vCJD, adapted from the WHO criteria*
Variably protease-sensitive prionopathy (VPSP)

This form of prion disease was first described in 2008\textsuperscript{97} when 11 patients with the codon 129 VV genotype and spongiform encephalopathy were found to have an unusual PrP\textsuperscript{Sc} subtype that was less resistant to protease digestion. In addition to this they were also found to have unusual neuropathology findings, with less spongiform change and gliosis occurrence than is typically seen in sCJD, but with heavy microplaque deposition in the thalamus and cerebellum. Initially the disease was called Protease-sensitive prionopathy, however further cases have since been identified in patients with the MM and MV genotypes\textsuperscript{98}, who were found to display much lower protease sensitivity, thus giving rise to the name of variably protease-sensitive prionopathy\textsuperscript{99}. The VPSP strain subtype has been found to display similar biochemical characteristics to the Nor98 scrapie strain but it is not known what the relevance of this association is\textsuperscript{100}, although VPSP is described as a form of prion disease transmissibility in animal models has as yet not been reliably demonstrated\textsuperscript{101,102}. However it may just be a matter of time before proof of VPSP transmissibility is established as transmitting some forms of inherited prion disease have also proved difficult\textsuperscript{103,104} and it is only recently that Laura Pirisinu et al. have been able to successfully transmit GSS with P102L, A117V and F198S mutations and produce distinct pathological phenotypes to bank voles\textsuperscript{105}. The disease duration in VPSP is longer than that seen in sCJD the average being around 2 years and age of onset older at around 70 years. As in sCJD the phenotypic features of disease also differ somewhat between the codon 129 genotypes, the clinical phenotype associated with the valine allele is predominantly dominated by psychiatric features, MM and MV genotypes have been associated more with myoclonus which has rarely reported in the VV genotype. Other symptoms common to all genotypes include Parkinsonism, ataxia and cognitive decline\textsuperscript{99}. 
Inherited prion disease

10-15% of human prion disease cases are inherited. In some of these a positive family history of prion disease is not identified; occasionally this is due to a de-novo mutation occurring and rarely it is secondary to reduced penetrance of the mutation. In the majority of cases with no family history it is because they cases of neurodegenerative illness were misdiagnosed as some other form of neurodegenerative disease or dementia as it was prior to the advent of molecular genetic testing being available.

All mutations occur on the prion protein gene (PRNP) and the pattern of inheritance is autosomal dominant. To date over 30 different pathogenic mutations have been identified (see figure 1), the different types of pathogenic mutations are octapeptide repeat (OPRI) expansion mutations or point mutations secondary to either amino acid substitution or a premature stop codon.

Worldwide the most prevalent point mutations are E200K, P102L, D178N and V210I; and of the repeat expansions the 6-OPRI mutation is most commonly reported\(^\text{106}\).

The majority of gene positive individuals at risk of inherited prion disease will develop symptoms between the ages of 30 and 70 years. The phenotype of the disease is characterised by the specific mutation, however even within families there can be significant variability in both the clinical characteristics of the disease as well as age of onset.

Similarly to sCJD and the acquired forms of prion disease, a prodrome of subtle symptoms and signs are often present before the onset of more definite features of the disease. These symptoms include fatigue, insomnia, behavioural change and sensory symptoms.
Inherited prion diseases are now more commonly defined by the specific PRNP mutation that causes them; however historically they had always been classified under specific clinical syndromes that are associated with a particular disease phenotype, these being: Gerstmann-Straussler-Scheinker (GSS), familial Creutzfeldt-Jakob disease (fCJD) and Fatal Familial Insomnia (FFI).

**GSS**

The GSS phenotype is most commonly caused by the P102L (proline to leucine) PRNP point mutation, although there a number of other mutations also associated with this disease phenotype including the A117V and P105L mutations. The P102L PRNP mutation has been identified as the second most common cause of inherited prion disease in the UK, after the 6-OPRI PRNP mutation. Age of disease onset is highly variable, between 20-68 years and similarly to sCJD the clinical phenotype can be affected by the codon 129 genotype present. Patients who are methionine homozygous at codon 129 typically have a slightly earlier disease onset time than those who are heterozygous. The average disease duration is 5 years, ranging from 1-10\(^7\). Typical features of the disease are early onset cerebellar signs, predominantly progressive ataxia and dysarthria, as well as accompanying peripheral sensory disturbance. Cognitive decline is not an early clinical feature but usually manifests later on in the disease process.

**FFI**

FFI as its name suggests is a form of inherited prion disease with sleep disturbance as a dominant feature of the clinical phenotype. It is caused by the D178N PRNP mutation, (on the methionine allele of codon 129). Methionine homozygosity at codon 129 is strongly associated with the FFI phenotype\(^{108,109}\) and was thought to be specific to it, however in more recent years there have since been patients identified of that genotype that have presented with symptoms and a disease course that is more similar to the CJD phenotype\(^{110}\);
therefore it appears that the 129 polymorphism/disease phenotype relationship is somewhat more complex than previously identified and it is possible that there are disease phenotype influencing factors that are yet to be identified that may have an additional effect. Disease onset is usually in the 4th or 5th decade of life, but ranges anywhere between 18-62 years, the disease duration is usually between 7-18 months. Clinical features of FFI include sleep disturbance: a progressively worsening insomnia with accompanying excessive daytime somnolence, in patients who have undergone polysomnography they have been found to have markedly fragmented deep sleep recordings. Autonomic dysfunction is also prominent, with temperature, blood pressure and pulse rate dysregulation, sweating, impotence, increased salivation and urinary retention. Patients will often go on to develop ataxia. Cognitive function is relatively well preserved until the end stages of the disease.

**Familial CJD**

Familial CJD is associated with a number of *PRNP* mutations. The typical phenotype is of rapidly progressive cognitive decline accompanied by myoclonus and ataxia, the duration of illness is usually weeks to months.

The E200K *PRNP* mutation is most commonly associated with the familial CJD syndrome. The clinical phenotype is almost indistinguishable from sCJD\textsuperscript{111}, with myoclonus, pyramidal, extrapyramidal and cerebellar symptoms and signs. The prevalence of E200K mutation is especially in Libyan Jews, median age of onset is 58 years and disease duration 7 months\textsuperscript{48}. Most patients follow a rapidly progressive course of illness; cognitive decline, cerebellar signs and myoclonus are all features of the clinical presentation. Some patients develop seizures and a few have been found to have a peripheral neuropathy present\textsuperscript{112} otherwise the clinical presentation is remarkably similar to that seen in sCJD. Although uncommon the D178N *PRNP* mutation, on the V allele, and the 4OPRI mutation also present with rapidly progressive illnesses that are both pathologically and phenotypically similar to that seen in sCJD.
The octapeptide repeat expansion mutations

In addition to the point mutation forms of inherited prion disease there are also octapeptide repeat expansion mutations (OPRI). The larger insertion mutations, ranging from 5 to 9 octapeptide repeats, present with an illness that is slowly progressive, the duration of disease can be over 2 decades in the case of those with 6OPRI, with an age of onset usually in the 3rd of 4th decades of life. Cortical cognitive impairment accompanied by psychiatric features are commonly the first symptoms of disease, other features include Parkinsonism, pyramidal signs, ataxia and myoclonus.

There are over 30 different mutations of PRNP and the clinical presentation is highly variable, even within families carrying a specific mutation the age of onset and features of the disease can differ dramatically.

1.5. Diagnosis in human prion diseases

Diagnosis of human prion disease relies on the combination of clinical features and investigation findings; the investigations most beneficial in supporting or reaching a diagnosis include EEG, CSF analysis, MRI brain imaging and genetics for diagnosing inherited forms of the disease.

Electroencephalogram (EEG)

In patients symptomatic with sCJD the most characteristic finding on the EEG is repetitive, triphasic, periodic sharp wave complexes (PSWCs). The sensitivity for making this finding increases with test repetition and occurs more commonly when myoclonus is also present. PSWCs may occur in other conditions too. Seizures can occur in sCJD and in some forms of inherited prion disease; it may be difficult to differentiate PSWCs from epileptic activity on the EEG. PSWCs
are not typically seen in patients with vCJD and many patients with inherited prion disease have non-specific encephalopathic changes present only.

**CSF analysis**

The cell count and protein are usually normal in patients with prion disease. There are certain proteins that may be raised in patients with prion disease, they are not specific to the disease itself, but are markers of neurodegeneration and therefore can be raised in other degenerative diseases of the central nervous system. 14-3-3 proteins are present in all eukaryotic cells and found in most patients with rapidly progressive sCJD. 14-3-3 is not specific to prion disease and may be elevated in patients with other conditions such as stroke, encephalitis, seizure disorders, some brain tumours, atypically in other neurodegenerative diseases, vCJD and inherited prion disease; however a positive test in a suspect case supports the diagnosis of sCJD.

S100b proteins are found in the central nervous system glia. Similarly to the 14-3-3 proteins they are raised in rapidly progressive CJD but again the specificity to the disease is low. Tau can be markedly elevated in rapidly progressive prion disease, however again it is a general marker of neurodegeneration and is also commonly raised in other neurodegenerative diseases such as Alzheimer’s disease.

The real time quaking-induced conversion (RT-QuiC) assay detects PrP\(^{Sc}\) in CSF, it has been found to have both high sensitivity and specificity in patients diagnosed with sCJD\(^{113-115}\).

**Brain imaging**

Signal change on brain MRI is a key diagnostic finding in most patients with prion disease. MRI is an accessible, non-invasive test that is highly sensitive in diagnosing both vCJD and sCJD; it should therefore be sought urgently in
suspected clinical cases especially if an alternative treatable neurological condition is suspected. Signal change is most evident on diffusion-weighted imaging (DWI); fluid attenuated imaging (FLAIR) and T2 weighted sequences (T2W).

**Genetics**

There are over 30 pathogenic mutations that can result in inherited forms of prion disease on the **PRNP** gene, located at chromosome 20. Sequencing the gene can also identify the polymorphism that occurs at codon 129, which is recognized to influence disease susceptibility, incubation and duration in human prion disease.

**1.5.1. The role of MRI in the diagnosis of prion disease**

Signal change on brain MRI is a key diagnostic finding in most patients with prion disease. MRI is an accessible, non-invasive test that is highly sensitive in diagnosing both vCJD and sCJD; it should therefore be sought urgently in suspected clinical cases especially if an alternative treatable neurological condition is suspected. Signal change is most evident on diffusion-weighted imaging (DWI); fluid attenuated imaging (FLAIR) and T2 weighted sequences (T2W). The signal distribution varies depending on the type of prion disease present, and it is included in the WHO criteria for diagnosing both vCJD and sCJD based on the specific characteristics of signal change present.

**Sporadic CJD**

The accuracy of MRI in the diagnosis of sCJD has been found to be high, at least over 80% in several studies. Vitali et al. found DWI and FLAIR imaging in sCJD to be both highly sensitive and specific; in a cohort of 48 sCJD and 29 non-prion disease rapidly progressing dementia patients, they reported
96% sensitivity and 93% specificity in predicting accurate diagnosis of sCJD\(^{116}\). In a large study by Zerr et al. with 436 sCJD patients, 83% of cases were identified based on the presence of signal change in the striatum and/or 2 cortical regions (temporal, parietal and occipital)\(^7\), this led to the inclusion of striatal high signal in the updated WHO diagnostic criteria for sCJD. Recently Forner et al. compared the diagnostic accuracy of DWI MRI with CSF biomarkers in the diagnosis of sCJD; MRI was found to be the best predictor (AUC of 0.97) and had a diagnostic accuracy of 97%\(^{117}\). On comparison of the different imaging sequences T2W, FLAIR and DWI, DWI has been reported to be the most sensitive in picking up both cortical and subcortical signal change in sCJD\(^3,116,118-120\).

The classical MRI appearances in sCJD are bilateral signal hyperintensity in the caudate and putamen on DWI, T2W imaging or FLAIR\(^{121,122}\), (see figure 5, image A). Thalamic signal change is also found, and can be an isolated imaging finding (see figure 5, image C), particularly in those with VV type 2 and MV type 2 subtypes (Parchi classification)\(^{116}\).

Basal ganglia hyperintensity is thought to be the most pathognomonic MRI sign of sporadic prion disease, Meissner et al. found it to be the most consistent finding in 71% of patients\(^3\). However as the use of the DWI sequence has increased the identification of cortical signal change has too, in a study by Carswell et al. cortical signal change was found to be most common, present in 74%, closely followed by caudate signal change (73%), then putamen (59%) and lastly thalamic signal intensity 37%\(^{123}\).

Variation in the presence and distribution of signal intensity on MRI has been found to occur. In a study of 39 patients with sCJD, Tschampa et al. looked at cortical signal change and reported signal intensity occurring most commonly in the insula, the cingulate gyrus, and the superior frontal gyrus in 95% of subjects. Other areas with high involvement included the cortical areas near the midline (the precuneus and paracentral lobe in 87%, and 77% respectively)\(^{124}\).
Other studies have found the neuroanatomical areas where signal change is present to coincide with both the clinical features and course of disease with Gao et al. reported patients with basal ganglia hyperintense lesions to have a shorter disease course and higher incidence of myoclonus, and those with occipital lesions to have a more rapid progression in symptoms and reach akinetic mutism earlier\textsuperscript{125-127}. In addition to this there are reports of lateralised MRI findings correlating with clinical symptomatology\textsuperscript{128,129}.

The association of MRI signal change with clinical features and disease duration is thought to reflect the underlying traits of the prion strain present. There have also been studies that have examined the relationship between strain subtype, codon 129 polymorphism and MRI signal change. The largest by Meissner et al. reported that patterns of MRI signal change correlate with specific sCJD molecular subtypes; with basal ganglia signal change more commonly being found in those with MV2, VV2 and MM1 subtypes, cerebral cortical signal change most evident in VV1, MM2 and MV1 subtypes and thalamic signal change in VV2 and MV2 subtypes\textsuperscript{3}.

Progression of disease has been found to correlate with change in the distribution and intensity of MRI signal. A study on 9 patients with sCJD, by Ukisu et al. reported increasing cortical signal change accompanied by cortical atrophy on serial MRI scans, there was disappearance of signal change in 1 patient\textsuperscript{130}. The most recent study by Eisenmenger et al. found degree and extent of DWI signal intensity to correlate with disease duration and that increased basal ganglia signal intensity held the most consistent correlation with progression of disease\textsuperscript{131} (see figure 5, image B). These changes in signal intensity undoubtedly reflect both the underlying microstructural pathology and the severity of disease that is taking place.
Figure 5
MRI scans performed in patients diagnosed with sCJD

Image A: Depicted is the classical appearance of caudate (small triangle), putamen (large triangle) and pulvinar (arrow) signal intensity accompanied by thalamic signal change (axial FLAIR)

Image B: Asymmetrical cortical signal change (axial DWI)

Image C: Thalamic signal change associated with striatal and cortical abnormalities (axial DWI)

Variant CJD

Bilateral symmetrical hyperintensity present in the pulvinar region of the thalamus, higher in intensity when compared to other grey matter, both cortical and non-cortical, is the characteristic finding seen in vCJD, this is known as the pulvinar sign. The presence of this characteristic signal change is most evident on FLAIR imaging, but can also be present on T2WI and axial DWI but with a lower sensitivity\(^2\) (see figure 6). The sensitivity of the presence of the pulvinar sign is close to 80% and the specificity over 90\(^%\).\(^{132,133}\)

In 75% of individuals there is accompanying signal change in the dorsomedial thalamic nuclei, this is known as the “hockey stick” sign. These imaging findings are included as part of the WHO diagnostic criteria for vCJD\(^{2,132,134}\).
Other MR findings include periaqueductal grey matter hyperintensity, hyperintensity of the caudate head and less commonly abnormal hyperintensity of the parietooccipital white matter\textsuperscript{135}. The typical cortical ribboning signal change seen on DWI in sCJD patients is much less prevalent in individuals with vCJD; this dissimilarity may reflect the difference in the entry and spread of disease of the vCJD prion strain or the variance in the underlying histological changes that are characterised by the specific strain that occurs in the different forms of prion diseases.

It is probable that the imaging changes present also reflect the underlying neuropathology; but there have been a limited number of studies, Mittal et al found that signal intensity present on the DWI sequence may reflect underlying spongiform change and Siddique et al. found a correlation with quantitative imaging\textsuperscript{9,136}. It is also possible that FLAIR is a more sensitive imaging sequence for signifying gliosis or neuronal loss, these being the common pulvinar histological changes identified in vCJD\textsuperscript{132}.
Figure 6

FLAIR and DWI scans from patients diagnosed with vCJD

Image A: Classical pulvinar sign (bottom arrow), striking signal hyperintensity in the pulvinar and dorsomedial thalamus that is greater than that seen in the basal ganglia (FLAIR sequence)

Image B: Pulvinar signal change seen on DWI

Image C: Cortical ribboning (DWI), an imaging finding sometimes seen in vCJD, but more commonly identified in those with the sporadic form of the disease

Iatrogenic prion disease

MRI signal change is also evident in iatrogenic forms of prion disease. In the case of transmission of prion disease through dura mater infected grafts, there are case reports of basal ganglia hyperintensity similar to that seen in sCJD\textsuperscript{137-139}. In a case series of 10 patients Meissner et al reported cortical and basal ganglia signal change followed by thalamic signal increase as the most common findings\textsuperscript{140}. Cadaveric pituitary derived human growth hormone cases, where the route of transmission is through peripheral inoculation, have also been reported to present with bilateral hyperintensity of the basal ganglia\textsuperscript{141}. A small cases series reported similar basal ganglia findings in 3 patients but with accompanying thalamic signal change in 2 out of the 3 patients. In a recent study by Rudge et al. all patients with basal ganglia hyperintensity (18 out of 20 subjects) were also found to have associated thalamic signal hyperintensity.
Lower limb pain, similar to that experienced in patients with vCJD, and sleep disturbance are symptoms seen in iCJD patients and as the author suggests may reflect damage occurring in the thalamus, as supported by the accompanying MR signal change. Other imaging findings in this study were signal intensity in the frontal cortex and superior cerebellar vermis, the latter finding may be explained by the typical clinical features of gait ataxia, which is characteristic of superior cerebellar vermis damage.

**Inherited prion disease**

Although the diagnosis of inherited prion disease in symptomatic individuals can be made with PRNP genotyping MRI is important in the exclusion of other possible diagnoses. In addition to this MRI can be used as an aid in diagnosing prion disease in asymptomatic PRNP gene positive individuals. The imaging findings in inherited prion disease include cortical and deep grey matter signal change as well as cerebellar and cortical atrophy these findings vary depending on the underlying PRNP mutation present.

In patients with the E200K PRNP mutation, the MRI features are often indistinguishable from that seen in sCJD with symmetrical basal ganglia signal change being present and most evident on DWI or T2WI.

In a study on the UK P102L mutation kindred, 15 of which had MRI scans, the typical MRI findings were cerebral atrophy, localised cerebellar atrophy and white matter lesions. There have also been a few case reports in this form of inherited prion disease that have described the presence of cortical and deep grey matter signal change similar to that seen in sCJD.

There are few published studies on MRI findings with the D178N mutation and FFI phenotype. Zerr et al. found no abnormalities on MRI, in a series of 8 patients. Haik et al. reported an increase in the apparent diffusion coefficient
of water (ADC) in the thalamus that matched gliosis that was restricted to the thalamus on post-mortem examination\textsuperscript{146}.

Signal change present on brain MRI is both a highly sensitive and specific marker of sporadic and acquired forms of prion disease, as our understanding of the association between clinical features of disease, strain type and imaging findings expands the possibility of using these combined features may not only prove to be an aid in diagnosing disease but could also be utilised as a valuable biomarker of disease progression in the advent of clinical trials.

1.5.2. Histopathological features of disease

A definitive diagnosis of prion disease can only be made histologically. Tonsil biopsy has proved useful in the diagnosis of vCJD; type 4 PrP\textsuperscript{Sc} can be identified by Western blot, abnormal PrP can be detected in the germinal centre of the tonsillar follicles after immunohistochemistry staining; with sensitivity and specificity of 100\% (Figure 7). In sCJD a definitive diagnosis would require examination of brain tissue; however this is usually only performed if there is a strong suspicion of an alternative potentially treatable cause or diagnosis. If a brain or tonsil biopsy is being done it is important to use disposable instruments.

![Figure 7](image)

\textbf{Figure 7}

\textit{Tonsil biopsy sections from a patient with vCJD. Tonsil follicles, denoted by the arrows, PrP\textsuperscript{Sc} deposits identifiable with immunohistochemistry staining in the germinal centre of the tonsil follicle (B)}
The neuropathology of prion disease is characterised by: grey matter spongiform change, neuronal loss, astrocyte and microglial activation and amyloid plaque formation. Although the anatomy and extent of the brain affected by these changes may vary depending on the underlying prion disease present, and these neurological processes may occur in other neurodegenerative disease, the combination of these histological features is distinctive to prion disease. Diagnosis is made by histological examination of multiple blocks of brain tissue, as the location of disease can vary substantially; immunohistochemistry is performed with anti PrP monoclonal antibodies and PRNP genotyping. The tissue blocks are decontaminated with formic acid and then embedded in paraffin prior to examination. They are stained routinely with haematoxylin and eosin and also with immunohistochemistry using anti-PrP monoclonal antibodies.

Spongiosis is typified by fine neuronal vacuolation that is predominantly located in the cortex, the deep grey matter nuclei and the molecular layer of the cerebellum; it can however occur throughout the neuropil, involve the white matter\textsuperscript{147-149} and even the spinal cord\textsuperscript{150}. The vacuoles usually occur in the axons and dendrites and less commonly in the cell bodies, which distinguishes prion disease from other neurodegenerative diseases such as Alzheimer’s disease and Dementia with Lewy bodies\textsuperscript{151}.

Vacuole size ranges from 2-200 micrometres. Vacuoles may coalesce to form large sponge-like voids or holes thus giving rise to the name spongiform encephalopathy. Gliosis and neuronal loss are predominantly found in the cortex and the granular layer, the cerebellum and the basal nucleus of Meynert\textsuperscript{152}.

Abnormal PrP deposition does not appear to correlate with gliosis and neuronal loss, deposition is predominantly synaptic or plaque-like, and the pattern of deposition usually reflects the underlying type of prion disease. Abnormal PrP can be found in both the grey and white matter, the spinal cord, ganglia,
intracranial vessel walls and even in the peripheral nervous system in variant CJD abnormal PrP is also present in the lymphoreticular system. Both sCJD and vCJD can cause deposition of abnormal PrP in the intracranial vessel walls. This may result in haematogenous spread and increased infectivity.

The anatomy and extent of neuropathology present varies substantially between the different subtypes of prion disease, each having distinctive histological features, this distinction is important in being able to differentiate between the form of prion disease present.

**Sporadic CJD histopathology**

There is marked heterogeneity in the pathological profile of sCJD and similar to the correlation seen between strain isolate and clinical phenotype the different neuropathological phenotypes appear to also relate to the characteristics and propagation of the dominant or specific strain type present and accompanying codon 129 genotype.

Using the London classification of strain type, the MM1 and MM2 types have prominent widespread cerebral spongiform change, with the occipital cortex being most commonly affected. There is less spongiosis present in the brainstem, cerebellum and in the basal ganglia in the MM1 type, the latter being a major difference between the two subtypes, the MM2 type has more evident basal ganglia involvement. PrP\textsuperscript{Sc} deposition is synaptic and/or perivacuolar, there are no amyloid plaques present. Synaptic deposition is most commonly seen in the MM1 type whereas perivacuolar deposition is more likely to occur in the MM2 subtype.

The MV2, VV2 and VV3 subtypes have prominent basal ganglia and parahippocampal gyrus spongiosis with more focal vacuolation in the cortex and cerebellum. Synaptic deposition of PrP\textsuperscript{Sc} is seen to occur in all 3 subtypes with perivacuolar deposition being present in the MV2 subtype as well as plaque like
deposits, less commonly seen in the basal ganglia and brain stem. The VV2 subtype has distinctive band-like plaques in layers 2 and 5 of the cerebral cortex. The MV3 type typically has little cortical spongiform change, but gross spongiosis is often present in the basal ganglia and rounded kuru like plaques are evident in the cerebellum\textsuperscript{154}. Also described is the MM6 subtype where there is widespread spongiform change seen in the cerebral cortex, most severe in the basal ganglia with focal changes evident in the cerebellum. Synaptic deposits of PrP\textsuperscript{Sc} most commonly occur in the cerebellum and brainstem and perivacuolar deposition in the cerebral cortex and basal ganglia\textsuperscript{154}, figure 8 shows the classical histopathology changes seen in sCJD.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{Classical histopathology changes seen in sCJD. A-spongiform change, B-astrocytosis, C-neuronal loss and D-PrP amyloid}
\end{figure}
Variant CJD histopathology

The histopathological hallmark of vCJD is the presence of abundant PrP amyloid plaques in the cerebrum, predominantly in the occipital cortex and cerebellar cortex, amyloid plaques are encircled by vacuolation, these are known as florid plaques. Peri-cellular PrP deposition also occurs in the cortex and smaller amyloid deposits are commonly found in the deep grey matter nuclei. Spongiform change is evident throughout the cortical grey matter and cerebellum but is most prominent in the basal ganglia whilst severe neuronal loss and gliosis are most evident in the posterior thalamic nuclei and midbrain. Diagnosis of vCJD is made on Western blot, by demonstrating the presence of type 4 (London classification) or type 2B (Parchi) PrP$_{Sc}$, pre-morbidly via tonsil biopsy or at post-mortem via lymphoreticular tissue (e.g. appendix, lymph node or spleen) and/or brain tissue, figure 9 shows the classical histopathology changes seen in vCJD.

![Figure 9](image)

**Figure 9**
*Histopathology in vCJD. A-Florid plaques (arrows) and spongiform change, B-PrP deposition*

Iatrogenic CJD histopathology

In human growth hormone associated iCJD, the neuropathology is similar to that seen in sCJD with cortical, basal ganglia and cerebellar spongiosis being
common along with synaptic PrP and plaque-like PrP deposition. Dural transmission cases can be divided into those that are plaque-like and those that are non-plaque-like, the difference between these subtypes probably relates to the underlying PrP isolate that has been transmitted. There have been recent reports of amyloid-β pathology in the brains of both recipients of human growth hormone and dural grafts, and the Aβ does not appear to co-localise with PrP so appears to be co-incidental iatrogenic transmission of Aβ too156. This is of concern for the general population as it is possible that transmission of Aβ and iatrogenic Alzheimer’s disease could be a genuine risk for healthy individuals.

**Inherited prion disease histopathology**

**E200K mutation**

The similarity between sCJD and E200K inherited prion disease also extends to the neuropathological findings, spongiosis, neuronal loss and gliosis are all seen, as well as PrP deposition; the presence of PrP plaques is less prevalent157. In E200K patients who are homozygous for methionine at codon 129, a distinctive PrP line of deposition is also seen in the molecular layer of the cerebellum.

**OPRI mutations**

The neuropathology seen in the OPRI mutation is highly variable158,159. Spongiosis, gliosis and neuronal loss are usually present as well as PrPSc deposition in the molecular layer of the cerebellum; in the larger octapeptide repeat mutations (8 and 9OPRI) there are also plaque-like deposits present.
P102L inherited prion disease

Histopathologically, classical GSS is associated with uni- or multicentric PrP plaques in the cerebellar and cerebral cortex with variable spongiform change and neurofibrillary tangles.

FFI-D178N mutation

The FFI clinical phenotype is associated with distinctive neuropathological findings; anterior and dorsomedial thalamic gliosis and neuronal loss is typically severe, there is also often inferior olive involvement, spongiosis is extrathalamic and predominantly accompanied by gliosis, but this is usually less prominent than that seen in the thalamus and olives, fluffy PrPSc deposition is seen and plaque deposition, this is variable in location, although usually absent or minimal in the thalamus; Llorens et al. recently reported in a small case series that PrPSc appeared to be associated with spongiform change.160

1.5.3. Correlation of MRI and neuropathology

Although MRI changes in prion disease are well documented, the underlying pathology associated with these changes is less well understood. There are a limited number of small studies that have examined the association between MRI signal change and neuropathology, some have reported positive findings of correlates with neuropathological change, however others haven't and the conflicting results remain difficult to interpret. Manners et al. looked at T2w imaging and ADC in brain regions of a case series of 20 patients (10 patients with sCJD and 10 control subjects), they found a positive association with neuropathology; a reduction in ADC correlated with spongiosis and PrPSc in the deep grey matter nuclei and with gliosis, PrPSc and spongiosis in the cortical grey matter.161 Siddique et al. carried out quantitative analysis of MTR in a small series of vCJD brains and reported an association between ex-vivo reduced MTR and spongiform change.9
In a study of 6 patients with sCJD, conducted by Russman et al. there was no neuropathological correlate identified with DWI signal change\textsuperscript{162}. However conversely Mittal et al. did find a positive correlation between spongiosis and DWI signal intensity as did Eisenmenger et al. in a case series of 7 patients, they reported degree of signal intensity on DWI correlating with degree of spongiosis\textsuperscript{131,136}. It is evident that MRI signal change reflects underlying microstructural change and spongiosis may correlate with diffusion-weighted imaging but at present results are inconsistent, a larger study may help elucidate things further. One of my projects has been to explore the association between MRI findings and their correlation with neuropathology; this was conducted in a cohort of patients with sCJD and is presented in chapter 5.

1.6. \textbf{MRI sequences and quantitative analysis}

Accompanied with clinical features, CSF and EEG findings, MRI is one of the main methods used to diagnose both the variant and sporadic forms of human prion disease. It is included in the WHO criteria\textsuperscript{1,2}. Over 90\% of patients that present with these forms of prion disease have imaging abnormalities evident on conventional magnetic resonance imaging (MRI)\textsuperscript{121}. Although conventional imaging may illustrate abnormalities present it is not quantitative and therefore may not be as sensitive or reliable in detecting longitudinal change.

1.6.1. \textbf{Background to DWI}

Diffusion weighted imaging was introduced to the clinical field in the mid-1980's by Denis Le-Bihan and Robert Turner. Denis Le-Bihan was attempting to find an imaging method that was able differentiate liver tumours from angiomas. Unfortunately there was little success in its use in liver imaging at that time. Le-Bihan decided to investigate its use as a neuroimaging sequence\textsuperscript{157}. Since then it has become an important imaging tool, it has been found to be
particularly valuable in the diagnosis of acute stroke, but has been used in the investigation and diagnosis of a number of other neurological disorders including human prion disease.

In human prion disease DWI is highly valued, it has both high sensitivity and specificity in differentiating CJD from other rapidly progressive dementias\textsuperscript{163} and is abnormal in up to 92\% of suspected cases of sCJD\textsuperscript{121}.

The changes seen on DWI scans in patients with sCJD include marked hyperintensity in the cortex, basal ganglia and thalamus. In patients with vCJD restricted diffusion is most evident in the striatum and thalamus. DWI is an MRI sequence that provides information on the mobility of water molecules. Water molecules are more mobile in the CSF, but in cell membranes, tracts and macromolecules diffusion is more restricted. Therefore DWI can be used as a means to measure microstructural change, cell swelling and tract changes. Although the acquisition of the DWI sequence can be affected by motion artefact, the DWI sequence can be obtained rapidly which is of great benefit in patients who have difficulty keeping still in the MRI scanner.

DWI depicts contrast change that is influenced by differences in water molecule mobility. It achieves this by the application of diffusion gradients during the preparatory phase of an imaging sequence, usually of the T2 weighted SE-EPI type (spin echo–ultrafast echo planar imaging preparation). Strong pulses are used to obtain diffusion-weighted images. The first pulse de-phases the proton spin; a second pulse is then applied which re-phases the spin. Some of the molecules within each voxel will have moved or diffused, the faster that they diffuse the more dephased the proton spin and the weaker the recorded signal.

Diffusion weighted imaging records differences in water mobility but not the direction of their displacement. Diffusion tensor imaging is able to determine the direction of proton motion (fractional anisotropy). The fractional anisotropy is measured on a scale of 0 to 1.0, with 0 equaling isotropic or unrestricted
diffusion and 1 representing diffusion restricted to one plane. Diffusion tensor imaging can be used to map neural tracts in the brain, this is known as tractography. In addition to measuring direction of diffusion, scalar indices can be calculated such as the mean diffusivity, this is a measure of the averaged molecular motion; this value is influenced by both cell integrity and size.

1.6.2. Background to magnetisation transfer imaging

Magnetisation transfer is a quantitative method of MRI that can characterise tissues at the microstructural level\textsuperscript{164}, due to its ability to detect change in tissue contrast it can indirectly measure fixed macromolecular structure. MTR has been found to identify abnormalities in patients with inherited prion disease that are not evident on conventional MRI\textsuperscript{9}. Detecting whether a change in the MTR correlates with disease progression is of importance, if a correlation is identified, then MTR may prove to be a suitable biomarker that could be used in therapeutic trials to measure response to pharmacological agents.

Dr Bob Balaban was the first to discover the potential use of MTR as an imaging sequence. The researchers were at the time performing a spin echo experiment that involved selectively saturating urea and looking for small signal suppression in water. They found instead that there was a significant reduction and loss of proton signal, which was not dependent on the offset frequency of the irradiation. The signal suppression came to be known as magnetisation transfer\textsuperscript{164}. The ability that magnetisation transfer has to identify a contrast between free and bound protons has led to its use as an in vivo marker of pathological change in a number of neurodegenerative diseases. The majority of MTR research has been performed in patients with multiple sclerosis (MS)\textsuperscript{165-167}. The magnetisation transfer sequence has been able to illustrate the natural history of white matter disease and magnetisation transfer abnormalities are evident in patients with MS prior to them being visible on conventional T2-weighted MRI\textsuperscript{168,169}. In addition to its ability to identify white matter abnormalities
Magnetisation transfer imaging has been able to demonstrate grey matter change too\textsuperscript{170,171}. Abnormal MTR measures have also been found in both patients with Alzheimer’s and Huntington’s disease\textsuperscript{172,173}; changes have been identified both in symptomatic patients and in pre-manifest Huntington’s disease carriers\textsuperscript{174}.

The application of MTR imaging in patients with prion disease has been investigated as a measure of underlying microstructural change. Siddique et al. found that on cross-sectional analysis of 18 patients with inherited prion disease MTR histogram parameters were significantly lower in symptomatic patients. Additionally on longitudinal analysis they identified a correlation between a decline in MTR histogram measures and a decline in clinical outcome measures, namely the Clinician’s Dementia Rating scale and the Barthel\textsuperscript{9}. De Vita et al. have also identified specific anatomical changes present on VBA of MTR in a sub-group of inherited prion disease patients with the 6 OPRI \textit{PRNP} mutation\textsuperscript{175}.

Conventional MRI sequences detect signal from mobile protons. The more mobile the proton, then the longer the relaxation time (T2) and the more MR visible they are. The T2 of bound macromolecules is too short to be detected by conventional MR sequences. MT is a means of making the bound macromolecular pool more MR visible. To obtain the MT, an off-resonance radio-frequency (RF) pulse that preferentially saturates the bound macromolecules is applied. The macromolecules have much broader frequency absorption than the free proton pool and therefore it is easier to saturate their spins. The transfer or exchange of the saturation from the bound macromolecules to the unbound proton pool is known as the magnetisation transfer and can then be detected by MRI (see figure 10). The liquid pool's spin number is normally given the value 1 (M\textsubscript{0A} =1). The macromolecular bound pool is less than this and given as a fraction of the liquid pool (M\textsubscript{0B}). After the application of the off-resonance RA pulse a transfer of spins from the bound to the liquid pool occurs, this is known as the M\textsubscript{SAT}.
The ratio of this transfer has been conventionally used as a method of measurement and can be calculated from knowing the value of the $M_{\text{SAT}}$ and the $M_0$. 

$$MTR = \frac{(M_0 - M_{\text{SAT}})}{M_0}$$

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**Figure 10**

*MRI sequences: $M_0$, $M_{\text{SAT}}$ and MTR images*

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### 1.6.3. Voxel based analysis

Voxel based morphometry (VBM) is a quantitative statistical method used to identify focal volumetric differences in brain anatomy, the areas of difference found are then represented on statistical parametric maps. VBM is widely used in neuroimaging research, not only in neurodegenerative diseases such as Huntington’s, Alzheimer’s and fronto-temporal dementia but also in psychiatric illnesses such as schizophrenia, it has been shown to pick up subtle volumetric changes that are not evident on conventional MR imaging.

When first introduced VBM analysis was initially confined to identifying regions of interest (ROIs) where structural anatomical changes were theorised to be occurring. The drawback with such an approach is that the extent of whole brain involvement in a particular disease or process is not measurable. Statistical
Parametric Mapping (SPM) was developed to measure global effects. Each voxel within the brain is statistically analysed, analysis of covariance (ANCOVA) of each of these voxels is applied, and in this way regional differences can be more specifically determined.

Voxel-based analysis (VBA) is a similar process to voxel based morphometry, however unlike volumetric analysis, voxel based analysis does not measure atrophy or volumetric brain matter change instead it measures voxel-wise differences of the quantitative method being analysed, i.e. in the case of voxel based analysis of MTR, within a concentration of grey or white matter the MTR values that differ the most on a voxel by voxel basis are compared and those regions of voxels that are found to differ significantly are depicted on statistical parametric maps.

![Image](image.png)

**Figure 11**

*The processing steps involved in voxel-based analysis*

### 1.6.4. Histogram analysis

MRI histogram analysis is a quantitative method of depicting the numbers of voxels within an area of the image analysed that all fall within the same signal intensity. Each of these sets of values is known as a bin and the bins are then
displayed as non-overlapping, but continuous sets of values on a frequency distribution graph. The following descriptive statistics can be obtained: the mean, median, peak height and peak location, the kurtosis, which is a measure of the shape and peak of the probability distribution and the skewness, the measure of how asymmetrical the probability distribution is, as well as measurement of the different percentiles, which represent the values in which a percentage of observations are calculated.

Histogram analysis can be used to analyse different imaging parameters including MT and diffusion weighted imaging, it is particularly applicable where pathology is likely to be diffuse and involve large less localised areas of tissue, such as that seen in diseases like multiple sclerosis or prion disease. It can be employed in measuring whole brain parametrics, or segmented grey or white matter regions, the major advantage of using this technique is that prior knowledge of the location of pathology is not required. Figure 12 is an example of the MTR histogram frequency distribution curves for 3 subject groups.

Figure 12
MTR histogram for 3 subject groups, illustrating the distribution curves i.e. the number of voxels falling within certain MTR values
1.6.5. Region of interest analysis

ROI analysis involves the segmentation of anatomical structures or areas within an image and selection of the neuroimaging data or voxels within that region and averaging of the voxels within it. The advantage of this technique is that detailed, localised information is obtainable when there is a priori knowledge of specific areas of tissue that are known to be affected by a disease process, for example the caudate in Huntington’s disease or the hippocampus in Alzheimer’s. Another way in which ROI’s are used are when specific anatomical regions have been identified on voxel-wise analysis as being of interest, ROI’s can then be used to explore those areas further, the benefits of using ROI analysis in this way are that the need for very strict multiple comparison corrections is mitigated as the number of voxel by voxel testing is less in the ROI and therefore the sensitivity of analysis is increased, however if there is no a priori hypothesis regarding a specific region being affected then it maybe that the area identified is a false positive. Other disadvantages of ROI analysis include accuracy of the application of the ROI, particularly if it is manually drawn, and also inter-user reliability and reproducibility.

1.6.6. Multi-atlas propagation and segmentation analysis (MAPS)

This is an automated method of segmenting the whole brain into individual regions of interest. It uses multiple atlases where specific anatomical structures have been delineated. The atlas template is non-rigidly registered and warped to the target (subject’s) image, the structure labels are then propagated into the target image and the mean voxel intensity within each region analysed. Because there is variability in brain anatomy between individuals, using a single atlas to segment a patient cohort is unlikely to be truly representative of all individuals and therefore the multiple atlas approach is likely to be a more precise method of analysis. The benefits of using MAPS in comparison to manual segmentation for ROI analysis are that is much less time consuming
and there is less inter-user variability, also if there is no a priori hypothesis of a specific area being affected by disease then multiple areas can be analysed. Figure 13 depicts the multi atlas segmentation maps.

**Figure 13**

*Region of interest segmentation maps illustrating anatomically segmented brain regions in axial, sagittal and coronal brain views (left to right)*
1.7. **Quantitative histopathology**

To date, histopathology vs. imaging correlations have relied on semi-quantitative methods of histology analysis such as the Braak and Braak staging for Alzheimer’s disease\(^\text{176}\). However there now exist quantitative software packages such as Definiens Tissue Studio that have been specifically developed to perform ROI histopathology analysis. Use of quantitative histopathology analysis has mainly been focused on extra-neural cancers and musculoskeletal tissue analysis, although there has been research conducted in the nervous system too. Several studies have assessed its reliability and found high levels of concordance with a pathologist’s evaluation\(^\text{177,178}\).

Definiens Tissue Studio is software that was developed for the analysis of a range of histopathology assays, including tissue slides, micro-arrays and microscope images. The process involves selecting areas for analysis using the ROI classification feature, allowing the user to customise the area for analysis. The digital image is uploaded after the slide has been scanned using Leica slidePath software. The imported Definiens digital image is a 2D raster image, with each image layer being composed of an array of pixels. The image is divided into tiles (smaller sections of the entire image), the analysis is conducted on each individual tile and then the images are sewn together.
1.8. The National Prion Monitoring Cohort (NPMC) study

In order to be able to enter into clinical therapeutic trials an understanding of the clinical aspects and natural history of prion disease is required and the development of biomarkers of disease onset and progression is hugely important in being able to assess the efficacy of therapeutic agents. The NPMC study is an observational study that was designed to do this, and a wealth of data have been collected on the course and clinical features in patients with all forms of prion disease as well as those at risk of developing the illness. One of the main outcomes of this study has been the development of clinical biomarkers such as the MRC Prion Disease Rating Scale (MRC Scale), which has proved to be an effective and reliable predictor of progression of prion disease\(^\text{15}\). Another aim of the cohort was to develop both quantitative diagnostic biomarkers as well as those that can monitor progression of disease. Both quantitative and conventional MR imaging sequences have been obtained in a large number of subjects recruited to the NPMC, I carried out a detailed analysis of quantitative MRI in a cohort of symptomatic and at risk asymptomatic patients, which is discussed and presented in chapter 3. I also investigated the utility of conventional MRI sequences as a combined biomarker with prion strain subtype and codon 129 genotype as a predictor of disease progression, this project is detailed in chapter 2.

The role of the NPMC has not only been confined to preparing for clinical trials but also aimed at recognising the diverse clinical features that exist in what is quite a heterogeneous group of patients, as well as further understanding the association between disease features and investigations such as MRI. The work in chapter 4, examines the correlation between MRI signal change and clinical features of prion disease most notably sleep disturbance, testing the hypothesis that sleep abnormalities are highly prevalent in all forms of prion disease, caused by thalamic pathology and depressive symptoms are more prevalent in those with sleep disturbance. In addition to testing this hypothesis, my aim was to develop the Prion Disease Sleep Questionnaire, a targeted and
quantitative method for collecting and analysing sleep symptomatology in prion disease.

An important aim of the cohort has been to assess what imaging findings represent on a microstructural level. In chapter 5 I test the hypothesis that DWI represents spongiform change in patients with prion disease and assess if there is a correlation between individual markers of neuropathology.
2. **Codon 129 combined with MRI signal change acts as a predictor of rate of disease decline in patients with sporadic CJD**

2.1. **Introduction - Literature review: Codon 129, strain and imaging changes and their correlation with clinical phenotypes of sCJD**

Prion diseases display a wide degree of both clinical and pathological heterogeneity, not only between the different forms of prion disease (inherited, sporadic and acquired) but also within the largest category of patients, those with sCJD. sCJD disease phenotypes are classically categorised according to the most dominant clinical symptoms and disease characteristics that patients present with; examples of these are: classical sCJD, Heidenhain and Oppenheimer-Brownell variants. As there are more patients identified and diagnosed with sCJD, it is recognised that there is clinical variability beyond these well-described subtypes. Other clinical phenotypes that have been described include: thalamic, psychiatric, cognitive, fatal insomnia, cerebellar and ataxic subtypes. In most cases of sCJD the disease duration is rapid, typically 4-6 months from symptom onset to death. However, variability in disease duration has been observed, both faster and slower than the typical 4-6 months, with approximately 10% of cases surviving over one year and in some over two years. In addition to the heterogeneity seen with the clinical phenotype, variability in the pattern of sCJD histopathology has also been described. This includes both the degree of spongiform change, gliosis, neuronal loss and amyloid deposition, and the distribution/areas within the brain where the pathology occurs. This heterogeneity can somewhat be explained by the identification of molecular mechanisms such as codon 129 genotype and the pathological scrapie prion protein strain that have both been found to act as powerful disease modifiers and have also been found to be associated with specific presentations of disease and histopathology findings.

The genetic polymorphism coding for methionine and valine at Codon 129 on the prion protein gene has been found to be both a powerful predictor of
disease progression in patients with sCJD\textsuperscript{15,182}. It has also been found to influence clinical phenotype and to be associated with specific investigation findings including those seen on MRI brain scans in patients with sCJD\textsuperscript{3,62,184-186}. There are 3 codon 129 genotypes, methionine homozygous: MM, methionine and valine heterozygous: MV and valine homozygous: VV. In the Caucasian population 52\% of patients are homozygous for methionine at codon 129 (MM), 36 \% heterozygous (MV) and 12\% homozygous for valine (VV)\textsuperscript{187}.

sCJD and iCJD are associated with the accumulation of 2, or 3 common scrapie prion strains, whether it is 2 or 3 depends on the classification system used to subtype them. In the Parchi classification the strains are 1 and 2a and under the London classification 1, 2 and 3, 1 of the Parchi classification corresponds to 1 and 2 of the London classification and 2a correlates with 3 of the London classification (see table 4 and figure 14). Prion strain variation has been found to not only affect the clinical phenotype, but also the incubation period to disease onset as well as neuropathology findings seen on post-mortem or brain biopsy\textsuperscript{65,69}.

<table>
<thead>
<tr>
<th>Parchi classification</th>
<th>London classification</th>
<th>Codon 129</th>
<th>Type of prion disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>MM</td>
<td>sCJD, iCJD</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>MM, MV, VV</td>
<td>sCJD, iCJD</td>
</tr>
<tr>
<td>2a</td>
<td>3</td>
<td>MV, VV (few cases of MM)</td>
<td>sCJD, iCJD</td>
</tr>
</tbody>
</table>

Table 4
Prion strain types Parchi and London classifications with corresponding codon 129 and prion disease type
Figure 14

*London Classification of PrP\textsuperscript{Sc} Western blot, depicting the different strain subtypes associated with sCJD (PrP\textsuperscript{Sc} types 1, 2 and 3) and vCJD (PrP\textsuperscript{Sc} type 4) (figure supplied by the MRC Prion Unit)*

Prion strain subtype can be identified on Western blot and is recognised by the size of the PrP\textsuperscript{Sc} region that is resistant to proteinase digestion. It has been observed that the PrP\textsuperscript{Sc} type is associated with specific disease phenotypes and it therefore may act as a modifier of disease, Morales et al hypothesised that smaller prion strains may be more toxic due to them aggregating more easily and thereby may contribute to shorter incubation periods and more rapidly progressive disease\textsuperscript{188}. Codon 129 and PrP\textsuperscript{Sc} strain type are unlikely to be completely independent of each other as the MM genotype is associated with Parchi strain type 1, (London strain types 1 or 2) in over 90 % of cases and MV and VV cases are associated with strain type 2a in over 80 % cases (London strain 3). There have been attempts to further classify and characterise specific clinical phenotypes and investigation findings, such as MRI signal change in sCJD by combining both the strain type and codon 129 polymorphism into 1 of 6 different subtypes (based on the Parchi strain classification), these being: MM1, MM2, MV1, MV2, VV1 and VV2.
The classically described clinical phenotypes aren’t 100% specific to 1 molecular classification. Broadly speaking the Parchi MM1 variant which is reported to occur in 60% of cases correlates most with the classic sCJD type, which is typically rapid cognitive decline, myoclonus and extrapyramidal/pyramidal signs, visual symptoms can also occur, typical of the Heidenhain variant\textsuperscript{189}, the disease duration is short (an average of 4 months) and periodic sharp wave complexes (PSWCs) are more likely to be present on the EEG\textsuperscript{68,154}. The Parchi MV1 subtype is less common and more likely to have a cerebellar presentation, similar to that seen in the Parchi MM1 genotype, it has a short disease duration of around 5 months\textsuperscript{190}.

The characteristic histopathology findings for both Parchi MM1 and Parchi MV1 variants are: fine spongiform change affecting the entire cortex, with gliosis and synaptic PrP\textsuperscript{Sc} deposition. Spongiosis is more likely to affect the cortex and particularly the occipital lobe rather than the deep grey matter nuclei\textsuperscript{154,189,190}. 16% of cases are the Parchi VV2 subtype, which is most commonly associated with a cerebellar clinical presentation and the disease duration is slightly longer, around 6 months. There are less likely to be PSWCs present on the EEG. In this variant, spongiosis characteristically affects the cortex, the frontal cortex and the deep grey matter nuclei being more severely affected than other cortical areas\textsuperscript{190}. There are also prominent histopathological changes reported in the cerebellum\textsuperscript{154}.

Parchi MV2 is responsible for around 9% cases and is more likely to have a cerebellar/ataxic presentation along with the usual classical sCJD symptoms of myoclonus, cognitive impairment and pyramidal/extrapyramidal signs. In this group the most distinctive finding is the extended disease duration of around 12 months\textsuperscript{191}. The characteristic findings at post-mortem are the presence of kuru like plaques that are predominantly situated in the cerebellum, in addition to this there are also more likely to be large confluent vacuoles present rather than the fine spongiosis that is present in the Parchi MM1 and MV1 subtypes.
The Parchi MM2 subtype also has a longer disease duration (median time of 16 months)\textsuperscript{192}; the more common cortical clinical presentation is that of cognitive decline with the development of classical CJD features as the disease progresses. Cerebellar or ataxic features are uncommon. PSWCs are not typically seen on the EEG. Widespread diffuse large confluent vacuoles are present on histopathology; with gliosis also being present both in the cortex and deep grey matter. PrP\textsuperscript{Sc} deposition is classically seen to be deposited in a perivacuolar pattern as well as being present as plaques.

In around 2% cases Parchi MM2 type is associated with a thalamic phenotype, which has also been described clinically as sporadic fatal insomnia (SFI). This has a similar disease phenotype to that seen in the inherited prion disease familial fatal insomnia (FFI), which is secondary to the D178N prion protein mutation, with methionine homozygosity at codon 129. Clinical features are: a longer disease duration (average 24 months); insomnia and autonomic dysfunction accompanied by classical CJD symptoms and significantly disrupted sleep architecture on EEG. Pathology is maximal in the thalamus; the characteristic findings are severe gliosis and neuronal loss\textsuperscript{193}; again these neuropathology findings are similar to what is seen in patients with FFI. PrP\textsuperscript{Sc} is present but to a lesser extent than that seen in the more usual MM2 presentation. The glycoform ratio is different in the SFI phenotype and may represent a sub-strain of type 2 PrP\textsuperscript{Sc} that is associated with this disease presentation.

Parchi VV1 is the least common subtype of sCJD, this subtype is seen in about 1% of cases. There are very few cases that have been reported in the literature. Patients who have this subtype have been described as presenting with a younger age of onset, and will often have a psychiatric clinical presentation that is followed by slow but progressive cognitive impairment. The average disease duration is around 21 months. There are also reported cases with shorter disease duration but due to the limited numbers of cases described it is difficult
to know what represents the truly typical phenotype. In a case series of 11 patients 9 of these had PSWCs present on EEG\textsuperscript{134}.

**MRI and association with codon 129 genotype**

Meissner et al.\textsuperscript{3} looked at specific MRI lesion profiles in sCJD in a cohort of 211 CJD patients. They categorised patients by codon 129 and strain type and then looked for a correlation between DWI changes in specific cortical, deep grey matter and cerebellar regions present on their MRI head scans. They did this for each codon 129 genotype and for codon129 combined with strain subtype. They found that basal ganglia signal was more common in the MV2, VV2, MM1, cortical signal change was more likely to be increased in VV1, MM2, MV1 and thalamic signal change was more predominant in the VV2, MV2 subtypes. They then looked at which areas were most affected for codon 129 genotype and also strain and codon 129 genotype combination, they found that those who were the MM genotype were most likely to have frontoparietal cortical signal change, MM1 were found to also have both basal ganglia and cerebral cortex involvement. MM2 subtypes had widespread signal change as well as thalamic and cerebellar changes. MV cases commonly had cortical signal change and the most affected region was the frontocingulate gyrus, MV2 had widespread cortical, basal ganglia, thalamic and cerebellar involvement, the cortical signal change predominantly affected the insular. VV2 was most frequently associated with basal ganglia signal change as well as affecting the cingulate gyri. The VV1 subtype was most commonly associated with cortical signal change in the parietal and temporal lobes. They also found that limited cerebral cortical hyperintensity and thalamic hyperintensity were related to PrP\textsuperscript{Sc} type 2 and valine homozygosity at codon 129, in addition to age at onset and having a long disease duration.

Although the sample size for this study size was not large many associations between prion protein subtype, codon129 and MRI lesion profile were looked at and the authors were able to find several associations between these
parameters. The overall findings appear to be that both cortical and basal ganglia signal change are associated with most of the genotype/strain subtypes, but there are certain brain regions that appear to be more associated with specific subtypes.

Collins et al performed a meta-analysis of a number of small studies with over 1000 patients with sCJD with MR brain imaging, EEG and CSF analysis, the aim of their study was to determine the overall diagnostic sensitivity of these investigations across the clinical spectrum of sCJD. They found that both MV and VV genotypes were more likely to have basal ganglia signal change and VV2 patients had the most typical MRI findings. In addition to this they also found that MRI signal change was not influenced by either the patient’s age at the onset of disease or with disease duration, whereas 14-3-3 presence on CSF analysis and PSWCs found on EEG were. These findings indicate that MRI could prove to be a less variable parameter than other investigation findings such as EEG and CSF and may therefore be a useful biomarker of disease progression for use across all patient age groups.

Other researchers have also commented on the association between genotype and imaging findings in sCJD, Macfarlane et al. state that patients with the MV genotype are more likely to have hyperintensity present in the basal ganglia on T2 weighted or DWI MRI sequences.

There have been a small number of studies that have looked at whether MRI signal change in patients combined with specific genotype and strain has been associated with a specific clinical phenotype. Krasnianski et al. looked at clinical findings and diagnostic tests in patients with sCJD who had the MV2 and MM1 subtype, they found that in patients with the MV2 subtype basal ganglia signal change was present in 90% on T2 weighted imaging, and cortical signal change was present on DWI in 88% with the frontal cortex being the most affected region. All patients had psychiatric symptoms. In the MM1 group 83% patients had basal ganglia signal change present. Other studies have examined
for the presence of both clinical disease progression and symptom association with MRI findings. Meissner et al. looked at patients with sCJD, specifically at the clinical features of the disease, codon 129 genotype, strain and MRI signal change in a total of 219 patients (153 definite confirmed cases of CJD, and the rest probable, the diagnosis based on clinical+- supportive MRI findings) they reported that patients with basal ganglia signal change on their MRI scan had a shorter survival period than those that didn’t (2.7 vs. 4.9 months). They went on to perform multiple testing which did not reveal a significant association between clinical symptoms and MRI findings, however on logistic regression analysis they looked at the presence of symptoms at onset including dementia, hallucinations, frontal lobe dysfunction, sensory abnormalities, depression, akinetic mutism, as well as age, sex and PRNP genotype as predictors of MRI signal abnormalities and found that basal ganglia signal change was associated with dementia in 80% cases and that an absence of basal ganglia signal change was more likely to be associated with depression and sensory abnormalities.

In conclusion there have been a number of studies that have attempted to examine the relationship between codon 129, PrPSc, MRI findings and clinical phenotype. To date there don’t appear to have been any larger studies in patients with sCJD that have looked at a range of typical sCJD MRI findings and analysed whether these could be used as a prognostic value, in acting as a predictor of clinical disease progression. There are studies that have looked at codon 129 +/- strain types and its influence on disease phenotype, incubation and duration, but none that have been used in combination with MRI signal change. Possible reasons a study like this has not been conducted include the fact that recruiting patients to such a study in large enough numbers is difficult to achieve given the rarity of the disease and clinical health of the patients. In addition to this a meta-analysis of studies has not proved possible with there having not being a universally adopted use of a clinical outcome measure that assesses rate of disease progression. The National Prion Monitoring Cohort has the largest number of patients with prion disease recruited to any cohort.
study, and they use the MRC Scale, which is a reliable and validated clinical outcome scale. It has therefore been possible to perform an analysis on a considerably sized dataset.

2.2. The National Prion Monitoring Cohort-Description of study, aims and achievements

Prion diseases are rare conditions, and the ability to fully understand the mechanism of pathology and measure clinical progression is important in being able to develop and trial disease modifying agents. There have been a number of prospective cohort studies conducted in other European countries but until 2008 there have been no large studies in the UK that have collected prospective data on the molecular and clinical phenotypes of all forms of prion disease. With the absence of neurological rating scales and biomarkers of both onset and progression it has been difficult to accurately measure response to therapeutic treatment.

In 1995 a new form of prion disease was identified, vCJD, this was caused by the same prion strain as BSE, the epidemic of prion disease that affected cattle in the mid-1980s to 1990s. With the knowledge that prion diseases can have long incubation periods there has been concern that a human prion disease epidemic may occur. With this in mind the National Prion Clinic was established in 2001 and in 2004 the MRC PRION-1 trial commenced with a UK national referral system for patients suspected of having prion disease also implemented at the request of the Chief Medical Officer, a coordinated approach was made with the aim being to not only provide specialist input in identifying, managing and caring for patients with all forms of human prion disease, but also to collect valuable epidemiological and clinical research data. PRION-1 was a drug therapy trial that examined the safety and efficacy of quinacrine use in patients with prion disease, however only a small number of patients were eligible for recruitment and treatment and there was difficulty in obtaining longitudinal data.
due to patients being too unwell to attend serial neurological assessments. Because prion disease is rare there was also a lack of knowledge on the variability of the clinical phenotypes of the different forms of prion diseases, which are now known to be a heterogeneous group and therefore further information on the natural history of each subtype would be required to reliably monitor and predict disease progression in the different subtypes of disease. It was appreciated that a structured study that examined the course of clinical disease was essential, in order to acquire knowledge of the phenotype of each prion disease subtype, and to identify possible biomarkers that could be used in future clinical trials.

The National Prion Monitoring Cohort (NPMC) is a longitudinal study that was initiated to collect prospective data on the natural history of both patients with and at risk of developing prion disease. Its main aim is to monitor all patients with a diagnosis of prion disease and those at a high risk of developing it. The main study objective was to obtain as much information as possible on clinical features of disease in order to develop staging systems and markers of change that could be used in future drug therapy trials.

All patients with probable or definite human prion disease were eligible for recruitment, including sporadic, variant, iatrogenic and inherited subtypes. Patients who were at risk of developing disease were also recruited, these included recipients of blood products from patients that later developed vCJD and relatives of patients with prion disease who were both gene mutation carriers and untested for PRNP gene mutation. A healthy control population of patients was also recruited to the study. The decision to include both asymptomatic and symptomatic patients was made in order to find possible biomarkers of onset in the asymptomatic patient group as well as to identify markers of disease progression.

The study was commenced in 2008, at November 2019, 836 patients have been enrolled with a recruitment rate of over 90%. Patients are recruited into
one of 4 different groups, those symptomatic with prion disease, those confirmed as either being gene positive for inherited prion disease or with known pre-clinical infection, those at increased risk of developing prion disease without confirmed diagnosis and healthy control or prion disease “mimic” participants. Patients eligible for recruitment were required to reside in or be independently able to travel to the UK. Symptomatic patients who were fit to travel or in close proximity to the National Hospital for Neurology and Neurosurgery (NHNN), in addition to asymptomatic and control participants attended cohort clinic days held at the NHNN for both enrolment to the cohort as well as clinical assessment and investigation.

The goal of the study is to collect as much information as possible on both clinical features and investigation findings in the cohort subjects and therefore the ideal acquisition of data included: an in depth clinical assessment, neuropsychological assessment and the following investigations: MRI, EEG, EMG, NCS and actigraphy as well as blood tests including molecular genotyping and CSF analysis. In those patients who had brain biopsies performed the histopathology results were also collected and post-mortem examination was carried out in consented patients. Not all patients would be able to have in depth investigations due to their clinical status and geographical location; however it was important to be able to collect as much clinical data possible, therefore the minimum data requirement for enrolment to the cohort was set as patient’s clinical history, management and neurological examination.

The standard follow up of all patients with symptomatic disease was set at 6 monthly intervals; optimum follow up of those with rapidly progressive disease was 0, 0.75-1.5, 3, 6 months and then every 6 months. Telephone follow-up was also conducted at 0.25-1.5 monthly intervals and repeated dependent on symptoms and clinical progression. The follow-up schedule was made flexible to accommodate for the heterogeneity of the different disease subtypes as well as the individual patient’s clinical status.
Diagnostic criteria and inclusion into the cohort depended on the subtype of prion disease. A confirmed/definite diagnosis of prion disease was made whenever possible, through demonstrating PrP\textsuperscript{Sc} in tonsillar tissue in cases of suspected vCJD and brain histology in those with sporadic or iatrogenic disease. In inherited prion disease cases detecting a pathogenic mutation on the PRNP gene would be used to make a definite diagnosis. In cases where histology/genetic diagnosis was not obtainable the cases would be presented to an expert panel and a decision made as to whether patients had probable prion disease. For the largest group of patients, those with sCJD, the WHO diagnostic criteria combined with supportive MRI imaging findings was used to categorise patients into having possible, probable or definite disease. Patients with probable or definite sCJD were deemed eligible for inclusion in the cohort, this criteria was also used for patients with iatrogenic disease and a history of exposure. Similarly patients with vCJD were also classified according to the WHO criteria. Patients with inherited forms of the disease required PRNP analysis for diagnosis.

Where appropriate, patients or their relatives were asked if they were willing to discuss and consent to a post-mortem examination and consent would be obtained either at enrolment or at a subsequent follow up appointment. If patients were found to have an alternative diagnosis on histological examination they would immediately be removed from the cohort.

Consent for enrolment in the NPMC was acquired from either patients or their relatives, when a patient was unable to consent. The study was approved by the Scotland A Research Ethics Committee. The clinical information obtained during the course of the patient’s disease was collected on clinical research forms (CRFs) and entered into a secure database. All patients who agreed to further tests such as blood tests, EEG or MRI scan would separately consent to each investigation that was conducted. Patients were stratified into specific groups depending on the type of prion disease and likely speed of disease progression.
Stratum 1 included those with sCJD, vCJD and the rapidly progressive inherited prion disease subtypes, those with E200K, 4 OPRI, D178N, E211Q and V2101 mutations. Stratum 2 included patients with inherited prion disease who were expected to have slower disease progression. The third group, stratum 3, included the at risk and control participants, patients from this group may be reclassified and move between strata, if for example an asymptomatic patient developed symptoms of prion disease they would be moved from stratum 3 to stratum 1 or 2.

In my role as a clinical researcher I visited patients around the UK, enrolled them to the NPMC and recruited 90 participants in total to the study. In addition to recruiting patients to the cohort I also visited them during the course of their illness to conduct further assessments and conducted virtual MRC Scales over the phone. The majority of patients I recruited were those with the sporadic form of the disease, but I also recruited patients with variant, iatrogenic and inherited forms of disease too, as well as those who are at risk and control participants. Data I present in this chapter includes patients recruited by co-workers.

Another of the key objectives and aims of the NPMC was to identify and develop markers of onset and progression in patients with or at risk of prion disease and therefore a number of forms of data have been collected, these include the clinical information, patient symptoms and clinical examination findings, in addition to the molecular genotype, neuropsychological parameters, MRI, EEG, CSF, serum and histopathology studies.

Besides collecting clinical data for the development of staging systems such as the MRC Scale, MRI data was also acquired. Both conventional scans and quantitative imaging sequences were obtained to aid in diagnosing the disease and to obtain important data that could be used to develop a biomarker to measure efficacy of drug therapies in future clinical trials. In addition to recruiting and following up NPMC study participants I also organised for patients to attend cohort days at the NHNN where they would be assessed by
myself or one of the other clinical researchers and would undergo a number of investigations, including an MRI brain scan, neuropsychology assessment, EEG and neurophysiology studies. I performed a considerable proportion of the work done both in organising and analysing the MRI sequences conducted on patients at the NHNN. I also obtained MRI scans on patients I recruited to the cohort from outside hospitals where the patients were scanned and which data have been used in a number of research projects; including the projects that I have conducted as part of my PhD. In addition to my role in recruiting new patients to the cohort, I also carried out regular clinical follow up assessments of patients and control subjects that were already recruited to the NPMC. I worked with Dr Harpreet Hyare, Dr Enrico De Vita and Dr John Thornton to categorise patients into the correct subgroups of prion disease. I conducted the anonymisation of the majority of scans acquired for patients recruited to the cohort. I also conducted the quantitative MRI analysis on all MTR sequences acquired on subjects scanned at the NHNN, which included both voxel-wise analysis and ROI analysis with fellow researcher Dr Enrico De Vita.

2.2.1. The Medical Research Council Prion Disease Rating Scale (MRC Scale)

One of the aims of the NPMC has been to collect information on the progression of prion disease in order to develop a clinical outcome measure that could be used to predict disease progression and to measure response to disease modifying treatment in future clinical trials.

The first analysis of the cohort data was conducted on 1337 clinical assessments and 479 telephone assessments in a total of 437 participants. A new outcome measure, the MRC prion disease rating scale (MRC Scale) was developed from the collection of clinical data from 3 scales: the Clinical Disease Rating Sum of Boxes, the Barthel and the Glasgow Coma Scale. This 20-point linear outcome measure was formulated from a combination of modified parts of
the 3 scales assessing cognitive function, speech, mobility, the ability to carry out personal care, feeding and continence. The scale was developed by using Rasch analysis for item modelling, correlation with other clinical scales and tested for inter-rater reliability. The MRC Scale is illustrated in the appendix on page 305. With the use of the MRC Scale as a clinical outcome measure different patterns of rate of disease progression were observed. Fast linear decline, a slow rate of decline, mostly seen in patients with inherited forms of prion disease and a more rapid decline followed by a plateau phase where patients scored lowly on the MRC Scale for a long time prior to death\textsuperscript{15}.

Data from the NPMC cohort have been able to illustrate the true heterogeneity of prion disease. Using a single outcome measure in both slowly and rapidly progressive disease will be difficult in a clinical trials scenario and the MRC Scale may not be able to pick up the subtle cognitive deficits that patients with some forms of inherited disease first present with. Therefore the MRC Scale is more likely to be of use in patients with rapidly progressive, stratum 1, type prion disease who have presented earlier on in the disease process before the ‘plateau phase’ where there is little further disease progression, to measure response to future disease modifying therapy.
Figure 15

The trajectories of disease progression seen in patients with inherited, variant and sporadic forms of prion disease

After the development of the MRC Scale, further analysis of the NPMC data has been conducted in order to establish an effective and powerful clinical trial design and to work out how many patients may be required to participate in a clinical trial measuring drug efficacy. Using a total of 2681 MRC Scale assessments in 598 participants, a number of linear mixed models were fitted to outcome measures to simulate clinical trials and identify which parameters could increase the power of a study in patients with sporadic CJD. It was found that a 50% decline in the MRC Scale would only require 120 participants assessed every 10 days to adequately power it. By restricting PrP codon 129 genotype to MM, VV or MV subtypes the power was increased even further.182
Additional analysis of specific MRI findings and strain subtype could influence disease progression and be included in a final model to be used in future clinical trials.

The collective achievements of the NPMC to date include: target recruitment with over 97% of eligible patients referred (see figure 16), and with under 1% drop out other than for mortality, an autopsy rate of around 65%, this is important, not only in being able to diagnose/confirm prion disease but also to aid the development of biomarkers, the development of a clinical outcome measure, the MRC Scale, the development of imaging biomarkers of disease progression, the development of EEG biomarkers of disease progression, the supply of serum samples for the development of both the Direct Detection Assay and other assays, engagement with patients and carers through the provision of teaching sessions and online web tools, and the acquisition of knowledge of specific prion disease symptoms, such as behavioural disturbance, myoclonus, and sleep disturbance which has aided in being able to provide better symptomatic care and specialist nursing support.

Figure 16
Recruitment figures for the NPMC with estimated (grey line) and actual recruitment figures (blue line)
2.2.2. The role of MRI in the diagnosis of prion disease

MRI is one of the key investigations used in helping to make a diagnosis of CJD in patients with both sporadic and variant forms of the disease. In variant cases the reported sensitivity is 78% and a specificity of 100%\textsuperscript{132,135}, for sporadic cases the sensitivity is reported as being between 83 to 92% with a specificity of up to 95%\textsuperscript{121,163,200}. In a recent study by Rudge et al. they looked at the role of imaging and CSF analysis in distinguishing CJD from possible mimics, diffusion weighted MRI was found to be the most useful and sensitive readily available test, (excluding brain biopsy) to classify cases correctly (92% CJD, 2% CJD mimics)\textsuperscript{89}.

The main MRI findings in patients diagnosed with sCJD are basal ganglia signal change, thalamic signal change is also seen and cortical ribboning, signal change seen in the cortical grey matter. Imaging sequences commonly acquired and used to identify the typical findings seen in sCJD are: DWI, ADC, T2 weighted imaging and FLAIR.

In patients with sCJD grey matter signal intensity is a characteristic finding on the DWI and FLAIR MRI sequences. High signal change is most evident on the DWI sequence (both with b-values of 1000s/mm\textsuperscript{2} and 3000s/mm\textsuperscript{2}) but also reported on FLAIR and T2 weighted imaging, correlation with restricted diffusion on ADC maps is important when reporting DWI signal intensity to eliminate the possibility of T2-weighted shine through. The deep grey matter nuclei are often affected including the caudate, anterior putamen and sometimes the thalamus. Cortical involvement is also a commonly reported finding, this is known as cortical ribboning, and is seen best on the DWI sequence, as signal hyper intensity with corresponding decreased apparent diffusion coefficient (ADC)\textsuperscript{120}. Other areas where signal change has been reported as being present are the cerebellum and hippocampus\textsuperscript{201}.
The benefits of using non-quantitative MRI brain scans as an outcome measure in patients with prion disease are: that it is non-invasive, that most hospitals in the UK have a 1.5 or 3T MRI scanner and patients with prion disease will usually have an MRI brain scan as part of their clinical work-up. There can be difficulties in acquiring scan data, and these are mainly due to acquisition issues. It can be difficult to transport patients with prion disease who are cared for in an out of hospital environment as they can severely symptomatic and due to the rapid progression in the disease can become rapidly moribund. The symptoms that patients have can affect the quality of the scans. Myoclonus is a clinical symptom frequently seen in patients with sCJD and patients may have myoclonic jerks causing movement of the head in the scanner, in addition to this patients may be cognitively impaired and therefore are not comprehend that they are required to lie still, both of these issues can result in movement artefact on the scans and result in uninterpretable data.
The following figures illustrate the typical MR imaging findings seen in sCJD patients.

**Figure 17**

*Axial DWI MRI sequence depicting typical signal change in a patient with sCJD - caudate and putamen signal change (upper arrows), in addition to thalamic signal change (lower arrows)*
2.2.3. Hypothesis: MRI signal change accompanied by codon 129 genotype predicts slope of decline in patients with sCJD

My hypothesis was that MRI signal change accompanied by codon 129 genotype can predict slope of decline in patients with sCJD and the aim of my research was to ascertain if this is the case and in addition to determine if PrP$^{Sc}$ strain type in combination with codon 129 genotype proves to be a better predictor of disease progression than solely codon 129 genotype in patients with sporadic CJD.

From the literature it is evident that codon 129 coupled with PrP$^{Sc}$ strain subtype is associated with a number of distinct disease phenotypes. It is also clear that
both clinical symptoms and investigation findings, such as MRI signal change, are more commonly associated with specific strains and codon 129 subtypes in patients with sCJD\textsuperscript{3,195,196}.

Using MRC Scale measurements as a measure of disease decline in patients with sCJD; linear mixed models can be used to estimate the rate of decline in each group (excluding the most severely impaired patients) Mead et al. found that codon 129 genotype is an important and powerful predictor of decline and disease progression in patients with sCJD\textsuperscript{182}. My hypothesis is that codon 129 combined with MRI signal change would act as a combined predictor of rate of decline in patients with prion disease and furthermore that MRI signal change coupled with both codon 129 genotype and PrP\textsuperscript{Sc} strain type would also act as a predictor of disease progression in patients with sCJD.

### 2.3. Methods

#### 2.3.1 Subjects and inclusion criteria

The total number of patients included in the study was 339. 406 symptomatic stratum 1 patients with sCJD that had been enrolled to the NPMC at the date of analysis were identified as potential subjects for this study. These patients all had more than one MRC Scale score recorded, that was over four at their enrolment visit. 369 of these patients had PRNP gene sequencing and a total of 339 also had MRI data available making the final number included in the study 339.

184 patients had brain tissue available, and of those 68 patients had Western blot performed, of those 62 had PrP\textsuperscript{Sc} strains identified.

Of the 339 patients included in the analysis, 187 of these were female, with a median age of disease onset of 67 years (range 45-85 years). 152 patients
were male with a median disease onset of 66 years (range 39-87 years). The majority of patients were methionine homozygous at codon 129. There were almost equal numbers of patients with the MV and VV genotype and there were no significant differences found in genotype incidence for each gender or for the rate of disease progression (MRC Scale slope) between male and female subjects. The age of onset was similar for patients with both the MM and MV genotypes and older in those with the VV genotype. See table 5.

<table>
<thead>
<tr>
<th>Codon 129 genotype</th>
<th>MM</th>
<th>MV</th>
<th>VV</th>
<th>MRC Scale Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>167</td>
<td>88</td>
<td>84</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>49%</td>
<td>(26%)</td>
<td>(25%)</td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>70</td>
<td>42</td>
<td>38</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>(47%)</td>
<td>(28%)</td>
<td>(25%)</td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>97</td>
<td>46</td>
<td>46</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>(51.6%)</td>
<td>(24.3%)</td>
<td>(24.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Median age at disease onset</strong></td>
<td>57</td>
<td>58</td>
<td>67</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(M=57,F=58)</td>
<td>(M&amp;F=58)</td>
<td>(M=69,F=67)</td>
<td></td>
</tr>
</tbody>
</table>

Table 5
Patient demographics table showing frequency of codon 129 genotype, MRC Scale slope, gender and age of disease onset for patients (total patient number =339)

The inclusion criteria were:

- Patients with a diagnosis of either probable or definite sCJD, this diagnosis was based on the WHO criteria accompanied by supportive imaging findings.
• Only patients who had an MRC Scale score of 5 or more at enrolment to the NPMC were included in the analysis. A cut-off value of 5 was chosen because there has been found to be variability in disease progression rate at end stage (MRC Scale score <5), some patients are found to plateau whilst others have a more linear decline. The slope of disease progression for those enrolled between MRC Scale Scores 20-5 in most patients follows a more predictable and linear course\textsuperscript{15}. Codon 129 genotype has been found to act as a predictor of decline at earlier stage of disease whereas when disease has progressed to an MRC Scale score of less than 5 the association with codon 129 has not been found to be significant. Patients with inherited prion disease, variant and iatrogenic forms were not included in this analysis. The reason for this is that depending on the underlying type of prion disease the disease phenotype can be very different and the rate of progression of disease can be highly variable.

• All patients had an MRI brain scan performed, which was reported by a neuroradiologist at the NHNN.

2.3.2. MRI acquisition

MRI brain scans were obtained from the majority of the 406 stratum 1 patients. Less than 10% (40 patients in total) did not have imaging available/performed. Most patients had their scans performed in their local hospital on a 1.5 Tesla scanner, which usually occurred at the hospital where they were diagnosed with sCJD. In these cases the scans were either copied onto a compact disc and sent to the NHNN or transferred electronically from the Picture Archiving and Communication System (PACS) via the Image Exchange Portal (IEP) to the receiving hospital’s PACS. Patients who attended the NHNN for a cohort assessment day also underwent an MRI brain scan at the NHNN on the 3 Tesla scanner, these scans were also included in the study. The majority of patients
recruited only had one MRI brain scan performed, but all MRI scan findings for each patient, regardless of the number of scans they had, were included in the study. All scans were reported by one of the NHNN neuroradiologists.

Specific sCJD MRI findings were recorded such as signal change, as well as non-specific ones such as atrophy in addition to any other abnormality that was observed. In this study the predictors that were picked were: atrophy, cortical signal change (cortical ribboning), basal ganglia signal change (caudate/putamen), the pulvinar sign and thalamic signal change, these findings were reported on the following sequences: T2W, FLAIR and DWI MRI.

2.3.3. Statistical analysis

IBM SPSS version 24 and STATA were used for conducting the statistical analysis.

- The 2 tailed student t-test was used to assess for the existence of significant differences between patient groups in those over the age of 65 years and disease onset time, page 98.

- The 2 tailed student t-test was also used to assess for significant differences in the rate of disease change (MRC Scale slope) in patients with and without signal abnormality in specific brain regions (table 8).

- Linear regression analysis was used to assess if codon 129, specific MRI parameters, age and gender acted as predictors of slope of change of the MRC Scale (dependent variable).

- Linear regression analysis was used to assess if codon 129 genotype and prion strain could act predictors of change in MRC Scale slope.
2.4. Results

Subjects whose disease onset occurred over the age of 65 years had more rapidly progressive disease, with a mean MRC Scale slope of 0.14 compared to 0.12 in those less than 65 years old. (t-test \( p\)-value 0.005).

The results for incidence of strain type are illustrated in table 6. There were no type 1 cases and similar numbers of type 2 and 3, with more female than male cases.

<table>
<thead>
<tr>
<th>Prion strain London classification</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Codon 129 genotype</th>
<th>Median age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MM</td>
<td>MV</td>
</tr>
<tr>
<td>Type 1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Type 2</td>
<td>56</td>
<td>20</td>
<td>36</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Type 3</td>
<td>47</td>
<td>21</td>
<td>27</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Average MRC Scale slope</th>
<th>MM</th>
<th>MV</th>
<th>VV</th>
<th>MM2</th>
<th>MM3</th>
<th>MV2</th>
<th>MV3</th>
<th>VV2</th>
<th>VV3</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>0.09</td>
<td>0.09</td>
<td>0.08</td>
<td>0.1</td>
<td>0.09</td>
<td>0.07</td>
<td>0.09</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Male</td>
<td>23</td>
<td>27</td>
<td>14</td>
<td>15</td>
<td>8</td>
<td>7</td>
<td>16</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>11</td>
<td>14</td>
<td>6</td>
<td>8</td>
<td>3</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>11</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 6

*Strain subtype, gender and codon 129 genotype (London classification of strain type), rate of disease progression measured by MRC Scale slope for each codon 129 genotype, and codon 129/PrPSc subtype*
Cortical signal change was the most frequently reported imaging finding, followed by basal ganglia signal change and then thalamic signal change and atrophy. There were only 4 cases of pulvinar signal change reported (see table 8).

<table>
<thead>
<tr>
<th>MRI findings</th>
<th>Total</th>
<th>Female</th>
<th>Male</th>
<th>Median age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal ganglia</td>
<td>221</td>
<td>129</td>
<td>92</td>
<td>66 (39-87)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>104</td>
<td>62</td>
<td>42</td>
<td>67 (45-87)</td>
</tr>
<tr>
<td>Cortex</td>
<td>255</td>
<td>140</td>
<td>115</td>
<td>67 (39-87)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>104</td>
<td>53</td>
<td>51</td>
<td>69 (47-85)</td>
</tr>
<tr>
<td>Pulvinar sign</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>58 (52-70)</td>
</tr>
</tbody>
</table>

Table 7
*Frequency of MRI imaging abnormalities for specific anatomical regions: basal ganglia, cortical ribboning, atrophy, the pulvinar sign and thalamic signal change*

Assessment of whether there was a difference in rate of disease progression for each MRI measure (cortical, basal ganglia and thalamic signal change in addition to atrophy) was conducted. Patients with thalamic signal change were found to have a statistically significant difference in rate of disease progression; this was longer than that seen in other MRI abnormalities (see table 8). There were less cases of MM homozygosity at codon 129 in patients with thalamic signal change than that seen with other signal change. Patients found to have atrophy on their brain scan were more likely to be MV or VV at codon 129.
<table>
<thead>
<tr>
<th>Region</th>
<th>MM (%)</th>
<th>MV (%)</th>
<th>VV (%)</th>
<th>Median MRC Scale slope</th>
<th>MRC Scale slope in patients with and without signal change for each anatomical region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>167</td>
<td>88</td>
<td>84</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>100 (60%)</td>
<td>60 (68%)</td>
<td>61 (72%)</td>
<td>0.11</td>
<td>0.93</td>
</tr>
<tr>
<td>Thalamus</td>
<td>34 (20%)</td>
<td>35 (40%)</td>
<td>35 (42%)</td>
<td>0.09</td>
<td>0.0056</td>
</tr>
<tr>
<td>Cortex</td>
<td>131 (78%)</td>
<td>61 (69%)</td>
<td>63 (75%)</td>
<td>0.11</td>
<td>0.45</td>
</tr>
<tr>
<td>Atrophy</td>
<td>47 (28%)</td>
<td>28 (32%)</td>
<td>30 (36%)</td>
<td>0.11</td>
<td>0.74</td>
</tr>
<tr>
<td>Pulvinar sign</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0.13</td>
<td></td>
</tr>
</tbody>
</table>

Table 8

Frequency of MRI signal change for codon 129 genotype and mean MRC Scale slope for each are of signal abnormality (2-tailed t-test to assess for statistically significant differences in rate of disease progression in patients with specific MRI scan finding vs. those without signal change)
2.4.1 Results of MRI signal change, \textit{PRNP} genotype and age as predictors of rate of disease decline in sCJD

None of the MRI parameters were found to be independent predictors of disease progression when included in the regression analysis model with codon 129 (see table 9 below). Codon 129 was the most powerful predictor. Age at disease onset also had an additional effect on rate of disease progression.

<table>
<thead>
<tr>
<th></th>
<th>Standardised coefficient beta</th>
<th>Significance (p-value)</th>
<th>95% confidence intervals</th>
<th>Correlation ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td></td>
<td>0.05</td>
<td>0.002</td>
<td>0.187</td>
</tr>
<tr>
<td>Codon 129</td>
<td>-0.436</td>
<td>$1.02 \times 10^{-14}$</td>
<td>-0.057</td>
<td>-0.04</td>
</tr>
<tr>
<td>MRI atrophy</td>
<td>0.029</td>
<td>0.58</td>
<td>-0.014</td>
<td>0.02</td>
</tr>
<tr>
<td>MRI basal ganglia SI</td>
<td>0.075</td>
<td>0.21</td>
<td>-0.008</td>
<td>0.04</td>
</tr>
<tr>
<td>MRI cortical ribboning SI</td>
<td>-0.058</td>
<td>0.30</td>
<td>-0.038</td>
<td>0.01</td>
</tr>
<tr>
<td>MRI thalamus SI</td>
<td>-0.054</td>
<td>0.34</td>
<td>-0.030</td>
<td>0.01</td>
</tr>
<tr>
<td>MRI pulvinar sign</td>
<td>0.021</td>
<td>0.41</td>
<td>-0.087</td>
<td>0.13</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.009</td>
<td>0.86</td>
<td>-0.019</td>
<td>0.02</td>
</tr>
<tr>
<td>Age</td>
<td>0.002</td>
<td>0.001</td>
<td>0.001</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 9

Linear regression analysis model, including codon 129 and MRI signal change as predictors, in addition to age and gender of MRC Scale slope (dependent variable)
2.4.2. Codon 129 and PrP<sup>Sc</sup> strain as combined predictor of rate of disease decline

To assess if codon 129 and prion strain subtype may be used as a combined predictor of rate of disease progression, the 68 subjects that had strain typing were included in a regression analysis model with codon 129 and age. Results show a better model fit with the inclusion of strain compared to only codon 129 genotype (see tables 10 and 11).

<table>
<thead>
<tr>
<th></th>
<th>Standardised coefficient beta</th>
<th>Significance (p-value)</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constant</strong></td>
<td>.082</td>
<td>0.159</td>
<td>-0.03 - 0.19</td>
</tr>
<tr>
<td><strong>Codon 129</strong></td>
<td>-.05</td>
<td>&lt;0.001</td>
<td>-0.07 - -0.003</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>0.002</td>
<td>0.02</td>
<td>0.001 - 0.003</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of squares</th>
<th>Deg of freedom</th>
<th>Mean square</th>
<th>Significance</th>
<th>Adjusted R&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>.184</td>
<td>2</td>
<td>.092</td>
<td>&lt;0.001</td>
<td>0.48</td>
</tr>
<tr>
<td>Residual</td>
<td>.189</td>
<td>59</td>
<td>.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>.373</td>
<td>61</td>
<td>.006</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 10**

Regression analysis model of codon 129 genotype as predictor of change in MRC Scale slope (dependent variable MRC Scale slope, predictors: codon 129 and age)
### Table 11

Regression analysis model of codon 129 genotype and prion strain as a combined predictor of change in MRC Scale slope (dependent variable MRC Scale slope, predictors: codon129 and PrP<sup>Sc</sup> strain and age)

<table>
<thead>
<tr>
<th>Model</th>
<th>Sum of squares</th>
<th>Deg of freedom</th>
<th>Mean square</th>
<th>Significance</th>
<th>Adjusted R&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>0.19</td>
<td>3</td>
<td>0.06</td>
<td>&lt;0.001</td>
<td>0.49</td>
</tr>
<tr>
<td>Residual</td>
<td>0.18</td>
<td>58</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.37</td>
<td>61</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Standardised coefficient beta</th>
<th>Significance (p-value)</th>
<th>95% confidence intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>0.04</td>
<td>0.004</td>
<td>0.32</td>
</tr>
<tr>
<td>Codon 129</td>
<td>-0.42</td>
<td>&lt;0.001</td>
<td>-0.06 - 0.004</td>
</tr>
<tr>
<td>Strain</td>
<td>0.03</td>
<td>0.145</td>
<td>-0.06 - 0.01</td>
</tr>
<tr>
<td>Age</td>
<td>0.25</td>
<td>0.05</td>
<td>0.00 - 0.01</td>
</tr>
</tbody>
</table>
2.5. **Discussion**

MRI signal change was not found to be an independent predictor of disease progression when included in the regression analysis model with codon 129. There were differences in disease progression seen on the analysis of patients with and without thalamic signal change; it could be the case that thalamic signal change may influence disease progression as there was a significant difference found between disease progression in the patients with thalamic signal change and those without on the student t-test (table 8). However this group of patients had far more cases with MV or VV codon 129 genotypes and this maybe what was influencing the progression of disease rather than the thalamic signal change itself. There was no association between signal change abnormality in the cortex and the basal ganglia. There was also no evidence of atrophy being a marker of rate of disease progression.

**Figure 19**

*The mean MRC Scale slope for each codon 129 genotype*
Codon 129 genotype was the strongest predictor of rate of disease progression, measured as the MRC Scale slope. The MM genotype was associated with a more rapid disease course, followed by MV and then VV genotypes (see figure 19).

Prion strain combined with codon 129 genotype was also a predictor of disease progression, and additional testing showed that prion strain appeared to have an additional independent affect separate to that of codon 129 on the regression analysis model, (See tables 10 and 11), with an improvement on the overall model when it was included. The adjusted $R^2$ increased in the model that included both strain and codon 129, implying that the model that included strain was a better fit than the one that only included codon 129 as a predictor of disease progression. In addition to this the root MSE (measure of individual variation) of the fit was marginally smaller with the addition of strain, which again favoured the combined strain and codon 129 mixed model.

Gender was not found to be a predictor of rate of disease progression, which was not unexpected given that there was no statistical difference in the MRC Scale slope between male and female subjects and they also had similar numbers for each codon 129 genotype (table 5). The age of the patient was found to be a predictor of rate of disease progression. This may in part be due to the fact that there were a higher percentage of patients who were 65 years old and above, with the MM genotype, which is known to be associated with a faster rate of disease progression, however from the mixed model (table 9) it appears that age at disease onset has an independent effect. Younger patients had a more slowly progressive disease, specifically in patients under 65 years the rate of progression was slower.

It is clear from this study that codon 129 is undoubtedly the best predictor of disease progression in patients with the sporadic form of CJD. The results indicated that there was no added benefit to a model that included specific imaging abnormalities.
Prion strain combined with codon 129 was seen to have a combined effect in predicting disease progression, however the number of subjects that had strain type was modest and inclusion of a larger dataset would be ideal. It is unlikely that prion strain will be used as a biomarker of disease progression as acquiring brain tissue from subjects when they are alive would be impractical. However it may be of use in understanding the pathogenesis of disease better. There may also be the potential for expanding the use of RT-QuIC analysis for identifying specific strains in CSF or using the total strain found as a marker of disease activity/progression. In addition to this it may be worth exploring the use of other CSF protein markers that are present in patients with prion disease such as 14-3-3 and tau, as these could be potential combined biomarkers of progression.

It was interesting to find that age was also an independent predictor of rate of disease progression in the regression analysis model (table 9); this may be due to younger patients having a greater brain volume than more elderly subjects and therefore having a greater brain reserve. This could be evaluated further by including the total brain volume or estimated total brain volume for subjects as a covariate and assessing if this affected rate of disease progression.

There were various limitations to the study. One of these was that the MRI scans were acquired from different scanners, which could lead to differences in quality of the scans. The scans were also acquired from scanners with differing magnetic strengths (both 1.5 and 3 Tesla scanners) and therefore this may have led to a disparity in picking up abnormalities seen on the scans, i.e. a reduction in the sensitivity of finding signal abnormality on scans acquired on the 1.5T scanner.

The patient scans were not acquired at the same time point(s) for each subject in the study and acquired at different time points during the course of their disease. Whilst imaging abnormalities can be seen early on in the disease process, scan findings are known to change with progression of disease. The location of DWI signal change has been found to vary depending on when in the
course of the disease the patient is scanned, and areas where signal change is first seen on DWI can disappear. Signal intensity on DWI is thought to represent spongiform change and with disease progression, gliosis occurs and neuronal loss and subsequently areas where signal change was seen to be present have been shown to be lost or disappear\textsuperscript{131}.

In addition to this, new signal abnormalities on repeat scans have been observed, which may be missed if a scan from only one time-point is available. In conclusion only having 1-2 scans acquired at differing times during the disease process, is unlikely to be truly representative of the imaging changes that occur at the different stages of the disease process.

Only 20% of patients had PrP\textsuperscript{Sc} strain results available at the time of analysis. The number of patients who had strain data was substantially less than the overall number of patients included in the study and although the number of patients recruited for post-mortem in the NPMC is over 60%, there were outstanding cases that still required strain subtyping. A correlation between strain, MRI signal change and codon 129 was not found. Although codon 129 and strain were able to predict disease progression, the numbers again were small and analysis of a larger dataset would be ideal in being able to confirm or refute this association.

The benefits of the study were: the large number of patients that were included, all of which had the same form of prion disease, definite or probable sCJD. Only analysing subjects with one type of prion disease meant there was less likely to be as much phenotypic heterogeneity than there would be if other forms of prion disease were included, in addition to this the predicted progression of disease was likely to be more accurate as the MRC Scale is optimised for use in patients with sCJD.

All scans were reported by a specialist Neuroradiologist, which means they should be more diagnostically accurate and have both a higher sensitivity and
specificity in identifying patients with typical scan findings seen in sCJD than scans being reported by a non-specialist.\textsuperscript{123}

I would recommend that a larger study be conducted ideally with the inclusion of more prion strain data. This may increase the chances of teasing out whether a relationship between strain type and codon 129 exists and whether codon 129 and strain type could have a cumulative effect on disease phenotype and would give us a better understanding of the association of strain type and codon 129 as biomarkers of disease.

If a larger study were conducted then it would be preferable for each subject's MRI scans to be acquired at the same magnetic field strength, ideally using the higher magnetic field of 3T. Scans in this study were acquired on both 1.5T and 3T scanners, 1.5T scans have been found to have a lower sensitivity for picking up abnormalities in other neurological conditions.\textsuperscript{202,203}

I would also recommend acquiring more than one scan during the course of patient’s illness could provide more data which may be a better representation of the abnormal changes that occur during the course of the disease process.
3. Magnetisation Transfer Ratio analysis in the National Prion Monitoring Cohort

3.1. Introduction to methods

In the introduction to my thesis I mentioned that MRI has become an important diagnostic tool in prion disease and conventional MRI findings are included in the World Health Organisation (WHO) criteria for diagnosing both probable vCJD and sCJD, in addition to the use of conventional MRI in diagnosing prion disease there has been a number of quantitative imaging studies that have looked for more sensitive measures for diagnosing and monitoring disease. Parameters that have been investigated include diffusion tensor imaging, volumetric studies and MTR imaging. Diffusion tensor imaging and volumetric studies have been conducted in inherited, sporadic and acquired forms of prion disease and MTR imaging in inherited prion disease patients, findings have been reported in both grey and white matter regions with all quantitative measures.4,8,198,204-208 However there are a limited number of serial studies in prion disease and only a few that have assessed for a correlation with clinical scores of function and disease severity9,175,198. In future therapeutic trials a quantitative measure of treatment response will be required and therefore it is of importance to be able to identify a marker that can deliver this. Although there have been limited numbers of studies looking at the role of MTR imaging in prion disease9,175, the significant findings identified by Siddique et al of MTR change, at 1.5T MRI, in a cohort of inherited prion disease patients9 make this parameter a suitable candidate for further quantitative analysis of its role as a biomarker of disease progression and/or onset in all forms of prion disease. In addition to this with the availability of 3T MRI scanning, which has increased sensitivity, findings in patients with prion disease may now be easier to detect.

My hypothesis is that MRI signal change can act as a predictor of rate of disease decline in patients with prion disease and my aim is to assess if certain quantitative MTR parameters would define microstructural change in patients with or at risk of developing prion disease and MTR measures would correlate
with clinical rating scores of disease severity both on cross-sectional and longitudinal analysis. To test this hypothesis I carried out multiparameter analysis of MTR, which was initially focused on voxel-based analysis (VBA), and region of interest analysis (ROI) and then later included cortical grey matter (GM) and white matter (WM) histogram analysis and multi-atlas propagation and segmentation (MAPS) regions of interest analysis. The study was conducted in a large dataset of both asymptomatic and symptomatic patients that were recruited to the National Prion Monitoring Cohort (NPMC) and the patient results were compared with those of healthy control subjects.

The primary study that I executed was VBA of MTR; I will describe the methods and results of this study first and then discuss the optimisation of the post-processing methods, which subsequently led to a change in the results on re-analysis. This initial study was an important step in the decision to further explore other analysis methods, these being MAPS and cortical GM and WM histogram analysis.

3.2. Materials and methods analysis study 1 - VBA and ROI analysis

3.2.1. Subjects-cross-sectional analysis

Magnetisation transfer imaging from 26 healthy controls (mean age 48.8 years, range 24-68 years, percentage male=42.3), 23 asymptomatic participants (composed from 20 asymptomatic gene positive participants and 3 subjects at risk of developing prion disease secondary to blood product exposure) (mean age 42 years, range 21-74, percentage male=42%) and 30 symptomatic patients (mean age 52, range 36-77 years, percentage male=53.3) was acquired. All participants were enrolled to the NPMC. Wherever possible the first scan acquired from each subject was the one included for the analysis, whenever this was not possible, for example if there was significant motion artefact present, the second scan from the series was used.
### Table 12

Demographics table for subjects included in cross-sectional VBA analysis total number =79 (controls, symptomatic and asymptomatic patients) showing gender ratio, mean age and range at disease onset

Participants who were gene positive for carrying a mutation on \textit{PRNP} had all undergone genetic testing prior to the commencement of the study. There were 2 participants who converted from being asymptomatic gene carriers to being symptomatic patients during the course of the study. All subjects underwent a detailed neurological assessment on enrolment to the study and at every scan date, in addition to this they also had their MRC Scale score calculated \textsuperscript{15}. Subjects who were classified as asymptomatic were those that had an absence of cognitive and neurological symptoms and signs on assessment and examination, all of which also scored the maximum score of 20 on the MRC Scale. Only healthy control participants were recruited, those who were found to exhibit neurological symptoms or signs were excluded from the study.

Figure 20 on page 111 illustrates the symptomatic subjects subdivided by type of prion disease. Figure 21 on page 111 shows the asymptomatic subjects subdivided by gene type accompanied by those at risk of acquired prion disease secondary to iatrogenic exposure.
Figure 20
Symptomatic subjects subdivided by type of prion disease. sCJD, vCJD, inherited prion disease mutations (E200K, 6OPRI, 5OPRI, D178N, Y163X, P102L and A117) and iatrogenic growth hormone patients=GH.

Figure 21
Asymptomatic subjects subdivided by type of prion disease. Inherited prion disease mutations=E200K, 6OPRI, D178N, P102L and A117V, vCJD= those at risk of developing vCJD (recipient of blood transfusion from vCJD patients)
3.2.2. MRI acquisition

All participants underwent MRI scans at the National Hospital for Neurology and Neurosurgery (NHNN). The MRI scans were acquired on a SIEMENS MAGNETOM Trio A Tim syngo MR B15 with a 32-channel head array coil. MT imaging consisted of a 3D-FLASH sequence acquired twice and sequentially without optional presaturation pulse yielding images referred to as $M_0$ (equilibrium image) and $M_{\text{sat}}$ (partially saturated image). (TE) of 3.0 milliseconds (ms) and a repetition time (TR) of 42ms, the flip angle used was 5 degrees. The total number of axial slices acquired was 60, with a voxel size of 0.9mm x 0.9mm x 3mm. The overall matrix size was 256 x 256 mm and the field of view 22.2 x 22.2 x18.0cm$^2$. The total magnetisation transfer acquisition time was 13'16". The pre-saturation pulse was a 6mm Gaussian pulse with duration of 12.8 ms, a bandwidth of 200Hz and peak amplitude of 23.2 mT, off water-resonance 2 kHz.

Scans with and without saturation were acquired sequentially, by acquiring the images in this way the later step of co-registration of the images would be as accurate as possible with less likelihood of motion artefact being present, this also ensured that the magnetisation transfer ratio calculation would be as precise as possible. Other scan sequences were also acquired included structural (T1) data; this was for the purpose of scan co-registration in the histogram and voxel based analysis post-scan processing. The T1 data was obtained by 3D-MPRAGE the TR was 2200ms and the TE 2.9 ms with an inversion time of 900ms. The flip angle was 10 degrees, 208 1.1mm partitions, and a field of view of 28.2 x 28.2 x 28.2cm$^2$. The matrix measured 256 x 256 mm. The acquisition time was 9’23”.

The additional sequences acquired during the scan were: diffusion weighted imaging, diffusion tensor imaging, fluid attenuated inversion recovery and structural T2 images. A neuroradiologist reviewed the conventional MRI images for the presence of signal abnormality and atrophy.
3.2.3. Data processing and image analysis

VBA methods

The magnetisation transfer data analysis involved the following steps:

- Co-registration of the $M_0$ and $M_{\text{sat}}$ images.
- Generation of the MTR maps by applying the formula $(M_0 - M_{\text{sat}})/M_0$ to the $M_0$ and $M_{\text{sat}}$ images.
- Affine registration of the magnetisation transfer data, the $M_0$, $M_{\text{sat}}$ and MTR maps to the T1 images.
- Warping of the magnetisation transfer data to the T1 generated VBM templates.
- Smoothing of the magnetisation transfer images.
- Grey and white matter mask generation from the summing of all VBM masks.

In the following paragraphs are more detailed descriptions and explanations behind the methods of analysis.

To reduce bias introduced by subject recognition the imaging data was anonymised. All scans were analysed in the Neuroimaging Centre Queen Square.

The initial post processing analysis for both the cross sectional and longitudinal subject analysis was the same. The analysis was carried out using processing scripts that were written in-house by Enrico De Vita and Gerard Ridgway; MATLAB was used to run some of the scripts and with SPM8 tools\textsuperscript{209}, FSLview was also employed to generate anatomical image maps and to view the individual subject scans for quality control purposes.
Image quality control and re-alignment

Head motion can result in a change in signal intensity and spatial normalisation may not succeed with markedly rotated or angled images, which may confound results. FSL, an imaging analysis program\textsuperscript{210}, was employed to assess the quality of the scans, by 2 independent operators. The scans were graded on a scale of 0-5, 0=excellent quality and 5=terrible, those scans with an agreed score of 4 or 5, were discounted and not included in the analysis.

Therefore before applying the automated steps of VBA any gross head movement was corrected for by manually re-aligning the MR images. All the T1 data was manually re-orientated to the anterior commissure-posterior commissure (AC-PC) line, the reference line between the superior surface of the anterior commissure to the centre of the posterior commissure, this was performed using SPM8 tools in MATLAB. Re-orientating the images to the AC-PC line corrects for any gross differences in the scan alignment.

Spatial normalisation

In order for VBA to be performed the images require a transformation that places them in the same stereotaxic space, this process is called spatial normalisation.

A template that conforms to a standard anatomical space is applied. Either the data series is co-registered to a T1-weighted image (created from the same subject data) or a mean image of the entire series is created and used to estimate warping parameters that map it onto the template\textsuperscript{211}. The averaged image can be created from warping multiple scans from an individual subject or warping the scans from the entire group of subjects and creating an averaged image to which the individual subject scans are overlaid. Spatial normalisation corrects for gross anatomical differences in brain structure, it does not correct for individual regional differences, if it perfectly corrected for these differences.
then there would be no findings observed. The resolution for the voxel-based analysis is around 1.5mm³. If the voxel size is too large then there is a higher risk of sampling error occurring outside the grey or white matter.

Spatial processing of the T1 data was first carried out to generate grey, white matter and cerebrospinal fluid segments. Grey and white matter templates were generated using DARTEL²¹², with a resolution of 1.5mm.

**Segmentation**

After the spatial normalisation step the images were segmented into cerebrospinal fluid (CSF), grey matter (GM) and white matter (WM) using cluster analysis. Because the different areas vary in voxel intensity segmenting them out allows for a more accurate comparison of voxels within each specific tissue analysed.

Each subject scan was then warped to the grey and white matter templates, to create individual segments.

**Smoothing**

The images are now smoothed using a Gaussian kernel. This process is a means of averaging the concentration of grey or white matter in a specified region by comparing each voxel with its neighbours and thereby creating multiple regions of interest. The smoothing kernel that is used is between 2-6mm and should correspond to the size of the anticipated effect.

Smoothing of the templates was performed using a 6mm Gaussian kernel.

Grey and white matter masks were generated for statistical analysis of the T1 dataset in SPM8.
**Statistical parametric mapping (SPM)**

SPM statistical analysis uses the general linear model; this is a means of identifying if there are covariates that influence findings and a way of making group comparisons. Statistical testing is done with either t-tests or F tests or if more than 2 groups are being compared an ANOVA. A statistical parametric map illustrates areas where there are statistically significant differences found between the groups compared. Multiple statistical tests are applied at each region of voxels. To control for errors incurred through multiple testing, strict statistical corrections are recommended to reduce the likelihood of false positive observations occurring. The most common corrections used are the False Discovery Rate Control (FDR) and the Family Wise Error Rate (FWE). Figure 22 illustrates the steps involved in VBA.

![Figure 22](image.png)

**Figure 22**

*Methods of Voxel-based analysis, showing image realignment, normalization, smoothing, application of the general linear model and SPM voxel map*

In MATLAB using SPM8 software, a group level ANCOVA was performed, the subject groups included were asymptomatic, symptomatic and control participants, age and total intracranial volume were included as co-variates. For multiple comparison correction, both the family wise error and false discovery
rate statistical tests were applied separately at a p<0.05. On group-wise comparison the voxels that were found to be significantly different were displayed on an averaged brain map.

**ROI analysis**

As a means to try and quantify the changes present on VBA of MTR, and to be able to visualise the individual subject values I performed a region of interest analysis on the right caudate and extracted the individual MTR values for each subject. ROI analysis required the first 3 steps for the VBA, co-registration of the images, generation of the MTR maps and registration of the individual MT data to the T1 dataset. After these steps were completed then an ROI was drawn free hand around the caudate of each individual subject, this was done with the use of FSL tools where an anatomical map was used as reference, the individual MTR values were then extracted from each ROI.

**Clinical scores and assessments**

During the course of their participation in the NPMC participants were clinically assessed at regular intervals, at each assessment a full cognitive and neurological examination was carried out and the MRC Prion Disease Rating Scale (MRC Scale) performed. The MRC Scale is comprised of questions that assess a decline in both cognitive and physical function and was devised to reliably measure and predict disease progression in prion disease patients.\(^{15}\)

At the time of MRI acquisition each subject underwent full cohort assessment, in addition to this whenever possible participants also had full neuropsychometry assessment carried out.
Statistical analysis

Cross-sectional analysis

The VBA statistical analysis was performed with SPM-8, in statistical parametric mapping multiple t-tests are performed at regions of voxels, in order to correct for errors incurred by these multiple comparisons being made strict multiple comparison statistical tests are used, both the FWE and FDR tests were carried out in SPM-8. Linear regression analysis was used to assess for an association between VBA and MRC scale.

For the cross-sectional caudate ROI analysis an assessment was made for group differences in MTR with the use of t-tests. Groups compared were: controls vs. asymptomatic patients and controls vs. symptomatic patients.

The cross-sectional relationship between subject MTR and the MRC Scale was assessed in the symptomatic patient group by plotting the individual subject MTR values against the corresponding MRC Scale.

Statistical tests were performed using IBM Statistical Package for the Social Sciences (SPSS, version 24).

3.2.4. MTR ROI results-cross-sectional analysis

On ROI analysis I found that there were significant differences present in the mean caudate MTR in the symptomatic patient group when compared to controls, see Figure 23.
Figure 23

*Right caudate ROI MTR results, control and symptomatic subjects, p-value = 0.00073 (mean and standard error bars shown)*

I also investigated whether a cross-sectional relationship existed between the ROI MTR in symptomatic patients and their corresponding MRC Scale score, the results of which are illustrated in figure 24.
3.2.5. VBA of MTR results-cross sectional analysis

On the statistical parametric map, VBA of MTR illustrated significant differences between symptomatic patients and control participants (see figure 25).

In the symptomatic patient group the anatomical areas that appeared most significantly affected were the perisylvian fissures, the fronto-temporal and fronto-parietal cortex as well as the caudate. In symptomatic patients the grey matter was more severely affected than the white matter; the cortical areas mentioned and the basal ganglia appeared the most affected. There were also white matter changes present although these were less significant.

On assessing for the presence of a correlation with the MRC rating scale I found no association present.
Figure 25

VBA of MTR. SPM-t maps of symptomatic patients with prion disease compared to controls. FDR $p<0.05$. The cortex is most affected. The most significant areas of reduction in MTR are the perisylvian fissures, the temporal-parietal cortex and the right caudate.

3.2.6. Discussion

The results of the voxel based analysis showed that reduced MTR was most evident in the cortex, the basal ganglia and thalami, these findings not only seem to correlate with conventional MRI findings seen in sCJD, vCJD and some forms of familial CJD but also with neuroanatomical areas that are found to have the most evident degree of spongiosis found on histopathology. The ROI MTR values also showed a significant difference between symptomatic subjects when compared to controls $p=0.00073$, which also appeared to support the VBA results of reduced MTR in the basal ganglia. However an area of concern was identified regarding the possibility of partial volume effects. When comparing the MTR data with that of the structural T1 images, to which it is warped to, the resolution is much lower and therefore the risk of partial volume effects is higher which would result in analysis of the surrounding CSF and an increased possibility of false positive results. During the course of my analysis a stricter mask, and therefore tissue sampling criteria was introduced, with this
more stringent method of analysis the results I obtained were not replicable with the same patient dataset or the subsequent new one. As there was uncertainty as to which method was the most accurate and I had found positive findings on the ROI analysis I decided that the best course of action was to adopt a newer method of analysis that reduced the likelihood of partial volume effect. I therefore adopted the multi-atlas based propagation and segmentation method of analysing the data where several anatomical regions could be examined. By using this method I could explore whether specific anatomical regions were more significantly affected than others, similar to the approach that VBA provides, but with a greater degree of certainty that the results I was acquiring were accurate. In addition to this I took the decision to use histogram analysis to assess for imaging change over the entire cortex and white matter, the reason for this was based on the fact that prion disease is a diffuse process that involves multiple areas of the brain, and this would give an overall picture of the microstructural change occurring in a heterogeneous group of subjects and therefore may provide a better biomarker of structural change that could be used in future clinical trials.

3.3. Materials and methods study 2-MAPS and histogram analysis

The second analysis was conducted on a slightly larger dataset, as more patients were recruited and scanned between the time of the first and the second analysis. Both cross-sectional and longitudinal analysis was conducted as well as the assessment for a correlation between change of MTR and clinical severity, the MRC Scale was used to assess for this relationship.

3.3.1. Subjects-cross-sectional analysis

Magnetisation transfer imaging from 85 subjects, 26 healthy controls (mean age 48.8 years, range 24-68 years, percentage male=42.3), 29 asymptomatic
participants (composed from 26 asymptomatic gene positive participants and 3 subjects at risk of developing prion disease secondary to blood product exposure) (mean age 47.8 years, range 21-78 years, percentage male=58.6) and 30 symptomatic patients (mean age 52, range 36-77 years, percentage male=53.3) was acquired. The symptomatic patient group was comprised of 8 sCJD patients, 3 patients with CJD secondary to human growth hormone exposure, one patient with vCJD and 18 patients with inherited prion disease. All participants were enrolled to the NPMC. Wherever possible the first scan acquired from each subject was the one included for analysis, whenever this was not possible, for example if there was significant motion artefact present, the second scan from the series was used.

Participants who were gene positive had all undergone earlier genetic testing. 2 participants converted from being asymptomatic gene carriers to being symptomatic patients during the course of the study. Subjects who were classified as asymptomatic were those that had an absence of cognitive and neurological symptoms and signs present on clinical assessment and examination.
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Controls</th>
<th>Symptomatics</th>
<th>Asymptomatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
<td>26 (33%)</td>
<td>30 (38%)</td>
<td>29 (29%)</td>
</tr>
<tr>
<td>Male to female ratio</td>
<td>1:1.3</td>
<td>1:0.9</td>
<td>1:0.7</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>48.8 years (24-68)</td>
<td>52 years (36-77)</td>
<td>47.8 years (21-78)</td>
</tr>
</tbody>
</table>

Table 13
Demographics table for subjects included in cross-sectional MTR histogram analysis total number =85 (controls, symptomatic and asymptomatic patients) showing mean age and range at disease onset

3.3.2. Subjects-longitudinal analysis

All the subjects that had two or more scans of acceptable quality were used for the longitudinal analysis; the total number of subjects was 52, 19 control participants (mean age 49.1 years, range 23-70, 50% male), 15 symptomatic patients (mean age 49.7 years, range 37-63, 55.6% male) and 18 asymptomatic gene positive patients (mean age 45.4 years, range 21-73, 46.7% male).

The median time between scans was 242 days in the symptomatic patient group, 413 days in the asymptomatic group and 406 days in the control group. The average number of scans acquired for the symptomatic and asymptomatic patient groups were 3 and for the control subjects 2.
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Controls</th>
<th>Symptomatics</th>
<th>Asymptomatics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (%)</strong></td>
<td>19 (36.5%)</td>
<td>15 (29%)</td>
<td>18 (34.5%)</td>
</tr>
<tr>
<td><strong>Male to female ratio</strong></td>
<td>1:1</td>
<td>1:0.8</td>
<td>1:1.1</td>
</tr>
<tr>
<td><strong>Mean age (range)</strong></td>
<td>49.1 years (23-70)</td>
<td>49.7 years (37-63)</td>
<td>45.4 years (21-73)</td>
</tr>
<tr>
<td><strong>Median time between scans</strong></td>
<td>406 days</td>
<td>242 days</td>
<td>413 days</td>
</tr>
</tbody>
</table>

**Table 14**

Demographics table for subjects included in longitudinal MTR histogram analysis total number =52 (controls, symptomatic and asymptomatic patients) showing gender ratio, mean age, range at disease onset and median time between scan dates

### 3.3.3. MRI acquisition, data processing and image analysis

As in the first study all participants underwent MRI scans at the National Hospital for Neurology and Neurosurgery (NHNN). The MRI acquisition was identical to the protocol detailed in study one. To reduce bias introduced by subject recognition the imaging data was anonymised. All scans were analysed in the UCL Academic Neuroradiological Unit neuroimaging analysis centre, Queen Square. The analysis was carried out using processing scripts that were written in-house with MATLAB and SPM12 tools\(^{209}\) as well as FSL\(^{213}\). The images were qualitatively assessed for the presence of motion artefact; those found to have moderate to severe motion were excluded from the analysis.
3.3.4. MT histogram generation

After the initial pre-processing and generation of the MTR maps. The histograms were generated using in-house scripts run in MATLAB with the help of Enrico De Vita. The MTR were co-registered to the re-orientated, warped T1 dataset and segmented into grey and white matter segments. The bin width of 3% was used; the brain tissue was normalised to the brain tissue grey and white matter segments. For each histogram, the mean, peak height, peak location, 10th, 25th, 40th, 50th, 60th, 75th and 90th percentiles were calculated for the grey and white matter brain regions, the histograms of the voxel MTR values for each patient were plotted, as well as averaged histograms for each subject group, the change in histogram shape reflects the change in MTR between subject groups and the tissue damage that may be occurring in symptomatic and asymptomatic subjects. Generating different percentiles in which the majority of MTR voxels exist allows for the identification of a shift in the values of the histogram between subject groups, i.e. in the symptomatic group, a reasonable hypothesis would be that there would be more voxels existing in the lower percentiles compared to the control participants.

3.3.5. Multi-atlas propagation and segmentation (MAPS)

The % MTR images were calculated as 100* (1- Msat/M0) after rigid registration of the M0 to the Msat images. The procedure to extract regions of interest was as follows:

To label the deep grey matter structures, each individual structural image was segmented through the propagation of anatomical labels from a set of 35 expertly annotated T1 weighted images (Neuromorphometrics inc., Somerville, MA, USA).
The set of labelled images were registered to each subject image using Niftyreg\textsuperscript{214}. The final labelled areas were estimated by majority voting of the registered labels\textsuperscript{215}. From the many binary labels generated the ROI’s selected were: head of caudate, putamen, globus pallidus, thalamus, hippocampus and amygdala.

The New Segment function in SPM12 using a refined Unified Segmentation\textsuperscript{216} approach (combining bias correction, normalisation and segmentation) was used to extract GM, WM and CSF partial volume fractions.

A cortical-only GM partial volume fractions was obtained by removing all deep grey matter structure identified in [1] from the GM partial volume fraction calculated in [2] Affine transformation between the structural data and the $M_{\text{sat}}$ was calculated and subsequently applied to all the calculated labels and partial volume fractions.

Given the lower resolution of the MTR data compared to the structural data in which space the labels and partial volume fractions were computed, once the labelled areas had been resampled in the MTR space, they were further trimmed to reduce contamination of each target structure from surrounding tissue.

All GM labels, including deep GM ROIs and cortical GM partial volume fraction were thresholded at 0.8. For each deep GM ROI, voxels were $P\text{VCSF} \geq 0.01$ were eliminated. Anything remaining was binarised. For cortical GM ROI, voxels were $P\text{VCSF} \geq 0.03$ were eliminated as well as voxels with $P\text{VWM} \geq 0.5$. Anything remaining was binarised. The WM partial volume fraction was truncated inferiorly at the level of the most inferior cortical GM voxel after binarisation.

MTR data was then extracted for all ROIs generated as explained above for
each dataset of each subject. Mean ROI values were extracted for deep GM structures.

Histogram analysis was conducted in the larger anatomical ROI’s, i.e. WM and cortical GM, to assess distribution of MTR values within these areas. Histograms were generated with a bin width of 3% and were normalised for brain volume. For each histogram, the mean, peak height, peak location, skewness, 10th, 25th, 40th, 50th, 60th, 75th and 90th percentiles were calculated for the grey and white matter brain regions, as well as the kurtosis and skewness. The histograms of the voxel MTR values for each patient were plotted, as well as averaged histograms for each subject group; the change in histogram shape reflects the change in MTR between subject groups and the tissue damage that may be occurring in symptomatic and asymptomatic subjects. Generating different percentiles in which the majority of MTR voxels exist allowed for the identification of a shift in the values of the histogram between subject groups, i.e. in the symptomatic group it could be hypothesised that there would be more voxels existing in the lower percentiles compared to the control participants.

3.3.6. Clinical scores and assessments

Each subject participating in the National Prion Monitoring Cohort was clinically assessed at regular intervals, at each assessment a full cognitive and neurological examination was performed, additionally the Medical Research Council prion disease rating scale (abbreviated to MRC Scale)\textsuperscript{15} was conducted. The 20-point MRC Scale was designed to assess domains of function affected in patients with prion disease; these include cognition, speech, feeding, personal care and mobility. It was devised to reliably measure and predict disease progression in patients diagnosed with or at risk of developing prion disease. The interval between obtaining each corresponding MRC Scale score and the MRI brain scan was no longer than 2 days. The mean MRC Scale
scored in in the cross-sectional control and asymptomatic patient groups was 20 and 16 in the symptomatic patient group.

3.3.7. Statistical analysis/ data analysis and evaluation

Statistical tests were performed using IBM Statistical Package for the Social Sciences (SPSS, Version 24, Chicago, Illinois) excepting the post hoc power calculations that were performed in STATA

Cross-sectional analysis:
An ANOVA was performed to assess for significant differences between the controls, symptomatic and asymptomatic patient groups. Regression analysis was used to assess for the relationship between subject group and brain region MTR with inclusion of age and gender and independent variables

Longitudinal analysis:
ANOVA performed to assess statistical differences between change in brain region metrics for subject groups. Regression analysis used to assess for change in subject group brain region MTR with inclusion of gender and age as independent variables

Post hoc power calculations were carried out by statistician Zoe Fox to determine the minimum sample size required in a therapeutic trial for a change in slope of 50, 80 and 90% for specific MTR parameters. The MTR parameters included in this analysis were those that were observed to have the most significant effect on the rate of disease progression and were identified via a backwards selection regression analysis model. Power analysis takes into account three factors: 1) The effect size, the larger the effect the less likely that the result is due to error, this is calculated as the difference between the means
of rejecting the null hypothesis and accepting the null hypothesis divided by the standard deviation 2) The sample size, the larger the sample size the easier to detect smaller effects, large samples are however difficult to recruit in rare diseases such as prion disease and therefore a smaller sample size would be preferable. 3) The variability of the sample data, more variability can result in random error, standard deviation is an example of a measure of variability. To determine the sample size required for a specific power the formula is:

\[ n = \left( \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{ES} \right)^2 \]

Where \( \alpha \) is the level of significance (0.05), \( Z \) is the value from the normal distribution holding \( 1- \alpha/2 \) below it, if \( \alpha=0.05 \), then \( 1- \alpha/2 = 0.975 \) and \( Z=1.960 \). The desired power of the test is \( 1-\beta \). \( Z_{1-\beta} \) is the value from the standard normal distribution holding \( 1- \beta \) below it. For estimating power of 80%. The \( Z_{1-\beta} \) values would be \( Z_{0.80} = 0.84 \).

3.3.8. Cross-sectional analysis methods

Prion disease is a diffuse process that typically causes symmetrical change\textsuperscript{134}, therefore the ROI MTR values for the 12 bilateral deep grey matter nuclei were analysed as 6 averaged ROIs, to check this was a valid approach, t-tests were used to assess for a significant difference between the bilateral ROIs before undertaking this approach. In addition to the ROI analysis, cortical GM and WM histogram analysis was performed, the following histogram indices were included for statistical analysis: the mean, median, 25\textsuperscript{th} and 75\textsuperscript{th} centiles, as well as the peak height and skewness.
Regression models assessing for the relationship between group and each brain region metric were used to analyse the MTR values from the ROI’s as well as grey and white matter histogram centiles for the 3 subject groups. The covariates included were age and gender.

3.3.9. Longitudinal analysis methods

Inter-group comparison of change in each brain region metric was calculated, with adjustment for age and gender. The ROIs and histogram parameters included for the longitudinal analysis were the same as those included in the cross-sectional analysis.

In the second stage of the statistical analysis a mixed model was employed to establish the best predictor of decline in the MRC Scale, age, gender and disease subtype were included as co-variates.

3.4. Results

3.4.1. Cross-sectional analysis results

Comparison of MTR values across subject groups

My findings for the cortical GM histogram analysis and for the WM histogram analysis were that there were significant differences present when comparing the symptomatic patient group and the control participants, in both of these regions analysed. The most significant findings were in the cortical grey matter, histogram parameters showing the greatest differences were: the 25th percentile, mean, median, peak and skewness, the white matter skewness (p<0.0001) (shown in table 15). In addition to this there were also significant differences evident in the ROIs too, with the caudate, hippocampus and putamen being the
areas that were significantly different between control participants and the symptomatic patients, these results are also shown in table 15 and figure 26, which illustrates the difference between control subjects MTR values and symptomatic patients for 8 of the ROIs/histogram parameters.

Multivariate regression analysis was also conducted, both unadjusted and adjusted values (for age and gender) are shown in table 16 on the following pages (135-136), significant results were found in several of the parameters analysed.

Figures 27 and 28 are group histograms for the white matter and cortical grey matter and show the differences of MTR values for the 3 groups and the leftward skew to lower MTR values in the symptomatic patient group, which is more evident in the grey matter.

On comparison of the control subjects with the asymptomatic/at risk patient group no significant differences were observed, though interestingly the hippocampus ROI MTR was reduced in the asymptomatic group.
### Table 15

Mean and standard deviation (SD) MTR values of brain region metrics for each subject group in addition to age, gender, MRC Scale score with interquartile range (IQR). NB: The ‘at risk’ group contain the asymptomatic IPD and the “at risk” iatrogenic; the patient group contains the iatrogenic, the symptomatic IPD and sCJD patients (WM= white matter)

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Controls (n=26)</th>
<th>At risk (n=29)</th>
<th>Patients (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (SD) years</td>
<td>48.8 (11.9)</td>
<td>47.8 (15.1)</td>
<td>52.0 (9.9)</td>
<td>0.40</td>
</tr>
<tr>
<td>Median MRC Scale (IQR)</td>
<td>20 (20, 20)</td>
<td>20 (20, 20)</td>
<td>17 (14, 19)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex n male (%)</td>
<td>11 (42.3%)</td>
<td>17 (58.6%)</td>
<td>16 (53.3%)</td>
<td>0.47</td>
</tr>
<tr>
<td>MTR % (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>30.19 (1.51)</td>
<td>30.24 (1.44)</td>
<td>28.79 (1.93)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>31.35 (1.37)</td>
<td>31.12 (1.10)</td>
<td>29.66 (1.91)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Putamen</td>
<td>31.53 (1.26)</td>
<td>31.70 (1.02)</td>
<td>30.55 (1.16)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Amygdala</td>
<td>32.12 (1.72)</td>
<td>32.38 (1.41)</td>
<td>31.52 (1.28)</td>
<td>0.08</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>32.91 (1.63)</td>
<td>33.25 (1.07)</td>
<td>32.80 (1.48)</td>
<td>0.45</td>
</tr>
<tr>
<td>Thalamus</td>
<td>35.72 (1.17)</td>
<td>35.80 (1.17)</td>
<td>35.43 (1.11)</td>
<td>0.44</td>
</tr>
<tr>
<td>Cortex mean</td>
<td>29.96 (1.39)</td>
<td>29.97 (1.00)</td>
<td>28.29 (1.80)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Peak Cortex</td>
<td>1.87 (0.49)</td>
<td>1.85 (0.44)</td>
<td>1.39 (0.43)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Skew Cortex</td>
<td>2.30 (0.29)</td>
<td>2.21 (0.27)</td>
<td>1.71 (0.38)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortex 25th centile</td>
<td>27.71 (1.64)</td>
<td>27.57 (1.23)</td>
<td>24.93 (2.38)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortex 50th centile</td>
<td>30.32 (1.38)</td>
<td>30.40 (0.99)</td>
<td>28.61 (1.92)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cortex 75th centile</td>
<td>32.60 (1.25)</td>
<td>32.78 (0.89)</td>
<td>31.85 (1.55)</td>
<td>0.01</td>
</tr>
<tr>
<td>WM mean</td>
<td>36.50 (1.07)</td>
<td>36.67 (1.01)</td>
<td>36.22 (0.96)</td>
<td>0.24</td>
</tr>
<tr>
<td>Peak WM</td>
<td>2.24 (0.48)</td>
<td>2.17 (0.43)</td>
<td>2.23 (0.61)</td>
<td>0.86</td>
</tr>
<tr>
<td>Skew WM</td>
<td>2.90 (0.30)</td>
<td>2.79 (0.29)</td>
<td>2.52 (0.31)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WM 25th centile</td>
<td>34.69 (1.21)</td>
<td>34.80 (1.12)</td>
<td>34.02 (1.11)</td>
<td>0.02</td>
</tr>
<tr>
<td>WM 50th centile</td>
<td>36.39 (1.07)</td>
<td>36.64 (0.9)</td>
<td>36.11 (0.97)</td>
<td>0.13</td>
</tr>
<tr>
<td>WM 75th centile</td>
<td>38.16 (0.99)</td>
<td>38.47 (0.95)</td>
<td>38.28 (0.95)</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Figure 26

Mean MTR for each individual ROI analysed in control subjects (black) and symptomatic patients (red), GPI=Globus pallidus internus, Hippo=Hippocampus
Table 16 (shown below and on pages 136-137)

Regression model showing the relationship between subject group and brain region metric, controls as reference, significant results were found in several of the MTR parameters analysed. Both unadjusted and adjusted analysis were conducted with* multivariable analyses adjusted for both age and gender (CI = confidence interval and WM = white matter)

<table>
<thead>
<tr>
<th>Group</th>
<th>Unadjusted Analysis</th>
<th>Multivariable analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>0.001</td>
</tr>
<tr>
<td>At risk</td>
<td>5.36 (-83.26, 93.98)</td>
<td>-139.08</td>
</tr>
<tr>
<td>Patients</td>
<td>-227.00, 51.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus:</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>-22.03 (-102.88, 58.82)</td>
<td>-169.13</td>
</tr>
<tr>
<td>Patients</td>
<td>(-249.34, -88.92)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Putamen:</td>
<td></td>
<td>0.0004</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>17.60 (-44.01, 79.20)</td>
<td>-97.13</td>
</tr>
<tr>
<td>Patients</td>
<td>(-158.25, -36.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amygdala:</td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>25.02 (-54.19, 104.24)</td>
<td>-60.27</td>
</tr>
<tr>
<td>Patients</td>
<td>(-138.86, 18.32)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Globus pallidus:</td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>33.57 (-42.04, 109.18)</td>
<td>-11.51</td>
</tr>
<tr>
<td>Patients</td>
<td>(-86.53, 63.50)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>At risk</td>
</tr>
<tr>
<td>------------------------</td>
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</tr>
<tr>
<td><strong>Thalamus:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>8.41 (-53.25, 70.06)</td>
</tr>
<tr>
<td>At risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td>-2.84 (-80.34, 74.65)</td>
</tr>
<tr>
<td><strong>Cortex mean:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.02 (-0.27, 0.22)</td>
</tr>
<tr>
<td>At risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td>-0.09 (-0.26, 0.08)</td>
</tr>
<tr>
<td><strong>Peak height cortex:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>18.60 (-49.25, 86.45)</td>
</tr>
<tr>
<td>At risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td>7.04 (-72.94, 87.03)</td>
</tr>
<tr>
<td><strong>Skew cortex:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>32.94 (-31.35, 97.22)</td>
</tr>
<tr>
<td>At risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td>14.83 (-59.32, 85.18)</td>
</tr>
<tr>
<td><strong>Cortex 25th centile:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>4.82 (-85.99, 95.63)</td>
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<tr>
<td>At risk</td>
<td></td>
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</tr>
<tr>
<td>Patients</td>
<td></td>
<td>-14.08 (-112.19, 84.03)</td>
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<tr>
<td><strong>Cortex 50th centile:</strong></td>
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<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>24.37 (-49.80, 98.54)</td>
</tr>
<tr>
<td>At risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td>7.04 (-72.94, 87.03)</td>
</tr>
<tr>
<td><strong>Cortex 75th centile:</strong></td>
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<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>18.60 (-49.25, 86.45)</td>
</tr>
<tr>
<td>At risk</td>
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<td>Patients</td>
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<td>At risk</td>
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<tr>
<td>-------------------------</td>
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<tr>
<td><strong>WM mean:</strong></td>
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<tr>
<td>Controls</td>
<td>Ref</td>
<td>17.01 (-37.33, 71.35)</td>
</tr>
<tr>
<td>At risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Peak height WM:</strong></td>
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<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.07 (-0.34, 0.21)</td>
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<tr>
<td>At risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
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<tr>
<td><strong>Skew WM:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.11 (-0.27, 0.05)</td>
</tr>
<tr>
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<tr>
<td>Patients</td>
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<tr>
<td><strong>WM 25th centile:</strong></td>
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<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>10.99 (-50.35, 72.33)</td>
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<tr>
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<td></td>
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<tr>
<td>Patients</td>
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<tr>
<td><strong>WM 50th centile:</strong></td>
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<td></td>
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<tr>
<td>Controls</td>
<td>Ref</td>
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<tr>
<td>At risk</td>
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<tr>
<td>Patients</td>
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<tr>
<td><strong>WM 75th centile:</strong></td>
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<tr>
<td>Controls</td>
<td>Ref</td>
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<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 27
Group histograms of the cortex, illustrating the reduced fraction and skew to the left of the bell curve of MTR in the symptomatic patient group. Co=Control group, As=Asymptomatic group, Sy=Symptomatic group, MTR $\times 10^2$

Figure 28
Group histograms of the white matter, illustrating the reduced white matter MTR in the Symptomatic (Sy) patient group. Co=Control group, As=Asymptomatic group, Sy=Symptomatic group, MTR $\times 10^2$
3.4.2. Longitudinal analysis results

Comparison of change in MTR values between subject groups

19 control subjects, 15 symptomatic patients and 18 asymptomatic patients had serial imaging and concurrent MRC Scale scores recorded.

On assessing if there was a significant change observed in brain region metrics, I found there was a significant change identified in all ROI’s apart from the thalamus, and all histogram parameters, bar the WM skewness, peak height and 75th percentile (see tables 17 and 18) in the symptomatic patient group when compared to controls. Tables 17 and 18 depict these results. Multivariate analysis was conducted; both the unadjusted and adjusted (for age and gender) results are shown in table 18.

There were no statistically significant differences identified in the asymptomatic patient group, however I did observe the presence of directional change in several MTR parameters that was in keeping with that seen in the symptomatic patient group i.e. a downward trend in MTR value.
## Table 17

ANOVA showing mean differences in change of brain region metrics per month and MRC slope for each subject group, and F test of significance. Age at enrolment to study and gender are not significantly different between groups.
<table>
<thead>
<tr>
<th>Group</th>
<th>Unadjusted analysis</th>
<th>Multivariable analysis*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Caudate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>0.01</td>
</tr>
<tr>
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<td>0.43 (-14.97, 15.84)</td>
<td>-21.51 (-37.69, -5.34)</td>
</tr>
<tr>
<td>Patients</td>
<td>-21.51 (-37.69, -5.34)</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>-1.74 (-23.11, 19.64)</td>
<td>-33.60 (-56.05, -11.16)</td>
</tr>
<tr>
<td>Patients</td>
<td>-33.60 (-56.05, -11.16)</td>
<td></td>
</tr>
<tr>
<td>Putamen</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
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<td>-14.07 (-24.97, -3.17)</td>
</tr>
<tr>
<td>Patients</td>
<td>-14.07 (-24.97, -3.17)</td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>-3.74 (-15.65, 8.17)</td>
<td>-21.38 (-33.89, -8.88)</td>
</tr>
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<td>-21.38 (-33.89, -8.88)</td>
<td></td>
</tr>
<tr>
<td>Globus pallidus</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>-1.26 (-21.67, 19.14)</td>
<td>-28.69 (-50.12, -7.27)</td>
</tr>
<tr>
<td>Patients</td>
<td>-28.69 (-50.12, -7.27)</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>-2.24 (-24.64, 20.16)</td>
<td>-18.32 (-41.85, 5.20)</td>
</tr>
<tr>
<td>Patients</td>
<td>-18.32 (-41.85, 5.20)</td>
<td></td>
</tr>
<tr>
<td>Peak height cortex</td>
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<td>0.06</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>0.93 (-18.15, 20.01)</td>
<td>-21.13 (-41.16, -1.09)</td>
</tr>
<tr>
<td>Patients</td>
<td>-21.13 (-41.16, -1.09)</td>
<td></td>
</tr>
<tr>
<td>Skew cortex</td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>At risk</td>
<td>0.00 (-0.01, 0.01)</td>
<td>-0.02 (-0.04, -0.01)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>At risk</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Cortex 25th cen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.38 (-9.37, 8.62)</td>
</tr>
<tr>
<td><strong>Cortex median</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.44 (-8.68, 7.79)</td>
</tr>
<tr>
<td><strong>Cortex 75th cen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.60 (-8.54, 7.34)</td>
</tr>
<tr>
<td><strong>Peak height WM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.01 (-0.06, 0.05)</td>
</tr>
<tr>
<td><strong>Skew WM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>0.00 (-0.01, 0.02)</td>
</tr>
<tr>
<td><strong>WM 25th cen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>0.35 (-7.83, 8.54)</td>
</tr>
<tr>
<td><strong>WM median</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>0.02 (-7.14, 7.18)</td>
</tr>
<tr>
<td><strong>WM 75th cen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>Ref</td>
<td>-0.35 (-7.07, 6.38)</td>
</tr>
</tbody>
</table>

**Table 18**

Regression model showing brain region slopes for each subject group (controls, symptomatic and at risk subjects). Showing both unadjusted and * Multivariable analyses adjusted for both age and gender (WM=white matter and cen-centile)
I plotted the trajectories for change in MTR over time for the different disease subtypes (sCJD patients, IPD growth hormone iCJD cases), for both the ROIs analysed and the grey and white matter histogram parameters. The following graphs a-p of figure 29 shows these data. It is evident that for several of the ROIs there is a downward trend in MTR over time in the symptomatic patient group, this is most obvious in the patients with sCJD and iCJD.

a)

![Caudate MTR Graph](image)

b)

![Hippocampus MTR Graph](image)
e)

![Graph showing changes in Gobus pallidus internus MTR over time.

f)

![Graph showing changes in Thalamus MTR over time.]
Figure 29 above (graphs a-p)

Trajectories of control subjects, asymptomatic and symptomatic subjects for ROI and histogram parameters
**Relationship between MTR change and disease severity**

I observed a correlation between decline in MTR histogram measures and the MRC Scale in the symptomatic group. The main parameters identified from the model were: the cortical GM 25\(^{th}\) centile, median, skewness and mean MTR, the WM 25\(^{th}\) centile and were the same ROI’s identified in the cross-sectional analysis, the caudate, hippocampus and putamen these results are illustrated in table 19 and include both an unadjusted analysis and an adjusted analysis that took into account the affect prion disease subtype may have.

Figure 30, graphs a-i, depicts the different ROIs analysed and the subject’s trajectories over time for MTR and MRC Scale, illustrating the correlation between the two for the symptomatic patients.

**Table 19** (on the following page -152)

*Mixed effects model - MTR change vs. disease severity. Each of the 18 multivariable models has been adjusted for gender, group and age – hence why estimates are not provided for these parameters. An adjusted analysis was conducted (right column) to take into account the affect disease groups such as iCJD and sCJD have on the results*
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unadjusted Analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Regression coefficient (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Gender; Female vs. male</td>
<td>-0.96 (-4.51, 2.59)</td>
<td>0.60</td>
</tr>
<tr>
<td>Disease group; iatrogenic/sCJD vs. IPD</td>
<td>-7.43 (-11.02, 3.84)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age; per year older</td>
<td>-0.09 (-0.28, 0.11)</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Brain Metric Parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>6.36 (1.77, 10.96)</td>
<td>0.007</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>4.36 (0.38, 8.33)</td>
<td>0.03</td>
</tr>
<tr>
<td>Putamen</td>
<td>8.91 (1.46, 16.35)</td>
<td>0.02</td>
</tr>
<tr>
<td>Amygdala</td>
<td>7.99 (1.42, 14.56)</td>
<td>0.02</td>
</tr>
<tr>
<td>Globus pallidus internus</td>
<td>5.44 (0.90, 9.97)</td>
<td>0.02</td>
</tr>
<tr>
<td>Thalamus</td>
<td>3.31 (-1.40, 8.01)</td>
<td>0.17</td>
</tr>
<tr>
<td>Peak height cortex</td>
<td>1.58 (-1.62, 4.79)</td>
<td>0.33</td>
</tr>
<tr>
<td>Cortex skew</td>
<td>3.19 (-0.24, 6.63)</td>
<td>0.07</td>
</tr>
<tr>
<td>Cortex 25th</td>
<td>7.07 (2.02, 12.11)</td>
<td>0.006</td>
</tr>
<tr>
<td>Cortex median</td>
<td>7.59 (1.28, 13.89)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cortex 75th</td>
<td>5.81 (-1.81, 13.42)</td>
<td>0.14</td>
</tr>
<tr>
<td>Peak height WM</td>
<td>-0.55 (-2.17, 1.08)</td>
<td>0.51</td>
</tr>
<tr>
<td>WM skew</td>
<td>3.94 (0.42, 7.47)</td>
<td>0.03</td>
</tr>
<tr>
<td>WM 25th</td>
<td>10.22 (1.17, 19.28)</td>
<td>0.03</td>
</tr>
<tr>
<td>WM median</td>
<td>8.00 (-2.84, 18.85)</td>
<td>0.15</td>
</tr>
<tr>
<td>WM 75th</td>
<td>3.37 (-7.86, 14.61)</td>
<td>0.56</td>
</tr>
</tbody>
</table>
The following graphs in figure 30 (a-i) depict each symptomatic patient’s MTR metric for the ROIs analysed caudate, hippocampus, amygdala, putamen and both cortical GM and WM histogram measures vs. their MRC Scale score, illustrating the correlation that exists between decline in MTR and MRC Scale score.

**Figure 30 (graphs a-i)**

*MTR ROIs vs. MRC Scale score in symptomatic patients*

![Graph showing MTR ROIs vs. MRC Scale score in symptomatic patients](image)
b) [Graph showing Hippocampus MTR % vs MRC Scale Score with data points for Growth hormone CJD, Inherited prion disease, and Sporadic CJD.]

c) [Graph showing Putamen MTR % vs MRC Scale Score with data points for Growth hormone CJD, Inherited prion disease, and Sporadic CJD.]
The following scatter plots in figure 31 on pages 158-160 illustrate the association between the MRC Scale slope, measure of rate of disease progression, and change in MTR (y axis) for symptomatic patients for grey and white matter histogram measures.

Cortex mean:

Peak Cortex:

Cortex 25th centile:

Cortex median:
Figure 31

Change in cortex and white matter histogram MTR vs. the MRC Scale slope for symptomatics
3.4.3. Post-hoc power calculations

Post-hoc power calculations were carried out to identify the sample size required to assess the efficacy of a therapeutic agent in a clinical trial and the effect on the slope of change for each of the MRI parameters analysed. Three targeted slopes of 50% decrease in disease progression, 80% decrease and 90% decrease were analysed. Statistician, Dr Zoe Fox, carried out this analysis. The parameters that were included were those that showed the most significant correlation with severity of disease progression on longitudinal analysis identified via a backwards selection regression analysis model; theses were: cortex 25\textsuperscript{th} percentile, cortex median, cortex 75\textsuperscript{th} percentile, putamen and white matter 25\textsuperscript{th} percentile (table 20). The minimum number of subjects required for each arm of the study was 30 for the cortex 25\textsuperscript{th} percentile for a 90% decrease in the MRC Scale slope and 100 for each arm for a 50% reduction in slope.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Slope (SD)</th>
<th>Targeted slope 1 (50% decrease)</th>
<th>Number needed per arm</th>
<th>Targeted slope 2 (80% decrease)</th>
<th>Number needed per arm</th>
<th>Targeted slope 3 (90% decrease)</th>
<th>Number needed per arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex 25&lt;sup&gt;th&lt;/sup&gt; centile</td>
<td>-22.1 (27.4)</td>
<td>-11.5</td>
<td>100</td>
<td>-4.42</td>
<td>38</td>
<td>-2.21</td>
<td>30</td>
</tr>
<tr>
<td>Cortex median</td>
<td>-17.2 (24.1)</td>
<td>-8.6</td>
<td>125</td>
<td>-5.16</td>
<td>49</td>
<td>-1.72</td>
<td>39</td>
</tr>
<tr>
<td>Cortex 75&lt;sup&gt;th&lt;/sup&gt; centile</td>
<td>-12.7 (21.9)</td>
<td>6.35</td>
<td>190</td>
<td>-2.54</td>
<td>74</td>
<td>-1.27</td>
<td>58</td>
</tr>
<tr>
<td>Putamen</td>
<td>-12.6 (31.9)</td>
<td>-6.3</td>
<td>390</td>
<td>-2.52</td>
<td>151</td>
<td>-1.26</td>
<td>119</td>
</tr>
<tr>
<td>WM 25&lt;sup&gt;th&lt;/sup&gt; centile</td>
<td>-7.1 (20.7)</td>
<td>-3.55</td>
<td>551</td>
<td>-1.42</td>
<td>213</td>
<td>-0.71</td>
<td>168</td>
</tr>
</tbody>
</table>

**Table 20**

*Predicted numbers needed for targeted reduction in disease progression for MRI parameters. The above table only focuses on information obtained from patients and not the at risk or control populations. All sample size calculations are based on 80% power and a two-sided alpha of 5%*
3.5. Discussion

On both cross-sectional and longitudinal analysis, significant differences in MTR metrics were observed in the symptomatic patient group when compared to control participants. A reduction in MTR was evident across multiple brain regions of the deep grey matter ROI’s, cortical GM and WM. The only two areas not to show cross-sectional change were the thalamus and globus pallidus internus.

On longitudinal analysis, change in MTR was identified in the cortical GM parameters (most significantly in the 25th centile, median, and skewness) the deep GM nuclei (the caudate, putamen, amygdala, globus pallidus internus and hippocampus) and the WM 25th centile and skewness. The slope of change in these parameters was also found to strongly correlate with the change of MRC Scale. A backwards selection model was used to ascertain which parameters were the key ones that were associated with the MRC Scale score, the key brain metrics identified were the cortex 25th percentile, the WM 25th percentile, putamen, the median cortex and the cortex 75th percentile. Because of the expected collinearity/co-dependence between the imaging parameters, secondary to the underlying diffuse microstructural neuropathological change that occurs in prion disease, the creation of a mixed model that included these key metrics was not carried out as it would be unlikely to be useful in achieving the best combined predictor of change in the MRC Scale score.

It is both positive and encouraging to find several MTR histogram parameters that are associated with severity of disease and may prove to be useful markers of disease progression in clinical trials and indicators of response to future therapeutics in sporadic, acquired and inherited forms of prion disease.

Although a significant change in MTR metrics was not identified in the asymptomatic patient group on cross-sectional analysis, there was an observed decrease in MTR on comparison to the control group in the cortical grey matter
MTR parameters that was in keeping with the significant directional changes present in the symptomatic patient group. Additionally on longitudinal analysis there was a trend of decline in cortical grey matter MTR parameters also in keeping with the decline seen in the symptomatic patient group. Even though this overall decline in MTR in the asymptomatic patients was not of statistical significance the directional changes observed are interesting and may represent the occurrence of early microstructural grey matter change. Another interesting finding observed in the peak white matter MTR metric (see figure 29, graph o) was an increase in the peak height in several asymptomatic patients with the P102L \textit{PRNP} mutation. Despite there being an absence of significant findings in the asymptomatic group I think these observations are interesting and warrant continued data collection in this group as they may reflect early microstructural pathology. Even though the most evident neuropathological change reported in prion disease is grey matter spongiosis\textsuperscript{190}, it is possible that early white matter change is occurring in some patients. A recent imaging study by Caverzasi et al showed reduced mean diffusivity within the white matter of patients with sporadic CJD which they thought indicated possible primary involvement of the white matter rather than secondary neuronal loss or degeneration\textsuperscript{204}, Lee et al also reported a progressive reduction in fractional anisotropy in the white matter pathways on DTI in patients with E200K inherited prion disease\textsuperscript{207}. Reiniger et al. conducted a neuropathological study in patients with inherited prion disease and compared the findings with sCJD patients, they reported the abundant deposition of filamentous prion protein in myelinated fibres, this was a feature that was identified in several inherited forms of prion disease in the subcortical white matter and was particularly abundant in those patients with N-terminal \textit{PRNP} mutations\textsuperscript{217}. MTR has been shown to be a particularly effective marker of white matter change in a number of neurodegenerative conditions most notably in multiple sclerosis so it may be sensitive enough to identify early structural change in prion disease. It is exciting to identify a possible candidate marker of disease onset but further data acquisition will be required before it is known whether this is a significant finding and can be used as a future marker of disease onset.
Prion disease is typically a rapidly progressive neurodegenerative disease and therefore it may not be surprising to have identified such positive results in the symptomatic patients recruited to this study. However there were a number caveats that could have presented as problems to the success of the study; one of these being the heterogeneity of the symptomatic and asymptomatic patient groups analysed. The symptomatic patient group included those with sporadic, acquired and inherited forms of the disease. This meant that rates of progression of disease were highly variable as well as clinical phenotype. However despite this heterogeneity a very strong correlation was identified between clinical measures of cognition and physical function (the MRC Scale) and MTR parameters. It is established that although there are different forms of prion disease they all share the hallmark pathological findings on post-mortem, that include spongiform change, PrP\textsuperscript{Sc} deposition and gliosis, and even within specific prion disease subtypes such as sCJD, there is a degree of variability in the extent of these changes being present which may or may not be secondary to PrP\textsuperscript{Sc} strain type. It is therefore likely that the reason for the strong correlation is that grey matter MTR change parallels pathological change and despite the heterogeneity in clinical phenotype and variable rapidity of disease progression the pathology of the disease is similar and is reflected in the imaging findings. The MRC Scale is a measure of clinical progression of disease and consequently is expected to also parallel the severity of neuropathology present, which would account for the strong correlation with the decline in cortical MTR metrics.

Another limitation to the study was the small sample size, prion disease is a rare neurodegenerative disease and recruitment to the study as well as the acquisition of multiple scans for the longitudinal analysis was challenging, added to this acquiring scans of good enough quality to undergo analysis was also tricky as the more symptomatic patients were liable to move during the scan and therefore a lot of data was found to be unusable. In future studies a shorter scan sequence may be of benefit in acquiring better scans. The small sample size may have also contributed to the absence of a statistical difference.
in MT imaging characteristics between carriers and non-carriers. Furthermore, based on probability of onset estimations, several of the asymptomatic gene carriers were deemed to be far from disease onset, again larger ‘at risk’ patient numbers may increase the probability of detecting early imaging abnormalities in this patient group. Additional strengths of the current study include the quantitative automated segmentation methods used for analysing the MRI data, a conservative approach was taken to eroding the MTR cortex, caudate and thalamus to avoid artefact caused by partial volume effect. Furthermore, we correlated MTR values with the MRC Scale a marker of functional and cognitive impairment.

**Further work**

As there are other candidate imaging biomarkers, these including DTI and quantitative assessment of atrophy with VBM, that have shown sensitivity for detecting progressive change in patients with prion disease, further work should be aimed at making a comparison between these parameters and assessing which imaging modality is not only the most sensitive but reliable marker of disease progression.

Movement artefact was a major issue in obtaining reliable data in this study, as many patients with prion disease were unable to tolerate the lengthy scan time. It would be advisable to therefore assess if there is the possibility of implementing a reduced scan time or whether patients may be eligible for taking a mild sedative during the scan.

Neither cross-sectional nor longitudinal differences of statistical significance were identified in the asymptomatic group when compared to control participants, however there were trends observed. Therefore further work is aimed at continuing data collection in the asymptomatic group and the plan will be to re-analyse the data when additional participants are recruited to this patient group and more data points have been achieved.
4. **Sleep symptomatology in the National Prion Monitoring Cohort (NPMC)**

4.1. **General background**

At present symptomatic treatment is the only therapy currently available for patients diagnosed with prion disease. Therefore knowledge of symptom prevalence, morbidity and response to implemented drug therapy is of importance. Sleep disturbance in patients with prion disease is a recognised symptom but the frequency in which it occurs, which subtypes of prion disease are most commonly affected and the pathogenesis is unknown. I aim to identify how common sleep disorders are and if sleep disturbance is associated with other features of disease such as: clinical symptoms, disease subtype, brain MRI changes and codon 129 genotype.

Most of the documented evidence of sleep disturbance in patients with prion disease has been in those with the clinical syndrome Fatal Familial Insomnia (FFI). FFI was first described in an Italian family who carried the autosomal dominant D178N prion protein mutation, a D (aspartic acid) to N (asparagine) variation at codon 178, patients who have the FFI clinical phenotype carry the mutation on the M allele at codon 129\(^{218}\). In patients diagnosed with FFI the predominant symptoms are insomnia, behavioural and autonomic disturbance and cognitive decline. Additional symptoms that present in other forms of prion disease can also occur in patients with FFI; however the most striking feature of FFI is the sleep disturbance, which is most commonly insomnia\(^{219}\).

**FFI neuropathology studies** have revealed selective atrophy of the thalamus\(^{220}\), from these findings and the results of polysomnography studies\(^{221}\) carried out in these patients it is thought that involvement of the thalamocortical tracts in this disease may be the cause of the profound sleep disturbance present\(^{221,222}\).
There have also been a number of reported cases of a clinical syndrome named sporadic fatal insomnia (SFI)\textsuperscript{223,224}, patients described with this syndrome have symptoms similar to that seen in FFI, intractable insomnia, broken sleep and daytime sleepiness, but in addition to the symptoms of sleep disturbance, also other features that classically present in sCJD, however in the SFI cases either the presenting feature or one of the most dominant symptoms has been disrupted sleep accompanied by autonomic abnormalities such as elevated blood pressure and heart rate, low grade pyrexia, increased salivation and diaphoresis. To date there have been around 10 cases of SFI reported, patients diagnosed with the syndrome have been homozygous for methionine at codon 129\textsuperscript{225}. As in FFI, the neuropathology has been found to be similar, with reported findings of severe neuronal loss (80-90%) accompanied by astrogliosis in the anterior-ventral and medial-dorsal thalamic nuclei and inferior olivary nucleus\textsuperscript{220,226}. PrP\textsuperscript{Sc} has been reported to be present at post-mortem, however deposition is variable\textsuperscript{220,227}. Polysomnography has been performed in a number of cases and 24 hour EEG recordings have shown varying results depending on the time point in the course of the disease, however most have shown reduced sleep efficiency, notably reduced stage 3 sleep as well as disrupted REM sleep\textsuperscript{228}. A recent study on patients with inherited prion disease secondary to the E200K mutation also found symptoms of insomnia to be prevalent in the early stages of disease and polysomnography confirmed the presence of disrupted sleep architecture\textsuperscript{229}.

The thalamus and its circuitry to the cortex are responsible for inducing slow wave, also known as deep, stage 3 sleep. In both FFI and SFI pathology of the thalamus has been implicated in the loss of sleep spindles and delta sleep that are pathognomonic of slow wave sleep, polysomnography studies in FFI have been reported as showing significant disorganization of sleep with reduced slow wave sleep and disturbance of slow wave oscillation\textsuperscript{222}. Figure 32 depicts the areas of the brain involved in sleep.
Figure 32
Areas and pathways of the brain involved in sleep induction and maintenance.
SCN= Suprachiasmatic nucleus, REM=Rapid eye movement and SWS=Slow wave sleep

As the thalamus and inferior olivary nucleus show the most neuropathology on post-mortem\textsuperscript{230-232}, it can be hypothesized that imaging abnormalities could be identified in these brain regions. In one FFI case report, thalamic signal change was found to be present on diffusion tensor imaging and in the same patient at post-mortem the thalamus was found to be markedly atrophic\textsuperscript{222}. In other FFI cases FDG-PET studies have shown hypometabolism that is again most marked in the thalamus\textsuperscript{233}. The reported polysomnography, imaging and autopsy findings in sCJD patients who have sleep disturbance have been found to vary\textsuperscript{234}. A recent study by Kang et al. looked at 28 patients with CJD, 26 of these were diagnosed with sCJD, they found a high prevalence of sleep symptomatology with 25 out of 28 patients reporting sleep dysfunction, the most
prominent polysomnography abnormalities were absent or poorly formed sleep spindles and K complexes and absence of slow wave sleep, the latter being evident in 79% of patients. This study very importantly highlights that sleep disturbance is a feature in prion disease, to investigate this further a larger study of sCJD patients would be helpful in not only validating Kang et al. results but also establishing if there is a response to implemented therapeutics, if there are any other associated symptoms and if there are accompanying imaging findings that may be representative of the underlying pathology responsible for the sleep disturbance. Figure 33 illustrates thalamic signal change in a patient with sCJD symptomatic with sleep disturbance.

Figure 33
Axial MRI scan, DWI sequence depicting thalamic signal change in a 64 yr. old patient with sCJD and insomnia
Sleep disturbance in the general population has been found to be associated with low-mood co-morbidity\textsuperscript{236}. There have been several studies that have evaluated the relationship between sleep disturbance and co-morbid depressive symptoms\textsuperscript{237,238}, and there is evidence to support a reduction in low mood morbidity in patients who have received treatment for insomnia\textsuperscript{239,240}. In a large meta-analysis of patients with Alzheimer’s disease and dementia with Lewy bodies, low mood and other behavioural disturbance more frequently occurred in those patients that were symptomatic with sleep disturbance\textsuperscript{241}. In Parkinson’s disease sleep disturbance is a commonly reported symptom and has been reported to co-occur with symptoms of depression and anxiety\textsuperscript{242-244}.

Sleep disturbance has been described in several forms of neurodegenerative disease\textsuperscript{245-248} and a number of scales have been devised to record the most common features of the disorder.

There are several clinical scales in existence that are designed to capture symptoms of sleep disturbance; these include the Epworth Sleepiness Scale (ESS), the Pittsburg Sleep Quality Index (PSQI) and the Sleep Quality Scale (SQS). Although these scales have been found to be both valid and reliable methods of measuring the frequency and severity of sleep abnormalities in those with sleep disturbance, they have not been explicitly created to monitor sleep abnormalities in one particular disease. The Parkinson’s Disease Sleep Scale (PDSS), a more recently developed scale, was specifically designed to monitor sleep abnormalities that patients with Parkinson’s disease are most symptomatic with\textsuperscript{249}; this scale was created by retrospectively collecting data on sleep symptomatology in patients with Parkinson’s disease and using the data to create a questionnaire. The scale has been found to be both a valid and reliable method of monitoring nocturnal symptoms of sleep disturbance and their response to drug therapy\textsuperscript{250,251}.

In symptomatic prion disease patients recruited to the National Prion Monitoring Cohort (NPMC), sleep disturbance appears to be a symptom that frequently occurs, the sleep symptomatology that patients with prion disease have
appears to be quite specific and could be better documented through the use of a questionnaire specifically designed to capture the most common complaints in this patient group. In addition to this, by monitoring sleep disturbance in symptomatic patients any implemented drug therapies could be monitored for their efficacy with the use of the questionnaire.

The aim of this project was to test the hypothesis that sleep abnormalities are highly prevalent in all forms of prion disease, they are primarily caused by thalamic pathology, and therefore correlate with thalamic MRI signal change in patients with prion disease that are symptomatic with sleep disturbance. After analysing the data acquired on the different types of sleep disturbance that were present in patients with prion disease it became evident that there were certain symptoms that commonly presented. I found that there wasn’t a sleep scale that could be used practically to specifically collect data on the sleep symptoms that were most prevalent in patients with prion disease; some of the other sleep scales were exhaustive in the number of symptoms they covered but many questions would not be applicable to patients with prion disease. Therefore as a second part of the project, I devised the Prion Disease Sleep Questionnaire, a quantitative method for collecting and analysing sleep symptomatology in prion disease.

4.2. Patients and methods

4.2.1. Recruitment criteria, patients and study methods

All patients included in the study were referred to the NHS National Prion Clinic and enrolled to the National Prion Monitoring Cohort (NPMC). The aim of the NPMC is to collect natural history data on symptoms and signs in all forms of prion disease, the cohort uptake is over 95% so the data collected from this patient group is highly typical of prion disease.
Patients included in the study were those diagnosed with probable or definite sCJD, using the WHO criteria, patients symptomatic with inherited prion disease (IPD), iatrogenic prion disease (iCJD) and vCJD. A total of 448 symptomatic patients recruited to the NPMC were eligible for the analysis, both controls and asymptomatic subjects were not included.

On enrolment to the NPMC all patients underwent a systematic clinical assessment, including cognitive and neurological examination. The patient and/or their carer were questioned regarding the nature of their symptoms including onset, severity and frequency. All clinical information including medications and indications for the prescription was also recorded. After the initial enrolment regular follow-up was then arranged and at each subsequent assessment the presence and severity of clinical features were documented, as well as any changes to drug prescriptions. In addition to this, as part of the clinical assessment, the MRC Scale (a functional outcome measure of clinical severity) was also recorded; the MRC Scale is illustrated on page 304 and 305 in the appendix.

4.2.2. Clinical data collection

Data collection of the prevalence of sleep disturbance was made using the clinical research data collection forms (CRFs) and the clinical notes. Recordings of sleep disturbance were on a scale of 0-3, 0=no sleep disturbance, 1 as sometimes symptomatic, 2 as often symptomatic and 3 as always symptomatic, the CRF with sleep specific questions (section 12) is illustrated on page 304 in the appendix.

For the purposes of the study, patients deemed to be symptomatic (with a sleep disturbance) were those that scored ‘often’, graded 2, or ‘always’, graded 3, for having a sleep disturbance on the CRFs. Most of the patients had been scored as either being symptomatic or not for having a sleep disturbance on the CRFs.
There was a small no of patients who were not assessable from the CRF for having sleep abnormalities, those classed as “U”, in order to capture as much data as possible, patients were also selected if they were receiving therapeutic drugs used to treat sleep disturbance that had been commenced during the course of their illness this data was acquired by performing a search of the clinical notes to assess if the medication had been commenced during the course of their current illness and the reason for it, if it was found that they were symptomatic for a sleep disorder they were also included in the analysis.

The specific sleep disturbances recorded on the CRFs were hypersomnolence, insomnia (difficulty falling asleep) and waking at night. In addition to this, further detailed information regarding the nature of the sleep disturbance was also collected from the clinical notes and letters, this information included any other types of sleep disturbance the patient was symptomatic with, and wherever possible when it had occurred during the course of the disease along with any evidence of therapeutic intervention, the aim was to identify as much clinical information that could be gathered regarding the nature and treatment of sleep disturbance present.

Data was collected to identify the prevalence and type of sleep symptomatology present at disease onset, and at time intervals during the course of disease to assess if there was longitudinal change occurring in both the frequency and type of sleep symptoms that presented. Additionally data on associated symptoms of low mood/depression co-morbidity was also collected on the CRF and scored in the same way as the sleep disturbance. Data on the commencement or cessation of drug therapy used for treatment of sleep symptomatology during the course of the study was also gathered.

Other data collected for the study included MRI head scan results, specifically the presence or absence of thalamic signal change, (all subjects’ MRI head scans were formally reported by an NHNN neuroradiologist). The reason for
collecting this data was to investigate if a relationship existed between thalamic MRI signal change and the presence of sleep disturbance.

Patient PRNP codon 129 genotype was also recorded. As mentioned in both the introduction and chapter 2, codon 129 genotype has been found to be a strong predictor of both disease phenotype and disease progression in mainly sCJD patients but also in other prion disease subtypes, therefore it seemed prudent to assess if it also had an impact on the presence of sleep disturbance. The MRC Scale mentioned in the introduction to my thesis is a 20-point scale that was developed to measure the disease severity in patients with prion disease based on measures of cognition and clinical function \(^{15}\). I used this scale to assess if prevalence of sleep disturbance changed with progression of disease and if specific sleep symptomatology was associated with rate of disease progression.

4.2.3. Statistical analysis

Statistical tests were performed using IBM SPSS statistics (version 24) and Microsoft Excel.

Chi-square tests were used for:

- The analysis of association between prevalence of specific sleep disturbance symptoms and the disease subtype present at disease onset and at any point in the course of disease.

- To assess for an association between the presence of symptoms of depression and sleep disturbance.

- To assess for an association between the presence of thalamic signal change and sleep disturbance.
Ordinal regression analysis was employed to assess if there was a correlation between grade of sleep score and the presence of depression, gender and age were included as covariates.

Logistic regression analysis was used for:

- Assessing for factors associated with the presence of sleep disturbance, including thalamic signal change, codon 129 and disease subtype covariates of age and gender were included in the models.

- To assess if the slope of disease progression (measured with the MRC Scale slope) acted as a predictor of presence of sleep disturbance, gender, age and disease subtype were included as covariates.

4.3. Results

4.3.1. Prevalence of sleep disturbance

Of the total of 448 patients included in the analysis, 273 sCJD patients, 49 with symptomatic IPD and 18 with acquired prion disease (patients with iCJD and vCJD) were symptomatic with sleep disturbance. Table 21 illustrates the groups of patients with and without a sleep disturbance divided by disease subtype.
Table 21

Patients with and without sleep disturbance divided by disease subtype (6OPRI, P102L and D178N refer to inherited prion disease gene mutations).

The prevalence of sleep disturbance in the NPMC was high with 76% of patients being found to be symptomatic with sleep problems (340 patients, median age 64 years, range 21-92 and male to female ratio of 1:1.1) vs. 24% of patients without (108 patients, median age 64 years, range 22-80 years, male to female ratio 1.4:1).

Table 22

Subjects with and without significant sleep disturbance enrolled to the NPMC total number of patients =448
In 8% of cases the first documented (presenting) symptom was sleep disturbance, this was either insomnia or hypersomnolence. In 44% of cases sleep disturbance featured as an early presenting symptom. Due to phenotypic heterogeneity duration of disease is highly variable, therefore “early presenting symptom” was defined as the symptom occurring within the first quarter of the total disease duration.

4.3.2. Phenomenology of sleep disturbance

The prevalence and phenomenology of sleep disturbance differed depending on the underlying prion disease diagnosis and stage of disease. Table 23 illustrates the prevalence of different sleep symptoms divided by subtype of prion disease at the onset of disease, table 24 shows the occurrence of sleep symptomatology in patients again divided by disease subtype at any time during the course of the disease. These data suggest that the prevalence of some sleep symptoms, such as hypersomnolence increases with disease progression and is more common in those with sCJD see tables 23 and 24. Also certain disease subtypes such as inherited prion disease were associated with nighttime waking at disease onset but this was less pronounced during the course of the disease, other symptoms i.e. shouting, wandering and toilet use was more common in the inherited prion disease patient group, see tables 23 and 24.
<table>
<thead>
<tr>
<th>Sleep disturbance</th>
<th>sCJD</th>
<th>Acquired prion disease</th>
<th>Inherited prion disease</th>
<th>Total</th>
<th>P-value (Chi-square statistic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insomnia (difficulty going to sleep)</strong></td>
<td>82</td>
<td>6</td>
<td>25</td>
<td>113</td>
<td>0.26 (2.7)</td>
</tr>
<tr>
<td></td>
<td>(87.6)</td>
<td>(6.0)</td>
<td>(19.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0.36]</td>
<td>[0]</td>
<td>[1.66]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hypersomnolence</strong></td>
<td>90</td>
<td>11</td>
<td>27</td>
<td>128</td>
<td>0.052 (5.9)</td>
</tr>
<tr>
<td></td>
<td>(98.6)</td>
<td>(6.8)</td>
<td>(22.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0.75]</td>
<td>[2.62]</td>
<td>[0.85]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Night-time waking</strong></td>
<td>83</td>
<td>7</td>
<td>29</td>
<td>119</td>
<td>0.04 (6.5)</td>
</tr>
<tr>
<td></td>
<td>(92.4)</td>
<td>(6.3)</td>
<td>(20.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[1.0]</td>
<td>[0.1]</td>
<td>[3.8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Night-time movement</strong></td>
<td>31</td>
<td>5</td>
<td>10</td>
<td>46</td>
<td>0.04 (6.26)</td>
</tr>
<tr>
<td></td>
<td>(37.2)</td>
<td>(2.63)</td>
<td>(6.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[1.04]</td>
<td>[2.1]</td>
<td>[2.4]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other (toilet use, shouting, wandering)</strong></td>
<td>27</td>
<td>1</td>
<td>18</td>
<td>46</td>
<td>0.0002 (16.99)</td>
</tr>
<tr>
<td></td>
<td>(35.44)</td>
<td>(2.51)</td>
<td>(8.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[2.0]</td>
<td>[0.9]</td>
<td>[12.3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>313</td>
<td>30</td>
<td>109</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 23**

The prevalence of sleep symptoms in patients with prion disease at disease onset. Observed patient totals, (expected cell totals) and [Chi-square statistic]. Chi-square test performed to assess for a significant prevalence of specific sleep disturbance present for each of the patient groups.
## Table 24

The total prevalence of sleep symptoms in patients with prion disease (at any time during the course of disease. Observed patient totals, (expected cell totals) and [Chi-square statistic]. Chi-square test performed to assess for a significant prevalence of specific sleep disturbance present for each of the patient groups.

<table>
<thead>
<tr>
<th>Sleep disturbance</th>
<th>sCJD</th>
<th>Acquired prion disease</th>
<th>Inherited prion disease</th>
<th>Total</th>
<th>P-value (Chi-Square test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insomnia (difficulty going to sleep)</td>
<td>148</td>
<td>7</td>
<td>37</td>
<td>192</td>
<td>0.26 (2.7)</td>
</tr>
<tr>
<td></td>
<td>(149)</td>
<td>(10.24)</td>
<td>(32.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0.0]</td>
<td>[1.0]</td>
<td>[0.52]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypersomnia</td>
<td>233</td>
<td>13</td>
<td>35</td>
<td>280</td>
<td>0.001 (13.39)</td>
</tr>
<tr>
<td></td>
<td>(217.4)</td>
<td>(15.6)</td>
<td>(45.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[1.11]</td>
<td>[0.43]</td>
<td>[2.38]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night-time waking</td>
<td>197</td>
<td>11</td>
<td>31</td>
<td>238</td>
<td>0.046 (6.16)</td>
</tr>
<tr>
<td></td>
<td>(187.3)</td>
<td>(11.1)</td>
<td>(40.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0.50]</td>
<td>[0.00]</td>
<td>[2.4]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Night-time movement</td>
<td>43</td>
<td>7</td>
<td>13</td>
<td>62</td>
<td>0.051 (5.93)</td>
</tr>
<tr>
<td></td>
<td>(48.9)</td>
<td>(3.4)</td>
<td>(10.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[0.7]</td>
<td>[3.9]</td>
<td>[0.46]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other (toilet use, shouting, wandering)</td>
<td>49</td>
<td>3</td>
<td>21</td>
<td>73</td>
<td>0.015 (8.39)</td>
</tr>
<tr>
<td></td>
<td>(56.6)</td>
<td>(3.9)</td>
<td>(12.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[1.0]</td>
<td>[0.2]</td>
<td>[5.8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>670</td>
<td>41</td>
<td>137</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The association between sleep disturbance and disease severity

To further assess the relationship between sleep symptomatology and disease severity, I looked at the association between how prevalent sleep disturbance was and if that changed with severity of disease, as measured by the MRC Scale (figure 34).

**Figure 34**

*Percentage of patients with sleep disturbance divided by severity of disease. Chi-square statistic for those with most severe disease vs. those with least severe disease 13.45, p-value=0.000244*

As there appeared to be a difference in the prevalence of specific sleep symptoms I decided to look at the most commonly occurring sleep symptoms and to assess how they varied with disease severity.
The most commonly recorded sleep symptom at any stage of disease was hypersomnolence, with a prevalence of 82%; this was followed by symptoms of insomnia/difficulty going to sleep (56%) and night-time waking (70%). Night-time movements occurred in 18% of cases and other symptoms that were reported were: night-time wandering, nocturia, nightmares, sleep talking, sleep apnoea and behavioural disturbance (cumulatively occurring in approximately 21%).

From tables 23 and 24 it can be seen that although there are a number of patients that are symptomatic with sleep related disturbance at disease onset, that there must be others that develop symptoms with progression of disease and in the case of some inherited prion disease patients those that become less symptomatic as disease progresses. With that in mind I explored how the prevalence of the most common symptoms: hypersomnolence, night waking and insomnia varied with disease progression using the MRC Scale score as the function of disease severity.
Hypersomnolence

Hypersomnolence was more frequently reported in patients with sCJD. In the early stages of the disease hypersomnolence was often accompanied by co-morbid insomnia. In the latter stages of the illness this was not found to be the case.

The most frequently reported symptom was excessive daytime napping. Patients were classified as being symptomatic with hypersomnolence if they or their relatives reported that they were napping excessively in the day or falling asleep during conversations, mealtimes or activities they were engaged in. Hypersomnolence occurrence increased with progression of disease being most commonly reported at an MRC Scale score of 3-5, interestingly at the end stage of the disease somnolence symptoms appear to decline, however this can probably be explained by the difficulty in defining somnolence symptoms at the end of disease as many patients are likely to be in a coma and therefore cannot be reliably scored for somnolence symptoms. Figure 35 depicts the distribution of somnolence prevalence by disease severity, as measured by the MRC Scale.
Figure 35

Prevalence of hypersomnolence vs. the MRC Scale score. Chi-square statistic for those symptomatic with severe disease vs. those with early onset disease = 15.46, p-value=0.00084
Symptoms of insomnia (difficulty falling asleep) and night-time waking

Symptoms of insomnia such as difficulty going to sleep and night-time waking were often reported to co-occur. These symptoms were also found to frequently occur alongside symptoms of anxiety, low mood and irritability, reported in the clinical notes and documented on the CRFs.

Patients with early onset, rapidly progressive sCJD and patients diagnosed with iCJD were often reported to have insomnia occurring as an early clinical feature.

In patients diagnosed with IPD difficulty falling asleep and night-time waking was frequently reported, in 76% patients, and most commonly occurred in the mid-stages of the illness. There were also reports particularly in patients diagnosed with 6OPRI inherited prion disease of co-morbid behavioural disturbance, such as shouting and aggressive behavior occurring at night, although behavioural problems are a common feature of this form of prion disease there were more reports of it occurring in those with significant sleep disturbance.

Difficulty falling asleep increased with disease progression and was most common at an MRC Scale of 6-8, at the later stages of disease the prevalence of this symptom decreased, this change in symptomatology might be explained by the increasing somnolence and reduced conscious level that occurs in patients towards the end of disease. Night-time waking was prevalent at all stages of disease but again gradually increased with disease severity being most commonly reported at and MRC Scale score of 3-5.

Figures 36 and 37 depict the change in prevalence of difficulty falling asleep and night-time waking with disease severity (measured by the MRC Scale).
Figure 36

Prevalence of difficulty falling asleep vs. the MRC Scale score

Figure 37

Prevalence of night-time waking vs. the MRC Scale score
Other symptoms

Night-time movements were reported in approximately 18% of cases. Myoclonus appeared to be the most frequently observed night-time movement disorder; but other movement disorders such as restless legs syndrome with accompanying periodic limb movements were also noted to occur.

Nocturia was found to occur in approximately 15% of patients and was more commonly reported in patients symptomatic with IPD.

A small number of patients were symptomatic with vivid dreams and/or nightmares. One patient diagnosed with P102L inherited prion disease was particularly symptomatic with vivid dreams accompanied by other sleep symptoms, he had difficulty in distinguishing his dreams from reality and shortly after the onset of the sleep disturbance became symptomatic with visual hallucinations. Other symptoms reported included wandering and night-time shouting, these were also more commonly reported in the inherited forms of prion disease, the latter being most prevalent in those with 5OPRI and 6OPRI mutations.

4.3.3. Pharmacological therapy

Data on current drug treatment and changes to medication were collected in all patients recruited to the NPMC. There were a number of different drug therapies used to treat sleep disturbance in the cohort. Pharmacological therapy included: benzodiazepines, Z drugs, antidepressants and melatonin for treatment of symptoms of insomnia and modafinil and sodium oxybate were used to treat patients that were symptomatic with hypersomnolence. There was a total of 91 prescriptions for drug treatment (in a total of 73 patients). Table 25 illustrates the different drug classes used, the number of patients prescribed treatment and the indication for use.
<table>
<thead>
<tr>
<th>Drug type</th>
<th>Number of patients</th>
<th>Indication for prescription</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z drugs</td>
<td>37</td>
<td>Insomnia and night-time waking</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>36</td>
<td>Insomnia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insomnia accompanied by myoclonus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REM sleep disorder</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>12</td>
<td>Insomnia and insomnia accompanied by low mood/depression</td>
</tr>
<tr>
<td>Hormones</td>
<td>3</td>
<td>Insomnia</td>
</tr>
<tr>
<td>Other-drugs promoting wakefulness</td>
<td>3</td>
<td>Hypersomnolence</td>
</tr>
<tr>
<td>(Modafinil and sodium oxybate)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 25  
*Pharmacological treatment of sleep disturbance in patients with prion disease*

Although hypersomnolence was the most frequently noted symptom, only 3 patients were prescribed medication to treat it, 2 patients were prescribed modafinil and one sodium oxybate, from the clinical notes there was some evidence of efficacy with the drug modafinil in reducing symptoms of somnolence in one patient.

The majority of patients symptomatic with sleep disturbance were prescribed medication for treatment of insomnia. The most commonly prescribed drugs were Z drugs, used in 41% of patients. Zopiclone was prescribed to 36 patients, and zolpidem in one case. The majority of patients tolerated treatment with zopiclone with only 2 cases being recorded of the medication being stopped, in one the reason for ceasing treatment was due to the side effect of daytime sleepiness, the patient in which this occurred was subsequently commenced on zolpidem and tolerated treatment with this.
Other drugs used for treating insomnia were benzodiazepines, these included temazepam (28 cases), clonazepam (8), lorazepam (2), diazepam (4) and midazolam (1), in 7 cases the medication was recorded as being prescribed for more than one purpose, to provide relief from other symptoms additional to the insomnia, these symptoms included agitation and myoclonus.

Another classes of medication used for treatment of insomnia were antidepressants, with amitriptyline being used in 10 cases and mirtazepine in 2. Both these medications were also prescribed as dual treatment for other symptoms including depression, low mood, headache and agitation. Melatonin the hormone therapy was also prescribed in 3 patients, there is evidence in one patient of a limited response to treatment on symptom re-assessment, however the response was not sustained on subsequent re-assessment.

There were 4 recordings of drugs being stopped due to the development of adverse effects, amitriptyline secondary to the side effect of dizziness, temazepam and zopiclone (2 accounts) secondary to the side effect of causing daytime sedation. The data regarding efficacy of drug therapy were limited, however there were several patients that were tried on a combination of different medications and therefore it is probable that the initial therapy implemented in these cases was not successful.

4.3.4. Associated predictors of the presence and phenotype of sleep disturbance

The factors that I assessed for whether there was an association with the presence of a sleep disturbance were: depression, thalamic signal change on MRI head scan, codon 129 and disease subtype. The results of which are detailed below in tables 26-30 and figure 38.
Co-morbidity of sleep disturbance with depression

Analysing the number of patients with and without depression vs. those with and without sleep disturbance I found that there was a significant association present. Chi-square test (p=0.0089). See table 26.

<table>
<thead>
<tr>
<th></th>
<th>Sleep score 2 or 3</th>
<th>Sleep score 0 or 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depression score 2 or 3</td>
<td>138 (121.37) [2.28]</td>
<td>87 (103.63) [2.67]</td>
</tr>
<tr>
<td>Depression score 0 or 1</td>
<td>300 (316.63) [0.87]</td>
<td>287 (270.37) [1.02]</td>
</tr>
<tr>
<td>Total</td>
<td>438</td>
<td>81</td>
</tr>
</tbody>
</table>

Table 26

*The association between depressive symptomatology and sleep disturbance, observed patient totals, (expected cell totals) and [Chi-square statistic]. Chi-square test: p-value=0.0089, Chi-square statistic 6.8416*

As mentioned previously sleep disturbance is graded from 0-4 (0=none and 4 =maximal/worst symptoms) on the CRFs, I conducted an ordinal regression analysis to assess if depressive symptoms correlated with the grade of sleep disturbance and to assess if other covariates such as age, gender and codon 129 also acted as predictors of sleep disturbance, the results of which are presented in table 27.
### Table 27

**Ordinal regression analysis of predictors of grade of sleep disturbance present**

**Res variable=Response Variable (CODON 129 MM, MV VV)**

<table>
<thead>
<tr>
<th>Res variable</th>
<th>Log odds coeff</th>
<th>S.E</th>
<th>Wald Chi-square test</th>
<th>Deqs of freedom</th>
<th>p-value</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>sleep = 0</td>
<td>-0.18</td>
<td>0.43</td>
<td>0.18</td>
<td>1</td>
<td>0.67</td>
<td>-1.1</td>
</tr>
<tr>
<td>sleep = 1</td>
<td>0.98</td>
<td>0.43</td>
<td>5.28</td>
<td>1</td>
<td>0.02</td>
<td>0.14</td>
</tr>
<tr>
<td>sleep = 2</td>
<td>2.87</td>
<td>0.44</td>
<td>42.32</td>
<td>1</td>
<td>&lt;0.001</td>
<td>2.01</td>
</tr>
<tr>
<td>Age at onset per yr.</td>
<td>0.02</td>
<td>0.01</td>
<td>8.70</td>
<td>1</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Depression</td>
<td>0.29</td>
<td>0.08</td>
<td>13.87</td>
<td>1</td>
<td>0.01</td>
<td>0.14</td>
</tr>
<tr>
<td>codon129 mm</td>
<td>-0.43</td>
<td>0.28</td>
<td>2.27</td>
<td>1</td>
<td>0.13</td>
<td>-0.99</td>
</tr>
<tr>
<td>codon129 mv</td>
<td>0.20</td>
<td>0.23</td>
<td>0.77</td>
<td>1</td>
<td>0.38</td>
<td>-0.25</td>
</tr>
<tr>
<td>codon129 vv</td>
<td>-0.18</td>
<td>0.22</td>
<td>0.68</td>
<td>1</td>
<td>0.41</td>
<td>-0.62</td>
</tr>
<tr>
<td>gender=0</td>
<td>-0.06</td>
<td>0.14</td>
<td>0.16</td>
<td>1</td>
<td>0.69</td>
<td>-0.34</td>
</tr>
<tr>
<td>gender=1</td>
<td>0a</td>
<td>.</td>
<td>.</td>
<td>0</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>

a. This parameter is set to zero because it is redundant.
Figure 38

The percentage of patients with depression graded by severity of sleep disturbance

The graph in figure 38 shows the percentage of patients with depression subdivided by grade of sleep disturbance.

The percentage of patients with depression increases with grade of sleep disturbance from 0-2, there is then a tail off in the percentage of patients with a sleep grade of 3 and depression, this observation could be caused by a number of factors, the most obvious one is that severe sleep disturbance is not associated with depressive symptoms. However, two thirds of patients with a sleep score of 3 have an MRC Scale score of 5 or less, i.e. they are at a more severe stage of disease and therefore it may not be possible to reliably assess symptoms of depression at this stage, indeed of a total of 155 patients graded 3 for sleep disturbance, 108 of those were given a U for grading the severity of depression i.e. ungradable. Another possible explanation for this observation is that grade 3 sleep disturbance may be over-represented by a specific type of sleep disturbance, which may not be the main factor associated with depression.
Thalamic signal intensity vs. presence of sleep disturbance

A significant association was found between the presence of sleep disturbance and thalamic signal change in the thalamus. 68% of patients with significant sleep disturbance had thalamic signal abnormalities present on the MRI scan. Chi-square test (p=0.0028), see table 28.

<table>
<thead>
<tr>
<th></th>
<th>Sleep score 2 or 3</th>
<th>Sleep score 0 or 1</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI: Thalamic signal intensity</td>
<td>103 (91.61)</td>
<td>22 (33.39)</td>
<td>124</td>
</tr>
<tr>
<td>MRI: No thalamic signal change</td>
<td>122 (133.39)</td>
<td>60 (48.61)</td>
<td>222</td>
</tr>
<tr>
<td>Total</td>
<td>225</td>
<td>82</td>
<td>307</td>
</tr>
</tbody>
</table>

Table 28
Contingency table showing numbers of patients with sleep disturbance (sleep score 2 or 3) and without sleep disturbance (sleep score 0 or 1) compared to numbers of patients with or without thalamic signal intensity on MRI head scan, expected cell totals are shown in brackets. Chi-square test: p-value=0.0028, Chi-square statistic 8.9393
Variables in the Equation

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Cons coeff</th>
<th>S.E</th>
<th>Wald Chi-sq test</th>
<th>Degs of fr’dom</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% C.I. for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thal</td>
<td>225</td>
<td>-0.82</td>
<td>0.28</td>
<td>8.11</td>
<td>1</td>
<td>0.004</td>
<td>0.44</td>
<td>0.25</td>
</tr>
<tr>
<td>Age at onset</td>
<td>0.01</td>
<td>0.01</td>
<td>0.31</td>
<td>1</td>
<td>0.58</td>
<td>1.00</td>
<td>0.98</td>
<td>1.0</td>
</tr>
<tr>
<td>Male</td>
<td>98</td>
<td>-0.36</td>
<td>0.27</td>
<td>1.82</td>
<td>1</td>
<td>0.18</td>
<td>0.69</td>
<td>0.4</td>
</tr>
<tr>
<td>Female</td>
<td>127</td>
<td>-0.2</td>
<td>0.27</td>
<td>1.44</td>
<td>1</td>
<td>0.23</td>
<td>3.27</td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.18</td>
<td>0.99</td>
<td>1.44</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 29

Predictors, (thalamic signal intensity, age and gender) associated with presence of sleep disturbance (total patient number 225) Thal=thalamus, N=number, Coeff=coefficient, S.E=standard error, Con=Constant, Degs of fr’dom=degrees of freedom

4.3.5. Assessment of other factors acting as predictors of presence of sleep disturbance

I assessed if there were any other factors that acted as predictors of the presence of sleep disturbance, these included codon 129 genotype, disease subtype, age and gender, the results are presented in table 30.
### Table 30

Factors not found to be associated with sleep disturbance. There was no association identified between codon 129 genotype, age, gender or diagnosis and the presence of sleep disturbance.
4.3.6. Logistic regression analysis to assess if rate of disease progression, disease subtype and other variables act as predictors of sleep symptomatology subtype

The MRC Scale slope of disease progression was calculated in 256 patients (212 patients with sCJD and 44 patients with inherited prion disease), logistic regression analysis was then conducted to assess if there was an association between specific sleep symptom type (difficulty going to sleep, night-time waking and hypersomnolence) and rate of disease progression, other variables included in the analysis included gender, diagnosis and age. Tables 31-33 illustrate these findings. Age at disease onset affected the presence of insomnia (p-value 0.001) and incidence of night-time waking (p-value 0.016). There were no factors that predicted the presence of somnolence (table 32).
<table>
<thead>
<tr>
<th>Variables in the Equation</th>
<th>Coefficient</th>
<th>S.E</th>
<th>Wald chi-square test</th>
<th>Degr of freedom</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% C.I. for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC Scale slope</td>
<td>-1.66</td>
<td>1.44</td>
<td>1.32</td>
<td>1</td>
<td>0.24</td>
<td>0.19</td>
<td>0.01</td>
</tr>
<tr>
<td>Diagnosis sCJD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ref</td>
</tr>
<tr>
<td>Diagnosis IPD</td>
<td>0.56</td>
<td>0.40</td>
<td>1.95</td>
<td>1</td>
<td>0.16</td>
<td>1.76</td>
<td>0.79</td>
</tr>
<tr>
<td>Gender male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ref</td>
</tr>
<tr>
<td>Gender female</td>
<td>0.31</td>
<td>0.23</td>
<td>1.80</td>
<td>1</td>
<td>0.17</td>
<td>1.36</td>
<td>0.86</td>
</tr>
<tr>
<td>Age at onset (per year)</td>
<td>-0.04</td>
<td>0.01</td>
<td>10.26</td>
<td>1</td>
<td>.001</td>
<td>0.95</td>
<td>0.93</td>
</tr>
<tr>
<td>Constant</td>
<td>1.53</td>
<td>0.7</td>
<td>4.71</td>
<td>1</td>
<td>.03</td>
<td>4.65</td>
<td>ref</td>
</tr>
</tbody>
</table>

**Table 31**

*Analysis of variables that may affect the presence of insomnia: MRC Scale slope, diagnosis, gender and age logistic regression analysis (number of patients=118)*
<table>
<thead>
<tr>
<th>Variables in the Equation</th>
<th>Coefficient</th>
<th>S.E</th>
<th>Wald Chi-square test</th>
<th>Degs of freedom</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% C.I. for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC Scale slope</td>
<td>0.50</td>
<td>1.31</td>
<td>0.14</td>
<td>1</td>
<td>0.70</td>
<td>1.65</td>
<td>0.12</td>
</tr>
<tr>
<td>Diagnosis sCJD</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>ref</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Diagnosis IPD</td>
<td>0.08</td>
<td>0.38</td>
<td>0.04</td>
<td>1</td>
<td>0.83</td>
<td>1.08</td>
<td>0.51</td>
</tr>
<tr>
<td>Gender male</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>ref</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Gender female</td>
<td>-0.16</td>
<td>0.21</td>
<td>0.55</td>
<td>1</td>
<td>0.45</td>
<td>0.84</td>
<td>0.55</td>
</tr>
<tr>
<td>Age at onset (per year)</td>
<td>0</td>
<td>0.01</td>
<td>0.03</td>
<td>1</td>
<td>0.85</td>
<td>1.00</td>
<td>0.97</td>
</tr>
<tr>
<td>Constant</td>
<td>0.18</td>
<td>0.66</td>
<td>0.07</td>
<td>1</td>
<td>0.78</td>
<td>1.19</td>
<td></td>
</tr>
</tbody>
</table>

a. Variable(s) entered on step 1: MRC Scale slope, diagnosis, gender and age.

**Table 32**

*Analysis of variables that may affect the presence of somnolence: MRC Scale slope, diagnosis, gender and age, logistic regression analysis (number of patients=205)*
### Table 33

*Analysis of variables that may affect the presence of night-time waking: MRC Scale slope, diagnosis, gender and age, logistic regression analysis (number of patients=115)*

<table>
<thead>
<tr>
<th>Variables in the Equation</th>
<th>Coefficient</th>
<th>S.E</th>
<th>Wald Chi-square test</th>
<th>Degs of freedom</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% C.I. for odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRC Scale slope</td>
<td>-2.58</td>
<td>1.49</td>
<td>3.01</td>
<td>1</td>
<td>0.08</td>
<td>0.07</td>
<td>0</td>
</tr>
<tr>
<td>Diagnosis sCJD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ref</td>
</tr>
<tr>
<td>Diagnosis IPD</td>
<td>0.07</td>
<td>0.4</td>
<td>0.03</td>
<td>1</td>
<td>0.87</td>
<td>1.07</td>
<td>0.49</td>
</tr>
<tr>
<td>Gender male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ref</td>
</tr>
<tr>
<td>Gender female</td>
<td>0.03</td>
<td>0.24</td>
<td>0.01</td>
<td>1</td>
<td>0.91</td>
<td>1.02</td>
<td>0.65</td>
</tr>
<tr>
<td>Age at disease onset</td>
<td>-0.03</td>
<td>0.01</td>
<td>5.82</td>
<td>1</td>
<td><strong>0.02</strong></td>
<td>0.97</td>
<td>0.94</td>
</tr>
<tr>
<td>Constant</td>
<td>1.41</td>
<td>0.71</td>
<td>3.97</td>
<td>1</td>
<td>0.05</td>
<td>4.10</td>
<td></td>
</tr>
</tbody>
</table>

a. Variable(s) entered on step 1: MRC Scale slope, diagnosis, gender, and age.
4.3.7. Patient case study

One of the patients recruited to the NPMC who was carrying the D178N PRNP mutation, methionine homozygous at codon 129 became symptomatic with prion disease during the course of the study, and his symptoms were compatible with those seen in the fatal familial insomnia phenotype. The patient was clinically assessed at regular intervals over the course of his illness, a polysomnography study was also conducted to assess if the sleep architecture was typical of that reported in the literature. Detailed below is his clinical history, polysomnography and post-mortem examination.

The patient was a 43 year old male who presented with a history of difficulty sleeping at night with several episodes of waking and episodes of not breathing; in addition to this he described symptoms of daytime somnolence, with feeling lethargic and falling asleep whilst having a meal. Other symptoms the patient complained of were dizziness, difficulty concentrating and slurred speech, unsteady gait and recurrent falls. As the illness progressed he developed worsening balance, significant cognitive impairment and seizures. The patient had 2 MRI head scans, with no evidence of abnormalities specific to prion disease.

The patient underwent polysomnography, approximately 6 months after the onset of his first symptoms (total disease duration 9 months).

**Polysomnography**

The background EEG was reported as being abnormal and slow. The ECG showed tachycardia with frequent ectopic activity. During the day the patient frequently fell asleep and appeared to enter REM sleep with loss of normal REM atonia.
Overnight sleep architecture was severely disrupted with a large proportion of REM sleep (48%) and only a small amount of deep sleep (8%). There was loss of normal atonia during REM sleep and frequent movements. There were K complexes and sleep spindles, but these were infrequent and poorly formed. There was also evidence of irregular Cheyne-Stokes/periodic breathing with occasional apnoeic episodes. The overall study demonstrated:

- Slow background EEG.
- Disrupted sleep architecture with very little deep sleep and periodic REM.
- Present but rare sleep spindles and K complexes.
- Excessive REM sleep with loss of normal REM atonia.
- Mild sleep apnoea with a pattern consistent with central sleep apnoea.
Stage I sleep with REM fragment towards end of epoch (21:06). Bipolar montage; 30 second epoch.

Stage II sleep with ill formed vertex sharp waves and rare sleep spindles (14:18). Bipolar montage; 30 second epoch.
Figure 39

*Patient sleep study, EEG traces from sleep stages 1-3. The hypnogram illustrates time spent in stages of sleep, only 5% of time was spent in deep sleep, the normal time spent in deep sleep is approximately 15-20% of the total sleep time*
The patient died approximately 9 months after the onset of his first symptoms and underwent post-mortem examination.

**Post-mortem findings**

The post-mortem did not show evidence of significant vacuolar change in the grey matter. There was widespread deposition of prion protein predominantly in the neocortex, but also to a lesser extent in the deep grey matter nuclei and cerebellum. In addition to this there was dot-like prion protein deposition in the thalamus and basal ganglia including the myelin sheaths that would be compatible with the known clinical syndrome of fatal familial insomnia. There was also widespread cerebral oedema of uncertain nature and widespread neuronal, cytoplasmic and perivascular labeling for the prion protein.

**Discussion**

The patient’s clinical syndrome was compatible with what is seen in the FFI phenotype, with autonomic symptoms and signs, a significant sleep disturbance and progressive neurological decline. The total disease duration, 9 months, was also in keeping with the disease duration range seen in patients with FFI. The polysomnography findings of severely disrupted sleep architecture, reduced REM sleep and markedly reduced sleep spindles and K complexes fit both with what is seen in FFI patients and the SFI cases reported in the literature\(^{252}\) the reduced sleep spindles and K complexes being generated by the thalamus may indicate pathological processes occurring in this case. The MRI scans did not show signal change compatible with what is typically seen in prion disease and interestingly the post-mortem showed very little evidence of vacuolar change, which is thought to correlate with DWI signal intensity in patients with prion disease\(^{161}\). The post-mortem examination showed evidence of dot-like \(\PrP^{Sc}\) deposition, typically seen in FFI cases, in particular in the thalamus and basal ganglia, which may explain the significant sleep disturbance the patient, was symptomatic with and the polysomnography findings. It would be prudent to be
cautious in interpreting this result as significant as it is a single patient case, however I think it would be of value to carry out further work in a larger study to test the hypothesis that pathology within the thalamus is responsible for some of the EEG findings, i.e. reduced sleep spindles and K complexes, that may indicate thalamic involvement in both patients with FFI and other patients with prion disease and a significant sleep disturbance.
4.4. The Prion Disease Sleep Questionnaire

Scales that are widely employed in clinical practice—include the Epworth sleepiness scale (ESS) and the Pittsburgh sleep quality index (PSQI) although these scales are well established and robust in obtaining data regarding sleep disturbance they were not designed to specifically address and quantify the different aspects of sleep disturbance in prion disease. I found when trying to use these scales in the NPMC patients that the ESS did not cover all symptoms and the PSQI was too lengthy for use in this patient group, if patients were answering questions themselves, due to the nature of their illness and difficulty with cognitive impairment and impaired concentration, they could not complete the questionnaire and many questions were not relevant to the nature of their sleep complaint. The length of the questionnaire could also pose a problem for patient’s carers to complete as at each patient assessment they have other CRFs to fill in and a lengthy questionnaire of sleep disturbance was likely to prove both unpopular and impractical. So I decided to construct a questionnaire that was designed to best capture the sleep symptoms that affected this patient group.

4.4.1. Construction of the Prion Disease Sleep Questionnaire

The aims of the questionnaire are:

- To be an effective but simple bedside screening instrument for the cross-sectional and longitudinal evaluation of sleep disturbances in patients with prion disease.

- To be a means of monitoring the efficacy and response of implemented drug therapy.

I studied other sleep scales, including the PDSS, the PSQI and the ESS to identify the best way of asking questions on specific sleep abnormalities and the
scoring systems they used. I then designed the Prion Disease Sleep Questionnaire to best capture sleep data that patients with prion disease appeared to be symptomatic with, this was not a straightforward task as knowing where were those symptoms were was not all identified on the CRFs. I therefore searched a database of over 300 patient’s clinical case notes, most of the information was obtained from the first clinical letters but some was obtained from later patient visits. In addition to the notes data I also conducted informal patient and carer interviews to identify and further characterise sleep abnormalities that patients were symptomatic with. I was therefore able to identify sleep disturbance symptoms that were most prevalent and specific in patients with prion disease. Using this information and referring to the established sleep scales, the ESS, PSQI and PDSS I created an item bank of these questions that I was then able to modify for use in patients with prion disease. The symptoms that were most commonly reported from the notes and CRF questions were: difficulty falling asleep, waking at night and daytime sleepiness. Waking at night to use the toilet, jerking movements at night and disturbing dreams were also reported symptoms although these were less commonly mentioned in the notes. Using this information and referring to the established sleep scales, the ESS, PSQI and PDSS I created an item bank of these questions that I was then able to modify for use in patients with prion disease. As daytime somnolence was a frequently reported symptom I included 3 questions to gauge the severity of this, these were daytime drowsiness, falling asleep during the day, and falling asleep during mealtimes or during a conversation. An expert panel of prion disease specialists then reviewed the questionnaire before it was approved for use.

An overall sleep quality question was included; this question was graded similarly to the PDSS. Questions were formulated to capture symptoms of both nocturnal sleep disturbance, daytime somnolence and to grade the severity of symptoms (falling asleep in conversations, at mealtimes). I also included an “other” box to ensure that there would be minimal missing data and that the questionnaire would capture any additional symptoms not included in the
specific sleep symptom questions. The sleep questionnaire is shown in figure 40.

**Figure 40**

_The Prion Disease Sleep Questionnaire (PDSQ)_)
4.4.2. Methods of analysis

After employing the use of the sleep questionnaire in a group of 132 patients with symptomatic prion disease (93 with sCJD, 33 with IPD, 5 with iCJD and one with vCJD) male to female ratio 1:1, mean age 52 years. The average number of observations for each patient was 1.9, with a range of 1-7. I performed a cross-sectional examination of the data to assess if there was a difference in overall severity of sleep disturbance in patients with early disease, defined as those with an MRC scale score of 10-20, vs. those with late disease, defined as those having an MRC Scale score of 0-9, and to assess if the sleep questionnaire was capturing these results effectively. I did this by comparing the overall sleep score in both groups, see figure 41. I then assessed if there was a difference in the prevalence and severity of specific sleep symptoms, captured by the different questions in the sleep questionnaire.

The cross-sectional observations of the prevalence and severity of each specific type of sleep disturbance present, as measured by total prion disease sleep questionnaire score are illustrated in figures 42-44 for those with more severe disease (MRC Scale score 0-10) and those with less severe disease in figures 45-47 (MRC Scale score 11-20).

A total of 54 patients had serial data available, I plotted the total sleep score in these patients against their MRC Scale score to look at the trajectories and assess whether there appeared to be a pattern or correlation between disease severity and severity of sleep disturbance, these results are illustrated in figure 48 (graphs a-c).
4.5. Results

Figure 41 illustrates the differences in severity and prevalence of symptoms in patients with early and late staged prion disease. The results show that there were more patients with severe disease (MRC Scale score 0-10) and in that group the sleep score was slightly worse, the largest percentage of patients had a total sleep score=11-15, versus 6-10 in the less severe group. This pattern is similar to that seen in the larger patient dataset presented earlier in the chapter (figure 34), where symptoms appear to become prevalent with severity of disease.

![Total sleep score vs. MRC Scale score in patients with prion disease](image)

**Figure 41**

*Total sleep score vs. MRC Scale score. Patients subdivided into advanced disease (MRC Scale score 0-10) and early disease (MRC Scale score 11-20) groups*

Looking at the following figures 42-47 where the sleep severity score for each of the sleep questionnaire questions is plotted against either early or late disease there are a number of interesting results.
The most striking difference is seen in the questions that address somnolence as a symptom. Falling asleep in the day was much more common in patients who had an MRC Scale score of 0-10 (p-value= 0.001). As was daytime drowsiness (p-value 0.008), falling asleep at mealtimes was also far more common in patients with severe disease (p-value <0.00001). Waking at night was common in the severe disease group, a larger percentage of patients (24% vs. 12%) reported the most severe symptoms earlier in the disease. Other symptoms didn’t appear to show a great difference depending on disease stage.

**Figures 42-44**

*PDSQ results: Frequency and severity of sleep related symptoms in patients with an MRC Scale score of 0-10*

![Graph showing results of sleep scale questions in patients with MRC Scale score 0-10](image)

**Figure 42**

*Frequency and severity of sleep related symptoms (sleep quality, difficulty falling asleep, waking at night and returning to sleep) in patients with low MRC Scale score 0-10*
Figure 43

Frequency and severity of sleep related symptoms (drowsy in the day, falling asleep in mealtimes and falling asleep in the day) in patients with an MRC Scale score of 0-10
Figure 44

Frequency and severity of sleep related symptoms (waking to use the toilet, disturbed dreams and woken by movement at night) in patients with an MRC Scale score of 0-10
Figures 45-47

PDSQ results: Frequency and severity of sleep related symptoms in patients with an MRC Scale score of 11-20

Results of sleep scale questions in patients with MRC Scale score 11-20

Figure 45

Frequency and severity of sleep related symptoms (sleep quality, difficulty falling asleep, waking at night and returning to sleep) in patients with higher MRC Scale score 11-20
Figure 46

Frequency and severity of sleep related symptoms (drowsy in the day, falling asleep in mealtimes and falling asleep in the day) in patients with an MRC Scale score of 11-20
Figure 47

Frequency and severity of sleep related symptoms (waking to use the toilet, disturbed dreams and woken by movement at night) in patients with an MRC Scale score of 11-20

Although the number of patients with several data points was small, I plotted the data to see if a trend could be identified between sleep severity and stage of disease in these patients (see figure 48, graphs a-c). I divided the patients into the different disease subtypes (sporadic, inherited and acquired forms of prion disease). From the graphs it is clear that there is a great deal of variation over time in individuals. This might relate to response to symptomatic treatments used in the course of the disease, the natural history of the symptom, or possibly the unreliability of the assessment tool.
Figure 48 (graphs a-c)

Total sleep score vs. MRC Scale score in symptomatic patients (patient number= 54)

a)

Total sleep score vs. stage of disease

Total sleep score vs. MRC Scale score in patients with acquired prion disease (iatrogenic CJD)
Total sleep score vs. stage of disease

Total sleep score vs. MRC Scale score in patients with inherited prion disease
Total sleep score vs. stage of disease

Total sleep score vs. MRC Scale score in patients with sCJD
4.6. Discussion

To date, this study is the largest to evaluate both the prevalence and phenomenology of sleep disturbance in human prion diseases. In the NPMC patient group I found that sleep problems were highly prevalent, occurring in 76% of patients. Sleep disturbance was found to be present in all types of human prion disease. Although the prevalence and phenotype of the sleep symptoms were diverse, the most commonly reported symptoms were insomnia, waking at night and somnolence; other reported symptoms included movement disorders, night-time behavioural problems and increased toilet visits. Of the most commonly reported sleep symptoms, hypersomnolence, was found to occur most commonly towards the end of disease with around 70% cases being reporting at an MRC Scale of between 0-8, the majority of reports of symptoms of insomnia and night-time waking occurred slightly earlier on in disease MRC Scale score 3-11.

Certain disease subtypes, such as the iatrogenic patient group, had a high prevalence of sleep problems with 13 out of 14 patients being symptomatic; however the sample size is small. There was also some variability between disease subtypes regarding specific symptom prevalence. Patients with IPD were more likely to have to get up to use the toilet, wander at night and display behavioural problems such as shouting, the latter being most prevalent in those with 5OPRI and 6OPRI mutations. Those with acquired prion disease (vCJD and iCJD) were more likely to be symptomatic with night-time movements (see Table 23).

Although there has been one recent study that has shown that sleep symptoms are prevalent in patients with prion disease\textsuperscript{235}, apart from patients with the FFI phenotype there had been very little focus on this symptom. It is possible that sleep symptoms have been previously under-reported due to their presence being masked by other more dominant and progressive features of prion disease. From this project it is also clear that sleep symptoms can vary over the
course of the illness, in a proportion of patients the sleep disturbance spontaneously resolved and in others the type of sleep disturbance was noted to change over the course of the disease and therefore if a symptom is not sustained it is possible this is the reason that it may be overlooked.

Despite the prevalence of sleep disturbance in this population very few patients were taking symptomatic drug therapy. In a few cases where there was successful treatment of the sleep disturbance there was also an anecdotal improvement in other symptoms, such as ataxia, cognitive function and behavioural disturbance. One of the recommendations I would make is that with increased awareness of both the prevalence and the morbidity associated with sleep disturbance drug treatment is commenced promptly, monitoring of response is also important as there is little known regarding the efficacy of implemented therapy.

Sleep disturbance was strongly associated with the presence of depressive symptoms. In the general population there has been reported improvement in depression when sleep disturbance is treated\textsuperscript{158,239,240}. It is unknown if depression is caused by the underlying disease process in prion disease or a psychological response to physical illness, and it is possible that it is secondary to both. Therefore treatment of sleep disturbance may lead to a resolution of depressive symptomatology and reduce the burden of morbidity experienced by the patient.

A significant association was also found between sleep disturbance and abnormal thalamic signal change identified on MR imaging. The thalamus is responsible for initiating and maintaining slow wave sleep and it is possible that thalamic pathology is responsible for sleep disturbance in those patients who had signal change present.

The pathology seen on post-mortem in patients with prion disease is diffuse; it is therefore likely that alternative parts of the sleep circuitry are pathologically
affected in patients who do not have thalamic signal change present or indeed the signal change reflects a specific form of pathology, i.e. spongiosis and other patients, such as the FFI case detailed in this work, may have thalamic pathology such as gliosis or PrPSc deposition that does not result in imaging changes on the MRI.

The data from the sleep questionnaire was more in depth and focused than that recorded on the CRFs. It concentrated on both prevalence and severity of symptoms as reported by the patient or carer and therefore was of benefit in gaining a greater understanding of specific types of symptoms and their frequency in the patient cohort analysed, a good example of data captured by the questionnaire was on the recording of insomnia symptoms. Although insomnia was reported more frequently at a severe stage of disease it was reported as being a more troublesome symptom earlier in disease. The ability to capture this kind of data is important as it can be used to implement drug therapy when most appropriate.

The sleep questionnaire questions were developed to target sleep symptoms that are most common in this patient group, informal feedback from practitioners on the usability of the questionnaire and from both patients and relatives has been positive. I would recommend the use of the prion disease sleep questionnaire to monitor the natural progression of sleep symptoms and their response to therapy not just in our patient cohort but also for it to be used by healthcare professionals looking after other patients with prion disease; I would welcome clinician feedback on the feasibility and usefulness of the tool.

Further work is aimed at continuing to collect data on the natural history of sleep symptomatology and correlating it with the MRC Scale clinical outcome measure to both determine onset of sleep problems in prion disease and variability of the symptom over the course of the disease.
With the implementation of the sleep questionnaire we have been able to start in depth monitoring of sleep disturbance in symptomatic patients. With this tool we hope to be able to prospectively monitor sleep symptomatology and response to implemented therapeutics.

To date we have performed polysomnography in one patient with a diagnosis of FFI, our aim is to repeat sleep studies in a small series of patients to assess the type of sleep disorder they are symptomatic with, if there is response to implemented therapeutics and whether accompanying MR signal change corresponds with both EEG findings and neuropathology.
5. Diffusion weighted imaging MRI signal change correlates with neuropathological parameters of prion disease found on quantitative histopathology analysis

Imaging abnormalities are one of the key findings seen in patients with prion disease and are used as a tool in aiding the diagnosis of those with variant, sporadic and iatrogenic forms of the disease. There is still limited understanding of what the imaging abnormalities seen on MRI represent in terms of prion disease pathology. This study investigates the correlation between specific parameters of neuropathology and diffusion weighted imaging abnormalities seen on MRI.

5.1. Introduction and background

Prion disease is described as being a rapidly progressive fatal neurodegenerative disease. The pathogenesis of prion disease is understood to be due to the accumulation and aggregation of the partially protease resistant scrapie prion protein, PrP\textsuperscript{Sc}, within the central nervous system (CNS). PrP\textsuperscript{Sc} is the abnormal or disease-causing form of prion protein; it can arise in a number of different ways. It can be acquired from exogenous exposure, i.e. from the environment, either via transmission from dietary exposure in cases of vCJD and Kuru or through iatrogenic exposure. In the majority of iatrogenic cases the main routes of transmission have been secondary to inoculation with cadaveric derived human growth hormone or through donor dural grafts. PrP\textsuperscript{Sc} can arise from an endogenous conformational change of the normal cellular prion protein, PrP\textsuperscript{C}, to the scrapie form; this can either occur sporadically or arise as a result of one of a number of recognised mutations of the prion protein gene. With the presence of PrP\textsuperscript{Sc} an autocatalytic process arises whereby normal prion protein, PrP\textsuperscript{C}, is converted to the scrapie form, with subsequent rapid accumulation and aggregation of abnormal prion protein. There is thought to be a latent phase before the onset of clinical disease with varying incubation times reported after
introduction of exogenous PrP<sub>Sc</sub>, this was first observed in patients infected with kuru, the human form of prion disease acquired through cannibalistic rituals, where infection to the time of onset of clinical symptoms was found to vary widely, reported to be between 5 and 60 years<sup>253</sup>. Similarly variable incubation times have also been reported in patients with iatrogenic CJD recipients of cadaveric derived human growth hormone and also in those patients with vCJD<sup>45,254</sup>. There are probably a number of factors that influence the incubation period, disease onset time and disease progression. Codon 129 genotype is recognised as one of these factors and is known to influence the onset, development and duration of human prion disease in variant, iatrogenic, sporadic and some inherited subtypes<sup>45,72,80,107,254,255</sup>. In patients diagnosed with kuru those with MV or VV genotypes developed the disease at a later date than those with the MM genotype<sup>253</sup>, all patients diagnosed with variant CJD have been methionine homozygous until more recently<sup>72</sup>, with one definite case of variant CJD occurring in a patient with the MV codon 129 genotype. Prion protein strain may independently influence onset time and there are possibly other unknown host susceptibility or genetic factors that influence disease onset and progression<sup>254,256,257</sup>.

5.1.1. Histopathology in prion disease

Prion disease is characterised by distinctive neuropathological findings: spongiform change, astrocytosis, aggregation of scrapie prion protein deposition as amyloid plaques and neuronal loss. Continued loss of cerebral tissue occurs with progression of disease and severe brain atrophy is often observed to occur in patients with longer disease durations. Depending on prion disease subtype, strain and codon 129 genotype there is seen to be variation in both the degree and anatomical location of spongiform change, gliosis, PrP<sub>Sc</sub> and neuronal loss.
The neuropathological hallmarks of prion disease are spongiosis, gliosis, neuronal loss and scrapie prion deposition, these features although not specific to prion disease occur in varying degrees and depend on the subtype of prion disease present as well as codon 129 genotype. These factors as well as others may influence the pathological findings seen on post-mortem. Including the type of neuropathology present, which brain regions are affected and the extent of pathology present (i.e. degree of protein deposition, neuronal loss, gliosis and spongiosis). The type of prion disease refers to vCJD, sCJD, iatrogenic and IPD subtypes, codon 129 genotype and prion strain present also influence underlying neuropathology. In addition to this there is phenotypic heterogeneity seen not only between the different subtypes of prion disease but also between subjects with the same underlying prion disease subtype, making it difficult to ascertain the exact relationship between all these factors and the association between pathological findings.

Prion protein deposition

Whilst we know that prion disease development is associated with a change of PrP<sup>C</sup> to PrP<sup>Sc</sup>. The exact mechanism of how neurotoxicity and neurodegeneration arises in the context of abnormal prion protein aggregation is still unknown. Hypotheses that have been put forward are: that the toxic gain of PrP<sup>Sc</sup> deposition itself directly results in neuronal death through the process of microglial activation and apoptosis/cell death<sup>258-260</sup>. The mechanism for how this might occur is not clear, studies in mice have shown that similar levels of PrP<sup>Sc</sup> have been found in those with end stage disease and in those in a subclinical carrier state, so a direct cumulative effect of PrP<sup>Sc</sup> as the mode of pathology, at least in this species, is less likely<sup>261</sup>. Another theory is that prions act as a catalytic surface for the production of toxic species, and this unidentified toxic species causes synaptotoxicity, neuroinflammation and neuronal loss<sup>262</sup>. Foliaki et al reported that the introduction of PrP<sup>Sc</sup> into the hippocampi of mice was found to cause synaptic disruption. It is possible that the process of conversion from PrP<sup>C</sup> to PrP<sup>Sc</sup> itself
results in the production of toxic species\textsuperscript{263} or that loss of normal PrP\textsuperscript{C} results in a reduction of neuroprotective function against toxic insults and oxidative damage. Although the mechanism for how this occurs or the exact physiological function of what PrP\textsuperscript{C} does in the central nervous system (CNS) is still not clear. Proposed functions of PrP\textsuperscript{C} include: a role in copper and iron homeostasis\textsuperscript{264,265}, myelination\textsuperscript{266}, protection against both oxidative stress and apoptosis\textsuperscript{267,268}, it may also have a fundamental role in the development of the CNS. Malaga-Trillo et al. noted that loss of PrP\textsuperscript{C} in zebra fish affected gastrulation resulting in malformation of the brain and eyes\textsuperscript{61}, knockout mice with gene deletions for PrP have been found to have mild phenotypic variation with abnormal hippocampal and synaptic function\textsuperscript{56,269,270}.

PrP\textsuperscript{C} is located in the synapses and may have a role in regulation of sleep, memory and neuronal excitability; influencing or regulating receptors such as NMDA and VGCC and therefore having effect on synaptic function and possibly also synaptic plasticity\textsuperscript{271-273}. Synaptic dysfunction is known to occur in other neurodegenerative diseases such as Parkinson’s disease and Huntington’s disease and may also be the mechanism for pathology in Prion disease.

It is generally accepted that the scrapie prion protein is involved in some way in the neurodegenerative process and this may be through direct PrP\textsuperscript{Sc} toxicity, or possibly through the production of a secondary related synaptotoxic species. If either mechanism is causative then one might expect to see a direct association between the occurrence of PrP\textsuperscript{Sc} deposition and either co-localisation or the close occurrence of other markers of neuropathology i.e. neuronal loss, spongiosis and gliosis. Although co-localisation of neuropathology may be seen and has been reported in patients with Gerstmann-Straussler-Scheinker syndrome\textsuperscript{274}, there are few studies that have explored the relationship further.

Being able to show that a correlation between PrP\textsuperscript{Sc} deposition and spongiosis/gliosis occurs may help to further our understanding of the role of PrP\textsuperscript{Sc} in causing prion disease.
There are various recognised patterns of PrP$^{\text{Sc}}$ deposition, related to a number of factors including prion disease subtype, PrP$^{\text{Sc}}$ strain and codon 129 genotype.

In sCJD for example, the MM2 subtype of sCJD are more commonly associated with perivacuolar deposition supporting perhaps a direct correlation between presence of PrP$^{\text{Sc}}$ having a more direct role in causing spongiform change, in other cases PrP$^{\text{Sc}}$ deposition is not found to co-localise with vacuolation and have been detected in the synapse, it is possible that the mechanism of neurodegeneration and direct or indirect possible toxicity varies on the subtype of prion disease present and possibly other influencing factors, figure 49 shows cortical PrP$^{\text{Sc}}$ deposition in the brain of a patient with sCJD.

**Figure 49**

ICSM35 stained section of brain tissue showing cortical PrP$^{\text{Sc}}$ deposition
**Spongiosis**

Spongiform change is a frequently observed and often an early pathological finding described in patients with prion disease. It is thought that spongiosis occurs after microglial activation and gliosis, this having been observed in experimental animal models\(^{258}\). Spongiosis typically occurs in the cerebral cortex and deep grey matter nuclei. It has also been described as occurring in the white matter, in the panencephalopathic form of CJD, which has been observed more frequently in Japanese patients\(^{275}\) but also described in case reports and in a case series of patients in the Netherlands with longer disease duration and therefore may reflect secondary degeneration from neuronal loss rather than primary white matter pathology\(^{149,276,277}\).

Spongiform change results from the formation of membrane bound vacuoles, in the cortex. They have typically been described as occurring in the deeper cortical layers, 4 and 5, and vacuoles observed in the superficial layers have been described as being artefact, and thought to be possibly secondary to neuronal loss. However more recent descriptions of neuropathological change have reported the observation of confluent vacuolation occurring in the superficial cortical layers and in particular being seen in patients with type 2 PrP\(^{Sc}\) deposition\(^{151,278,279}\). Other types of PrP\(^{Sc}\) strains such as type 1 PrP\(^{Sc}\) deposition are more commonly associated with fine vacuole type spongiosis seen in the deeper cortical layers. These confluent vacuoles in the superficial layers associated with PrP\(^{Sc}\) type 2 have also been reported to show higher signal intensity on DWI MRI brain sequences\(^{278}\).

Vacuoles are typically round or oval in shape and their size ranges from 2-200 micrometres in diameter, they can merge to form large clusters or cavities within the neuropil. Cerebellar as well as deep grey matter nuclei spongiosis is also frequently seen in most forms of prion disease to varying degrees, figure 50 shows spongiform change in the brain of a patient with sCJD.
Gliosis

Gliosis is reactive change of the glial cells, most commonly astrocytes and microglia, which occurs predominantly in the deeper cortical layers of the cerebral cortex. Detection of gliosis is generally carried out with the use of glial fibrillary acidic protein (GFAP) immunostaining. Gliosis and spongiosis have been often observed to co-localise in the deep cortical layers towards the end stages of disease severe gliosis and cell swelling can mask the degree of spongiosis present, figure 51 is brain tissue from a patient with sCJD showing the blue stained areas of gliosis.
Neuronal loss

Neuronal loss in prion disease is thought to occur primarily through apoptosis and apoptotic neurons are found in association with microglial activation. Activated microglia are thought to cause oxidative stress with the release of inflammatory cytokines as well as reactive oxygen species. As mentioned previously PrPSc itself may exert a direct toxic effect on the cell or synapse itself and cause cell death.

5.1.2. Imaging and histopathology

MRI in patients with prion disease is an important tool that is used to help diagnose patients with the disease. Imaging abnormalities are most often seen in the deep grey nuclei and cortex and the abnormalities seen of restricted diffusion on diffusion-weighted sequences are often helpful in making a diagnosis of prion disease. DWI abnormalities seen in patients with prion disease have both high sensitivity and specificity in differentiating CJD from other rapidly progressive dementias, with restricted diffusion being observed...
in up to 92% of suspected cases of sCJD\textsuperscript{121} and these changes are most evident in the deep grey matter nuclei and cerebral cortex.

It is unknown exactly how imaging abnormalities reflect or correlate with the underlying histopathology associated with prion disease.

There have been several small studies that have assessed whether a correlation exists between pre and post-mortem MRI findings and neuropathological change identified on autopsy. The findings have been variable. Siddique et al. analysed frontal lobe blocks from 6 variant CJD patient’s brains, the frontal blocks were all imaged on a high strength magnetic field, 9.4T, scanner. She found a significant association between reduced frontal grey matter MTR and spongiosis, but no significant correlation was noted between gliosis or prion protein deposition with MTR value in either the grey or white matter. This study, although small, is promising in that it found a strong association between imaging abnormalities and neuropathology in vCJD patients however there is the caveat that post-mortem MTR in brain tissue is lower than that seen on in-vivo imaging\textsuperscript{9}, and therefore post-mortem imaging may not give an accurate representation of the underlying pathology that it is observed to correlate with.

Other studies have analysed if DWI abnormalities correlates with prion disease neuropathology. Mittal et al. reported a correlation between the degree of spongiosis present and restricted diffusion\textsuperscript{136}, Manners et al. reported a significant correlation between restricted ADC and spongiosis\textsuperscript{161}, but they also found a weaker correlation between gliosis and PrP\textsuperscript{Sc} deposition. Haik et al. claimed a significant correlation between PrP\textsuperscript{Sc} deposition and DWI signal change\textsuperscript{281}.

To date, histopathology reporting and staging has relied on semi-quantitative methods of analysis such as Braak staging, which was developed as criteria for use in the diagnosis of staging in both Alzheimer’s disease\textsuperscript{176} and Parkinson’s
disease. There is no specific staging used in prion disease, tau pathology that can occur in vCJD and is staged using the Braak method, otherwise degree of neuropathology is graded semi-quantitatively, by counting the number of vacuoles within an area, the degree of staining for gliosis or PrP<sub>Sc</sub> deposition and then grading the pathology present as mild, moderate and severe. More recently there has been increased use of quantitative software analysis packages in the field of histopathological research. These have been specifically developed for quantitative tissue analysis, with the aim being to identify different tissue patterns, morphology and pathological change, the identification of which may be used to find sensitive and specific biomarkers that can be correlated with disease progression and patient outcome. The data analysis software Definiens Tissue Phenomics is a pioneer of this technology; its greatest use has been in improving the identification of biomarkers in tumour tissue but with its superior imaging analysis is also finding use in other tissue analysis, including brain tissue<sup>177,178,282</sup>

From reviewing the current literature and research, it appears that the weight of evidence is that cortical ribboning seen on DWI probably correlates with spongiosis; however there are studies that have reported contrary findings. I therefore decided to investigate if a correlation exists between restricted cortical diffusion seen on conventional DWI, and quantitatively analysed neuropathology parameters: spongiosis, gliosis and prion protein deposition, to try and identify and establish which parameter is most strongly associated with restricted diffusion.

There is on-going research being conducted to assess how scrapie prion protein aggregation in prion disease causes disease, whether this is through a toxic gain in function, loss of normal protein function or other means. Whether the aggregation of protein, if this is pathological, is associated with other neuropathology findings and cellular damage. As there is still uncertainty regarding how PrP<sub>Sc</sub> causes neurodegeneration I investigated if I could find an association between the occurrence of spongiosis, gliosis and PrP<sub>Sc</sub> and if there
was any evidence for a co-localisation of pathology or a correlation that existed between the degree of neuropathology seen to occur, i.e. PrP\textsuperscript{Sc} deposition, gliosis and spongiosis, which may then indicate a possible direct role for the abnormal scrapie protein in causing neurodegeneration.

In order to do this I looked at graded areas across the cortex as percentiles and assessed if there was a correlation between the extent of vacuolation and intensity of staining for GFAP and ICSM35 for each percentile across the cortex as well as for the overall mean. I also analysed if a correlation existed between grade of GFAP staining and ICSM35, indicating an association between underlying gliosis and PrP\textsuperscript{Sc} deposition.

**Diffusion weighted imaging**

Please refer to section 1.6.1. for a detailed description of diffusion weighted imaging (DWI).

**5.1.3. Quantitative histopathology analysis software**

Definiens Tissue Studio is software that was developed for the analysis of a range of histopathology assays, including tissue slides, micro-arrays and microscope images. The process of the examination of tissue, involves selecting areas for the analysis using the ROI classification feature, thus allowing the user to customise and select the specific areas for analysis. The digital image is uploaded after the slide has been scanned using the high-resolution software SlidePath Gateway, developed by Leica Biosystems. The imported Definiens digital image is a 2D raster image, with each image layer being composed of an array of pixels. The image is divided into tiles, which are smaller sections of the entire image, the analysis is conducted on each individual tile and then the images are sewn together.
5.2. Aims

- To determine if diffusion weighted imaging (DWI) signal change on MRI brain scans in patients with prion disease correlate with quantitative analysis of histopathology on autopsy.

- To determine if an association exists between deposition of PrP$^{Sc}$, gliosis and spongiosis in both patients with and without signal change on DWI.

5.3. Methods

Patients recruited to the NPMC who had had both a post-mortem performed (consent was acquired for brain tissue to be used for research purposes from the patient or their relatives) and neuroimaging that included a DWI were selected as participants in the study.

The DWI sequences were obtained from hospitals within the UK where the patient had undergone an MRI brain scan. The scans were both 1.5T and 3T scans. All scans were reviewed for quality purposes and only those that were of high enough quality were included in the study. Dr Harpreet Hyare, consultant neuroradiologist at the National Prion Clinic reported the scan findings.

The scans were then divided into two groups: 1) The cortical ribboning group: defined as scans that had been reported as showing the presence of diffuse cortical ribboning i.e. restricted diffusion in the cortical grey matter and 2) The non-cortical ribboning group, scans where no diffusion weighted imaging changes were reported.
5.3.1. Exclusion and inclusion criteria

Scans acquired more than one year prior to the patient’s death were excluded from the analysis, this decision was based on the assumption that changes present on DWI one year before death would be unlikely to reflect the underlying pathology present after such a long period of time. If more than one scan was acquired on a patient, then the one that was performed closest to death was the one that was included in the analysis.

In total 33 patients with a diagnosis of prion disease were selected, these included sporadic, iatrogenic, variant and inherited (E200K mutation) forms of the disease, 21 with diffuse cortical signal change identified on DWI and 12 patients without any signal change present. In the cortical ribboning group the median time between scan date and death was 53.5 days (range: 2-333 days), median age of disease onset 63 years, range 22-85 years and male to female ratio of 1:13, 45% of patient were methionine homozygous at codon 129, 36% heterozygous and the remainder valine homozygous.

In the non-cortical ribboning group the median time between scan date and death was 89.5 days (range: 23-269 days), median age of disease onset 67 years, range 24-77 years and male to female ratio 1:1, 50% were methionine homozygous, 30% heterozygous and the remainder valine homozygous. Prion protein strain was not known for all subjects.

Frontal lobe section tissue blocks acquired from each subject were used for the purposes of the histopathology analysis. The frontal lobe tissue sections were embedded in paraffin and then stained with three different stains before analysis. Three histopathology parameters were analysed. Spongiform change, measured as both the number of vacuoles and as a percentage of tissue area affected, this was performed on haemotoxylin and eosin (H&E) stained slides. Detection of scrapie prion protein deposition (PrP$^\text{Sc}$) was assessed by using the immunohistochemical monoclonal antibody stain, ICSM 35, to bind to PrP$^\text{Sc}$, and
the amount of tissue stained was measured. The degree of gliosis was measured by using the process of staining tissue sections with glial fibrillar acidic protein (GFAP) stain. GFAP stain binds to this protein, which is present in glial cells and is released in gliosis. Therefore measuring the amount GFAP stain present can assess the degree of gliosis.

Prior to performing the analysis, neuropathologist Dr Zane Jaunmukatane reviewed all slides. For quality control purposes, slides that were found to be of poor quality, i.e. those that were inadequately stained or damaged, were replaced with newly acquired ones or excluded from the analysis. The slides were scanned into SlidePath Gateway and then loaded into the Definiens workspace.

5.3.2. Region of Interest selection (ROI)

The ROI was manually selected; although an automatic ROI can be applied it was decided that by drawing a polygon manually a more accurate area of the cortex could be selected, and areas where artefact was found to be present could be more easily excluded. ROIs were identified with the guidance of neuropathologist, Dr Zane Jaunmukatane. For the initial/pilot analysis ROIs were drawn on each of the different slides and as similar an area as possible was selected for each individual subject’s slides (the GFAP stained slides, ICSM-35 and H&E). However in order to compare like with like the second analysis used the translational software in Definiens, so one ROI was applied to the H&E stained slide for each individual patient and then the same area translated onto the GFAP and ICSM35 stained slides, therefore obtaining as similar an area as possible to compare. After translation each of the slides were inspected and if needed adjusted to ensure both that the ROI fit and was not affected by artefact.
5.3.3. Analysis builder

Overview of steps of the analysis builder:

- Definition of the magnification strength: available options include: 5x, 6.6x, 10x, 20x and 40x. Stain and nuclear detection are adequately detected on a lower resolution image, whereas membrane detection requires higher magnification strengths of 10x and 20x.

- Application of a background to tissue separation in order for the analysis to be confined purely to the brain tissue.

- Selection of the layer of tissue being analysed.

- Application of homogeneity/brightness thresholds.

- Selection of the stain being analysed e.g. H&E, GFAP or ICSM-35.

- Application of a stain threshold; objects below and above a specific contrast intensity can then be de-selected from the analysis.

- Additional inclusions/exclusions were applied for the purposes of determining the degree of vacuolation present, these included:
  
  - The exclusion of “vacuoles” in close proximity to both nuclei and blood vessels.
  - The exclusion of vacuoles under 3 micrometres in diameter.
  - Vacuoles with a width over x3 the length.

These criteria were decided upon after consulting with Dr Jaunmukatane on the classification of a vacuole. Further quality control was implemented after an
initial “pilot analysis” of the H&E stained slides, there was concern that vacuoles in the outer layer of the cortex maybe artefact and therefore erosion of the outer cortical layer to 10% was deemed necessary to minimise false identification of spongiform change that may be secondary to hydropic cellular change and not vacuolisation.

Spot detection was used to quantify the degree of gliosis and PrP Sc deposition present.

We first performed a pilot analysis of the group at magnification strength of x10. On review of the selected ROI we found that micro-vacuoles were not being identified at the x10 magnification and therefore the degree of spongiosis present was being under-valued on some slides. Magnification of x20 had higher resolution and was therefore used for the study.

5.3.4. Digital image analysis

Digital image analysis of whole slide images was used for the evaluation of immunohistochemical staining and spongiform vacuoles in Prion samples, to measure the character of relevant histological markers and identify features then characterise how they are distributed at different depths in the cortex. Analysis was implemented using Definiens Developer XD 2.6 (Munich); whole slide images were generated using a Leica SCN400F at resolution equivalent to x20 magnification.

Throughout this section, bold text denotes an image object type, for example background and tissue, italicised text denotes an image layer, such as the red, green and blue layers that make up a colour picture, and underlined text denotes dynamic thresholds, based on the image being analysed, that are used to separate, classify, or in some way manipulate image objects based on their visual properties.
The analysis is carried out using multiple ‘object levels’, which contain the classification of object types. These levels form a stack with increasing fragmentation from the top to the bottom, i.e. minimum fragmentation at the top is the whole image whilst the maximum fragmentation at the bottom is individual pixels. Any fragmentation in a level is maintained in all layers below, as the structure is hierarchical. This analysis will result in three object levels, containing the selected cortex region, the distance classes and the cellular separation. Temporary object levels will also be used during the analysis.

**Summary of methods of analysis**

Regions of cortex were manually selected on H&E stained sections; these were then translated to the GFAP and ICSM stained sections using an automated tissue registration process. All translated regions of cortex were reviewed and corrected as necessary. The inner and outer borders, describing the interface between the cortex and meninges or white matter, respectively, were identified and the relative distance between these borders calculated.

**Vacuoles** or brown chromogenic staining were then identified through a series of thresholding and morphometric measurements. The cortex was then segmented to 10% depth measurements \((0-10, 10-20, \ldots, 80-90, 90-100)\) and the number and area of cellular features in each depth class was exported.

**Technical description**

**Tissue identification**

Separation of tissue and background areas was performed on an image at resolution equivalent to x10 magnification using thresholding on a composite image containing the lowest values, representing the darkest, from the three images that make a colour image (red, green and blue). This image of darkest values was then filtered by applying a moving average of 25x25 pixels, giving
darkest (25); this ‘smooths’ the image to remove peaks and troughs towards the average value.

The image was then segmented into a 15x15 pixel ‘chessboard’ and the threshold that describes the highest (lightest) 1% of blocks was identified (99th centile) for the red, green, blue and darkest layers giving R99, G99, B99 and D99, respectively; the average of R99, G99 and B99 was then calculated to give C99. The image was then segmented on darkest to give ROI < D99 < Temp < C99-5 < background. Any area of ROI with area less than 100 µm² was removed into background; and any area of Temp with standard deviation in darkest < 2 was reassigned as background. All remaining Temp was removed into ROI.

The ROI was then segmented to give a 10x10 chessboard and the 95th centile of mean darkest was calculated to give D95. The absolute difference between D95 and the mean of darkest in ROI regions, was calculated and, if the difference was below 10, ROI was segmented to ROI and background on darkest(25) using C99-5, or if the difference is greater than 10, the same segmentation was performed using D95.

Background and ROI were then segmented to a 10x10 chessboard and the 10th centile of mean darkest(25) in background calculated to give D(25)10; any region of ROI with mean darkest greater than D(25)10 and standard deviation of darkest less than 5 was then removed into background.

All blocks were then merged by type and ROI objects with area less than 500 µm² and standard deviation in darkest less than 10, or area less than 50 µm² were removed to background, and any background objects with area less than 500 µm² and standard deviation in darkest greater than 5, or area less than 50 µm² were removed to ROI. ROI was then grown into background by 1 pixel and the area of ROI recorded to give a ROI.
Any ROI with area less than 0.1% of a ROI; with area less than 1% a ROI and compactness (relates the object width and length with its area) were removed to background.

All remaining ROI were then shrunk by 20 pixels, then grown by 20 pixels where darkest (25) < C99-5. ROI were merged and any with area less than 1% of a ROI were removed.

**Selection of regions of interest (ROI)**

Definiens Tissue Studio (Munich) allows the translation of selected regions from one sample to another, enabling the analysis of comparable regions between different stains. Manual selection of cortex was performed on H&E sections, followed by automated image orientation and ROI translation. These translated regions were then inspected and, where necessary, modified to mirror the original ROI.

**Distance analysis set-up**

To measure the relative distance into the cortex from the external surface to the grey/white matter interface, the two surfaces, external and interface, were manually added. This was performed by shrinking the cortex by 4 pixels, giving a new class outline; where the ROI describes the sulci, the outline was modified and expanded if necessary to split the two gyri. This outline, which encircles the cortex, was then manually cut at the ‘corners’ of the ROI, with the outline object at the grey/white matter interface reclassified as interface and the outline object at the external surface reclassified as external. For each pixel the distance to interface and external were measured to give distanceExternal and distanceInterface. Relative distance from the external surface to the interface was then calculated as \( \frac{1}{(\text{distanceExternal} + \text{distanceInterface})} \times \text{distanceExternal} \), giving distanceRelative.
Analysis of histological features

Feature analysis was performed on images at a resolution equivalent to x20 magnification. Image layers were generated using the HSD model$^{283}$ that represent the blue and brown character of the image, and object density (OD) which is the sum of the two. These were then filtered to give blue(7), brown(3) and OD(7). These were then combined to give maxStain, composed of the highest values from blue and brown, and maxStain(filtered), composed of the highest values from blue(7) and brown(3).

Multiple thresholding calculations were then performed to identify suitable thresholds for analysing the given image. For brown and OD the following method was applied to all cortex; for blue(7) the cortex with values below 0.1 au on maxStain(filtered) were temporarily excluded. OD(first), OD(last) and OD(max), Brown(first), Brown (last) and Brown (max), Blue(first), Blue (last) and Blue (max). These thresholds are calculated based on a percentile comparison method that identifies increases in threshold significantly above a projected linear model.

For each image layer (active layer), the following processing is performed: The ROI is segmented into 1500x1500µm cubes, with a maximum size of 2.25mm$^2$. Any ROI with area less than 0.5mm$^2$ was merged with the ROI object that it shares most border with. These ROI objects were then reclassified as thresholding and the threshold Th-min calculated as the mean of active layer within thresholding, plus the standard deviation of active layer within thresholding. Any thresholding with area less than 1.5mm$^2$ was then reclassified as excluded (if all thresholding has been reclassified, any excluded with maximum pixel value in active layer > Th-min and area greater than 1% the area of ROI is reclassified as thresholding). Any thresholding with maximum pixel value in active layer < Th-min was then reclassified as excluded.
For each **thresholding** object, the following process is performed:
The current **thresholding** object, which will be called **current**, is then segmented into 2x2µm (maximum area of 4µm²) objects. These will be used for all centile calculations, i.e. for a series of 100 segments; the 50\(^{th}\) centile gives a threshold that separates the 50 **current** objects with the highest mean intensity of *active layer* from the lowest 50 with the lowest intensity.

To allow calculation of the significance measurement used in the identification of potential thresholds, the range of intensity values is measured. Thresholds that identify the highest and lowest 1% of **current** (99\(^{th}\) and 1\(^{st}\) centile), according to the mean intensity of *active layer*, are calculated and applied to temporarily exclude the **current** objects with mean *active layer* intensity above or below these thresholds. The minimum and maximum mean *active layer* intensity within the remaining **current** objects were then used to calculate the range of intensity values, e.g. a minimum intensity of 0.2au and maximum intensity of 2.2au gives a range of 2au.

This **range** is then divided by the number of centile measurements to be compared, e.g. if a step size of 5% is to be used (0%, 5%, ..., 95%, 100%) there are 20 measurements and comparisons. Dividing the **range** by 20 gives the increase in threshold that could be expected if there is a linear distribution of stain intensities. Since the stain distribution is not linear but sigmoidal or multimodal, significant increases in threshold between centiles are identified as those three times higher than the linear projection. For example, if the **range** is 2au and the centile step is 5%, 2/20=0.1, so a **significance** threshold at 3 times this value is 0.3au.

The series of centile comparisons are then performed starting from 1% (0% would give a value of 0) then increasing first to the value of the step size (e.g. 5) then by the step size up to 100%. For each centile the **current** threshold is compared with the **previous** threshold and if **current**–**previous**>**significance** the current centile is recorded as a potential threshold, the difference between current and previous is recorded if it is higher than the existing maximum.
difference. Consecutive significant differences are counted twice, for the first series of significant increases and for the last series of significant increases. If no significant increases have been identified the step size is doubled to a maximum of 20, significance is recalculated and the centile comparison repeated.

The resultant data from this centile comparison is a centile representing the first significant increase and the number of subsequent significant increases, the last significant increase and the number of preceding significant increases, and the centile representing the highest single increase between centiles. For the first and last runs of significant centiles further tuning is performed using centile comparisons at 1% step size. For example, if the 10th-15th centile comparison was the first significant increase in threshold for active layer and the 15th-20th and 20th-25th comparisons also had significant increases; centiles 10-25 would be compared to identify the 1% centile increase that gives the greatest increase.

These three numbers, the threshold derived from the first, last and highest centile comparison, are summed for each thresholding object then divided by the number of thresholding objects that have been processed to give first, last and highest thresholds for the given active layer. Adjusted thresholds were then calculated for each active layer as last – first.

**H&E - identification of vacuoles**

The cortex was segmented on blue to give cortex < blue(adjusted) < nucleus; this adjusted blue threshold allows inclusion of lighter nuclei as they have a varied appearance. Nuclei with area greater than 150 µm² were segmented on blue to give cortex < blue(last) < nucleus, then any remaining nuclei with area greater than 150 µm² were segmented on blue to give cortex < blue(max) < nucleus. Any nucleus with maximum value of maxStain less than OD(last) was removed to cortex.
Nuclei were then ‘shrunk’, meaning they were decreased in size by the outermost layer of pixels where given criteria are met, producing a new temporary object; the criteria used is OD less than OD(first). Any nucleus with area less than 10 µm² was removed into temporary. Nuclei were then grown into temporary by 5 pixels, then into temporary and cortex by one pixel, then into temporary and cortex where blue(7) is greater than blue(last). Temporary was then removed into the object it shares the highest proportion of its border with. All nuclei were then merged with neighbouring nuclei if the resulting object has an elliptic fit (proportion of pixels that fit in an enclosing ellipse) of greater than 0.5.

The minimum distance of each pixel of cortex to nucleus was then recorded in distanceNucleus. Vacuoles were then identified by segmenting cortex on brown(3) using brown(first). Vacuoles were then grown into cortex where OD less than OD(adjusted), then shrunk by one pixel to give temporary, and grown by 2 pixels into temporary; this removes thin areas of vacuole with width less than 3 pixels. Areas of cortex enclosed by vacuole with area less than 3 µm² were removed into vacuole.

Vacuoles were then excluded based on a number of criteria:

- Border to ROI boundary.
- Maximum value of distanceExternal less than 170 (this removes the molecular layer which can contain a high number of vacuolar structures not associated with spongiform disease).
- Minimum value of distanceNucleus less than 3 as close proximity to nuclei indicates a shrinkage artefact rather than a vacuole – any potential shrinkage with elliptic index (elliptic fit/shape index (border length/4√area) where 1 represents a perfect ellipse) of greater than 0.75, or relative border to nucleus less than 0.2 and area greater than 50 µm² were reclassified as vacuoles.
• Area less than 3 µm².
• Mean \textit{maxStain} > OD(first).
• Mean \textit{brown(3)} > \textit{brown(first)}.
• Length/width measurement > 3 (elongated object uncharacteristic of vacuoles) and standard deviation in \textit{blue} < \textit{blue(first)}.
• Shape index > 2 and standard deviation in \textit{brown} < \textit{brown(first)}.

IHC – Identification of chromogenic stain

The \textit{cortex} was segmented on \textit{brown+ve} to give \textit{cortex} < 0.2 < \textit{brown}; \textit{brown} was segmented on \textit{brown(3)} to give \textit{cortex} < OD(adjusted) < \textit{lowbrown} < OD(last) < \textit{brown}; \textit{cortex} was segmented on \textit{blue} to give \textit{background} < OD(first < \textit{cortex} < \textit{blue(last)} < \textit{nucleus}, with a minimum size of 7 pixels.

\textbf{Nuclei} with area less than 5 µm² were removed into \textit{cortex}, and then remaining \textbf{nuclei} were grown by 3 pixels into \textit{cortex} where the \textit{nuclear} object occupies greater than 40% of a 5x5 pixel box (this surface tension requirement prevents arbitrary increase in object size, limiting growth towards a more elliptic shape through smoothing of invaginations or removal of enclosed regions). All regions were then merged and any \textbf{nucleus} with area less than 10 µm² was removed into \textit{cortex}.

Distance based segmentation

Following identification of histological features, i.e. vacuoles and IHC staining, the \textit{cortex} was segmented at 10% intervals on \textit{distanceRelative} to give 0-10 < 0.1 < 10-20 < 0.2 < ... < 0.8 < 80-90 < 0.9 < 90-100. The area of \textit{cortex}, the area of each distance class (0-10, 10-20, ..., 80-90, 90-100), and the number and area of \textbf{vacuoles} or area of \textbf{lowbrown} and \textbf{brown} in each distance class were then exported.
Figure 52

*Slide image showing ROI analysis area (orange) and borders (inner – blue, outer – green)*
Figure 53
Slide image showing relative depth from outer border (0/dark) to inner border (1/light)
5.3.5. Statistical analysis

IBM SPSS version 24 was used for conducting the statistical analysis. A p-value of under 0.01 was taken as significant, as multiple testing was carried out.

**H&E analysis**

Univariate analysis of variance was performed, to assess for differences between the cortical ribboning group and the non-cortical ribboning group. The model used included age and gender as covariates. Comparison of the mean number of vacuoles between the groups was conducted for the selected ROI, in addition to this the number of vacuoles for each 10th percentile: 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90 and 90-100 was conducted. See table 34.

**GFAP analysis**

Univariate analysis of variance was performed, to look for differences between the cortical ribboning group and the non-cortical ribboning group, the model included age and gender as covariates. Comparison of the mean degree of staining (gliosis) identified across the ROI was made as well as for each 10th percentile: 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90 and 90-100. See table 35.

**ICSM 35 (PrPSc deposition) analysis**

Univariate analysis of variance was performed, to look for differences between the cortical ribboning group and the non-cortical ribboning group, the model included age and gender as covariates. Comparison of the mean degree of staining (PrPSc deposition) identified across the ROI and then for each 10 percentile was conducted: 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90 and 90-100. See table 36.
The second statistical analysis conducted was performed to assess for a correlation between each histopathological measure for each percentile across the cortex outer to inner edge. Pearson’s correlation was done with two tail significance testing.

5.4. Results

A two-tailed student t-test was used to assess whether the time between the last MRI brain scan and death was significantly different between the groups. There was no significant difference found between groups, the p-value was 0.51. The Pearson correlation was used to assess if an association existed between the timing between the last MRI brain scan and death and degree of spongiosis. The correlation coefficient (r) was 0.14, with a p-value of 0.38, there was no statistically significant difference identified.

5.4.1. Analysis of group differences

The tables 32-34 illustrate the results of the univariate analysis, corrected for age and gender, performed to assess for a significant difference between the cortical and non-cortical ribboning groups for the parameters of vacuolation, GFAP staining and PrP\textsuperscript{Sc} deposition (as measured by ISCM-35 staining) at each of the 10\textsuperscript{th} percentiles measured across the cortex to the white matter (0-outer cortex and 100 WM interface). Mean number of vacuoles, GFAP and ICSM-35 staining with confidence intervals are also included.

Highlighted are the p-values that cross the statistical significance threshold of p-value <0.01. Number of subjects: 33 (12 non-cortical ribboning, 21 cortical ribboning cases).
<table>
<thead>
<tr>
<th>Percentile</th>
<th>Corrected model p-value</th>
<th>Gender</th>
<th>Age</th>
<th>Group</th>
<th>Mean number of vacuoles (Confidence intervals CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-CR</td>
</tr>
<tr>
<td>Mean</td>
<td>0.002</td>
<td>0.12</td>
<td>0.44</td>
<td>0.00</td>
<td>126 (84, 173) 252 (218, 291)</td>
</tr>
<tr>
<td>0-10</td>
<td>0.092</td>
<td>0.63</td>
<td>0.83</td>
<td>0.03</td>
<td>141 (73, 221) 298 (233, 379)</td>
</tr>
<tr>
<td>10-20</td>
<td>0.004</td>
<td>0.32</td>
<td>0.96</td>
<td>0.00</td>
<td>160 (91, 246) 361 (303, 420)</td>
</tr>
<tr>
<td>20-30</td>
<td>0.005</td>
<td>0.18</td>
<td>0.61</td>
<td>0.002</td>
<td>141 (86, 209) 301 (259, 370)</td>
</tr>
<tr>
<td>30-40</td>
<td>0.003</td>
<td>0.13</td>
<td>0.44</td>
<td>0.003</td>
<td>126 (90, 166) 282 (231, 346)</td>
</tr>
<tr>
<td>40-50</td>
<td>0.01</td>
<td>0.05</td>
<td>0.26</td>
<td>0.027</td>
<td>129 (89, 175) 261 (202, 324)</td>
</tr>
<tr>
<td>50-60</td>
<td>0.04</td>
<td>0.26</td>
<td>0.85</td>
<td>0.02</td>
<td>125 (82, 175) 265 (199, 335)</td>
</tr>
<tr>
<td>60-70</td>
<td>0.03</td>
<td>0.22</td>
<td>0.99</td>
<td>0.009</td>
<td>117 (72, 165) 256 (200, 320)</td>
</tr>
<tr>
<td>70-80</td>
<td>0.02</td>
<td>0.18</td>
<td>0.97</td>
<td>0.006</td>
<td>112 (70, 156) 231 (186, 286)</td>
</tr>
<tr>
<td>80-90</td>
<td>0.29</td>
<td>0.63</td>
<td>0.63</td>
<td>0.12</td>
<td>121 (73, 176) 187 (150, 229)</td>
</tr>
<tr>
<td>90-100</td>
<td>0.47</td>
<td>0.34</td>
<td>0.54</td>
<td>0.38</td>
<td>91 (48, 139) 126 (95, 161)</td>
</tr>
</tbody>
</table>

**Table 34**

*Group differences in degree of spongiosis (number of vacuoles) for cortical and non-cortical ribboning subjects. Univariate analysis, corrected for age and gender*
<table>
<thead>
<tr>
<th>Percentile</th>
<th>Corrected model p-value</th>
<th>Gender</th>
<th>Age</th>
<th>Group</th>
<th>Mean staining GFAP (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-CR</td>
<td>CR</td>
</tr>
<tr>
<td>Mean</td>
<td>0.48</td>
<td>0.332</td>
<td>0.79</td>
<td>0.24</td>
<td>71.0 (61.7-79.8)</td>
</tr>
<tr>
<td>0-10</td>
<td>0.17</td>
<td>0.10</td>
<td>0.31</td>
<td>0.2</td>
<td>77.8 (64.2,90.3)</td>
</tr>
<tr>
<td>10-20</td>
<td>0.15</td>
<td>0.11</td>
<td>0.3</td>
<td>0.14</td>
<td>70 (60,79)</td>
</tr>
<tr>
<td>20-30</td>
<td>0.21</td>
<td>0.17</td>
<td>0.59</td>
<td>0.12</td>
<td>67.9 (59.4,75.9)</td>
</tr>
<tr>
<td>30-40</td>
<td>0.37</td>
<td>0.29</td>
<td>0.75</td>
<td>0.17</td>
<td>67.9 (58.6, 75.8)</td>
</tr>
<tr>
<td>40-50</td>
<td>0.51</td>
<td>0.38</td>
<td>0.82</td>
<td>0.23</td>
<td>68.1 (58.4, 76.4)</td>
</tr>
<tr>
<td>50-60</td>
<td>0.63</td>
<td>0.46</td>
<td>0.94</td>
<td>0.29</td>
<td>69.6 (59.6, 79.9)</td>
</tr>
<tr>
<td>60-70</td>
<td>0.77</td>
<td>0.48</td>
<td>0.98</td>
<td>0.45</td>
<td>73.7 (58.9, 87.1)</td>
</tr>
<tr>
<td>70-80</td>
<td>0.83</td>
<td>0.57</td>
<td>0.92</td>
<td>0.49</td>
<td>74.2 (60.0, 87.6)</td>
</tr>
<tr>
<td>80-90</td>
<td>0.84</td>
<td>0.63</td>
<td>0.94</td>
<td>0.46</td>
<td>72.0 (56.5, 86.8)</td>
</tr>
<tr>
<td>90-100</td>
<td>0.81</td>
<td>0.79</td>
<td>0.91</td>
<td>0.36</td>
<td>68.8 (54.1, 83.7)</td>
</tr>
</tbody>
</table>

**Table 35**

*Group differences in degree of gliosis (GFAP) for cortical and non-cortical ribboning subjects. Univariate analysis, corrected for age and gender*
<table>
<thead>
<tr>
<th>Percentile</th>
<th>Corrected model p-value</th>
<th>Gender</th>
<th>Age</th>
<th>Group</th>
<th>Mean staining ICSM-35 (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-CR</td>
<td>CR</td>
</tr>
<tr>
<td>Mean</td>
<td>0.33</td>
<td>0.12</td>
<td>0.65</td>
<td>0.36</td>
<td>57.8 (32.5, 84.8)</td>
</tr>
<tr>
<td>0-10</td>
<td>0.29</td>
<td>0.13</td>
<td>0.96</td>
<td>0.27</td>
<td>54.8 (31.6, 77.5)</td>
</tr>
<tr>
<td>10-20</td>
<td>0.28</td>
<td>0.09</td>
<td>0.75</td>
<td>0.34</td>
<td>57.9 (33.1, 81.3)</td>
</tr>
<tr>
<td>20-30</td>
<td>0.23</td>
<td>0.08</td>
<td>0.68</td>
<td>0.29</td>
<td>57.2 (33.2, 82.1)</td>
</tr>
<tr>
<td>30-40</td>
<td>0.25</td>
<td>0.08</td>
<td>0.62</td>
<td>0.34</td>
<td>58.2 (32.5, 82.7)</td>
</tr>
<tr>
<td>40-50</td>
<td>0.28</td>
<td>0.09</td>
<td>0.58</td>
<td>0.37</td>
<td>58.9 (33.6, 86.2)</td>
</tr>
<tr>
<td>50-60</td>
<td>0.33</td>
<td>0.11</td>
<td>0.56</td>
<td>0.38</td>
<td>58.7 (33.4, 86.9)</td>
</tr>
<tr>
<td>60-70</td>
<td>0.29</td>
<td>0.10</td>
<td>0.49</td>
<td>0.37</td>
<td>58.8 (32.1, 85.8)</td>
</tr>
<tr>
<td>70-80</td>
<td>0.40</td>
<td>0.14</td>
<td>0.51</td>
<td>0.45</td>
<td>59.1 (31.9, 88.0)</td>
</tr>
<tr>
<td>80-90</td>
<td>0.61</td>
<td>0.25</td>
<td>0.65</td>
<td>0.52</td>
<td>58.1 (29.1, 87.5)</td>
</tr>
<tr>
<td>90-100</td>
<td>0.74</td>
<td>0.35</td>
<td>0.76</td>
<td>0.58</td>
<td>56.8 (27.2, 87.3)</td>
</tr>
</tbody>
</table>

**Table 36**

*Group differences in degree of PrPSc deposition (ICSM35) for cortical and non-cortical ribboning subjects. Univariate analysis, corrected for age and gender*
Figure 54

Scatterplot illustrating the mean number of vacuoles for each subject in the cortical ribboning and non-cortical ribboning groups (with mean value and confidence intervals)
### 5.4.2. Correlation between histopathological parameters across the cortex

All patients were included in the analysis to assess if a correlation existed between number of vacuoles and degree of gliosis staining, number of vacuoles and PrP\(^{Sc}\) deposition and between degree of gliosis and PrP\(^{Sc}\) deposition. The results of analysis for each percentile across the cortex are illustrated in the following tables. P-values <0.01 are in bold and moderate to strong correlations are starred.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vac 0_10</td>
<td>PCC (r)</td>
<td>0.40(^*)</td>
<td>0.47(^**)</td>
<td>0.21</td>
<td>0.11</td>
<td>0.14</td>
<td>0.1</td>
<td>0.04</td>
<td>-0.05</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.03</td>
<td>0.008</td>
<td>0.25</td>
<td>0.6</td>
<td>0.57</td>
<td>0.45</td>
<td>0.61</td>
<td>0.83</td>
<td>0.81</td>
</tr>
<tr>
<td>Vac 10_20</td>
<td>PCC (r)</td>
<td>0.28</td>
<td>0.48(^**)</td>
<td>0.32</td>
<td>0.24</td>
<td>0.24</td>
<td>0.24</td>
<td>0.19</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.12</td>
<td>0.007</td>
<td>0.08</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
<td>0.3</td>
<td>0.46</td>
<td>0.81</td>
</tr>
<tr>
<td>Vac 20_30</td>
<td>PCC (r)</td>
<td>0.38</td>
<td>0.59</td>
<td>0.45</td>
<td>0.36</td>
<td>0.35</td>
<td>0.34</td>
<td>0.29</td>
<td>0.22</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.04</td>
<td>0.001</td>
<td>0.01</td>
<td>0.04</td>
<td>0.05</td>
<td>0.06</td>
<td>0.11</td>
<td>0.22</td>
<td>0.68</td>
</tr>
<tr>
<td>Vac 30_40</td>
<td>PCC (r)</td>
<td>0.21</td>
<td>0.41(^*)</td>
<td>0.39(^*)</td>
<td>0.35</td>
<td>0.34</td>
<td>0.31</td>
<td>0.28</td>
<td>0.23</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.26</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.06</td>
<td>0.09</td>
<td>0.13</td>
<td>0.21</td>
<td>0.68</td>
</tr>
<tr>
<td>-------</td>
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<td>-----------</td>
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<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Vac 40_50</td>
<td></td>
<td>0.19</td>
<td>0.29</td>
<td>0.27</td>
<td>0.23</td>
<td>0.22</td>
<td>0.21</td>
<td>0.18</td>
<td>0.16</td>
<td>0.04</td>
</tr>
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<td></td>
<td>p-value</td>
<td>0.3</td>
<td>0.11</td>
<td>0.15</td>
<td>0.22</td>
<td>0.23</td>
<td>0.26</td>
<td>0.33</td>
<td>0.38</td>
<td>0.85</td>
</tr>
<tr>
<td>Vac 50_60</td>
<td></td>
<td>0.19</td>
<td>0.3</td>
<td>0.28</td>
<td>0.23</td>
<td>0.22</td>
<td>0.2</td>
<td>0.19</td>
<td>0.18</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.32</td>
<td>0.1</td>
<td>0.13</td>
<td>0.21</td>
<td>0.24</td>
<td>0.28</td>
<td>0.3</td>
<td>0.33</td>
<td>0.76</td>
</tr>
<tr>
<td>Vac 60_70</td>
<td></td>
<td>0.23</td>
<td>0.39</td>
<td>0.38</td>
<td>0.34</td>
<td>0.33</td>
<td>0.31</td>
<td>0.29</td>
<td>0.29</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.21</td>
<td>0.03</td>
<td>0.03</td>
<td>0.06</td>
<td>0.07</td>
<td>0.1</td>
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**Table 37**

*Pearson’s correlation (PCC), vacuoles (vac) vs. GFAP (Gli) staining across the cortex*
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<th>Vac 20-30</th>
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**Table 38**

*Pearson’s correlation (PCC), PrP<sup>Sc</sup> deposition (ICSM-35) vs. number of vacuoles (Vac) across the cortex*
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**Table 39**

*Pearson’s correlation (PCC) gliosis (Gli) vs. PrPSc (ICSM-35) deposition across the cortex*
The following figures 55-58 are from all subjects. The figures illustrate the trend of each histopathology parameter measurement across the cortex: the degree of vacuolation, PrP\textsuperscript{Sc} deposition (ICSM-35 staining) and gliosis (GFAP staining).

0=outer edge (eroded by 10% to minimise artefact) 100 =grey/white matter border. Red lines= subjects from the no cortical ribboning group and the blue lines= subjects from the cortical ribboning group.

**Figure 55**

*Number of vacuoles per millimetre across the cortex for all subjects*

0-10= *Outer border of cortex (eroded by 10%)*

90-100= *Inner border of cortex/white matter*

*Blue graph lines= patients with cortical ribboning on DWI sequence*

*Red graph lines= patients with no cortical ribboning on DWI sequence*
Figure 56

Relative area of tissue (%) affected by spongiosis

1 = 10th percentile outer border of cortex

10 = 100th percentile inner border of cortex/white matter

Blue = patients with cortical ribboning on DWI sequence

Red = patients with no cortical ribboning on DWI sequence
**Figure 57**

*Degree of ISCM-35 staining (% of PrP\textsuperscript{Sc} deposition) across the cortex*

1 = 10\textsuperscript{th} percentile outer border of cortex

10 = 100\textsuperscript{th} percentile inner border of cortex/white matter

Blue = patients with cortical ribboning on DWI sequence

Red = patients with no cortical ribboning on DWI sequence
Figure 58

Degree of GFAP staining (% of gliosis) across the cortex

1 = 10th percentile outer border of cortex
10 = 100th percentile inner border of cortex/white matter

Blue = patients with cortical ribboning on DWI sequence
Red = patients with no cortical ribboning on DWI sequence

GFAP staining across the cortex
(measure of gliosis)
The following figures 59-61 illustrate the mean values for all subjects for each histopathological parameter, spongiosis, gliosis and PrP$^{Sc}$ deposition across the cortex.

**Figure 59**

*Spongiosis across the cortex for all subjects, 0=outer edge, 100=GM/WM border*
Figure 60

PrP\textsuperscript{Sc} deposition across the cortex for all subjects, 0=outer edge, 100=GM/WM border
Figure 61

_Gliosis staining across the cortex for all subjects, 0=outer edge, 100=GM/WM border_
5.4.3. Interpretation of results

There was a significant difference (p <0.01) between the two patient groups for spongiosis/number of vacuoles present. In the patients with cortical ribboning present on the DWI MRI sequence there was a higher degree of spongiosis present than in those with no cortical ribboning present. The spongiform change was most evident in the outer cortex up to the 40th percentile in both patient groups and the difference between the groups was most pronounced at the exterior of the cortex, 0-50th percentiles, but significantly different to the inner 20th percentile. It may be the case that diffusion weighted change seen on the MRI scans of these patients not only reflects the larger amount of spongiosis present but may also be a result of spongiosis localising predominantly within the exterior part of the cortex.

The cortical ribboning group had a higher degree of staining for GFAP and ISCM-35, indicating more gliosis and PrPSc deposition than the group with no cortical ribboning present, but there was no statistical difference found for either the mean or at any percentile across the cortex.

A correlation between GFAP staining (degree of gliosis) in the outer layers of the cortex was observed at several percentiles with the degree of spongiosis most pronounced at the exterior of the cortex, but also evident at deeper level too (see table 37). There was also a correlation between gliosis and PrPSc deposition; this was more evident in the deeper cortical layers at percentiles 50-70 for PrPSc deposition and 40-60 for gliosis (see table 39). There was also a degree of correlation between spongiosis and PrPSc deposition (see table 38). This was most evident for PrPSc deposition in the deeper cortex correlating with spongiosis occurring in the outer cortex, differing from what was seen with gliosis and PrPSc where there was more evidence for the pathology occurring closer together, with the strongest correlation being for gliosis in the 50th-60th percentiles and PrPSc in 50th-70th.
5.5. Discussion

The most striking result was that spongiform change was more pronounced in the group of patients with diffusion weighted imaging change present in the cortex on MRI brain scan this supports the theory and evidence from other’s researchers’ work that spongiform change results in restricted diffusion on brain MRI\(^9,121,136\).

Even in a small group the results were highly significant. The cortical ribboning group had a larger amount of gliosis and PrP\(^{Sc}\) deposition present that also correlated with degree of vacuolation indicating that there is likely to be an association between PrP\(^{Sc}\) deposition, gliosis and spongiosis, although correction for multiple testing was not conducted and therefore any correlations need to be interpreted with caution. Overall there was no significant difference found in the occurrence of gliosis or in PrP\(^{Sc}\) deposition between the two groups. There was a trend towards there being slightly more gliosis and PrP\(^{Sc}\) deposition in the cortical ribboning group which along with the correlation in gliosis and PrP\(^{Sc}\) deposition in certain cortical areas points towards the likelihood that all the neuropathology parameters are interrelated but as mentioned no statistical difference.

One of the aims of this project was to identify if there was evidence of pathology co-localisation, i.e. PrP\(^{Sc}\) deposition occurring in association with gliosis and spongiosis. There was some evidence for a correlation existing between the different pathological parameters but at different percentiles/ levels within the cortex. Again correction for multiple testing was not carried out, so the significance of there may be over-interpretation of the significance of these findings. Overall spongiosis was maximal in the outer layers of the cortex (see table 34 and figures 55, 56 and 59) this was most marked in the cortical ribboning group, but also present although to a lesser extent in the non-cortical ribboning group, there was a reduction in vacuoles across the cortex maximal at the exterior to the white matter. Similarly gliosis was also maximal at the
exterior of the cortex decreasing to the 30th percentile and remaining fairly uniform across the cortex. There was more variability evident in the non-cortical ribboning group and in some subjects an increase in gliosis at the inner cortex. The pattern of PrPSc deposition was a gradual increase from the exterior of the cortex to the white matter border with maximal deposition in the 60-80th percentiles.

Although a direct co-localisation of pathology was not observed, an association was found at different percentiles.

Limitations of the study were that this is a relatively small study, the numbers in each group were not equal, with more patients in the cortical ribboning group that the non-cortical ribboning group. The interval between the last MRI brain scan and post-mortem was quite long and therefore knowing if the post-mortem findings accurately represent what is seen on the scan is unknown. MRI scans were acquired on different hospital scanners and the quality of images varied, all the scans were assessed for quality control purposes, those with movement artefact were not included, however imaging artefact is always possible. All the histopathology slides were reviewed by a neuropathologist as part of the quality control process and specimens that were not deemed to be of high enough quality were not included in the analysis but it is difficult to be absolutely sure that artefact is completely excluded.

One of the main points of this research was to identify what imaging findings correspond to on a microstructural level. From this study it is most likely that restricted cortical diffusion seen on MRI represents spongiform change. This is an important finding not only as we can better understand the imaging findings, but also as this can add to the value of imaging in its use as a biomarker.
Further work

As the subject group numbers were small further analysis with a larger subject number to investigate if the results are reproducible would be advisable. In regard to assessing for correlations between the histopathological parameters at different cortical layers, it would be advisable to conduct correction for multiple testing. In addition to this repeating the study in a group of patients with the same subtype of prion disease, for example, only analysing patients with sCJD patients would reduce the possibility of factors such as disease subtype having an influence on the analysis outcome.

Prion strain type and codon 129 genotype were not tested in all subjects included in this study and therefore it was not possible to include these in the final model as possible confounders, repeating the analysis with these factors included would be helpful in being able to justify a true result.

In addition to having a larger sample size and homogenous patient group examining additional brain regions to assess if similar findings are evident in other anatomical areas would be worthwhile. Including the deep grey matter nuclei, the caudate, putamen and thalamus as well as other cortical areas, this would not only help in demonstrating if the results are reproducible but also identifying if similar pathology is evident in other grey matter regions and that spongiosis is found to correlate with restricted diffusion seen on diffusion weighted imaging. White matter abnormalities are also reported in prion disease and using quantitative histopathology analysis may be of help in further categorising and determining if spongiosis truly occurs there or if the reported white matter findings seen in panencephalopathic CJD are solely a result of neuronal loss and not true spongiosis.

As further work, and part of this project, my aim was to also compare quantitative MR imaging findings, namely magnetisation transfer ratio, MTR, with quantitative histopathology analysis. The aim of the project was to
ascertain if there was an association between quantitative analysis of degree of spongiosis and the quantitative imaging parameter MTR, in patients with all subtypes of prion disease, Siddique et al reported that patients with variant CJD that had post-mortem 9 Tesla quantitative MR imaging had reduced MTR that paralleled spongiosis\(^8\) and being able to test if this finding was also present in a larger sample size of patients with other forms of prion disease would have been helpful in showing how or what quantitative imaging findings reflect on a molecular level. Unfortunately there was not a sufficiently large sample of patients who had post-mortem results and adequate quality quantitative imaging available to analyse. I think further work that looks at comparing quantitative imaging with quantitative histopathology would be of great benefit in being able to ascertain if MTR correlates with spongiosis and understanding how imaging parameters such as MTR and also DTI reflect underlying neuropathology. In addition to this subset analysis comparing different types of prion disease, sporadic vs. iatrogenic, variant and inherited cases could help with understanding how the pathology may differ between the specific forms of prion disease.
6. Conclusion

The aim of my PhD has been to investigate whether brain imaging can act as a biomarker of both onset and progression in patients with prion disease. In addition to this to confirm if clinical features of disease correlate with specific signal abnormalities identified on MRI brain scan. Finally to assess if specific histopathological findings on post-mortem correlate with cortical imaging abnormalities in patients diagnosed with prion disease.

In chapter 2 I hypothesised that specific MRI signal change could predict disease activity and rate of progression and may in combination with codon 129 genotype acts as a powerful outcome measure. However this hypothesis was not proved correct and conventional MR brain imaging does not help to predict disease progression in patients with sCJD. I was able to show as previous researchers have that codon 129 remains the main predictor of disease progression in this patient group. In addition to this that prion strain type had an added effect on predicting rate of disease progression.

The aim of my second project was to test the hypothesis that sensitive quantitative imaging parameters such as MTR would predict disease progression in symptomatic patients, the main findings in this project supported that hypothesis and there was a strong correlation with the MRC Scale score and MTR value in patients with symptomatic disease which could be used as a clinical biomarker in combination with the MRC Scale to predict response to therapeutics in future clinical trials.

The hypothesis of my third project was that sleep abnormalities are highly prevalent in all forms of prion disease, which they are primarily caused by thalamic pathology and are associated with co-morbid mood disturbance. I found that sleep disturbance was highly prevalent in all forms of prion disease. I also found that sleep disturbance was strongly associated with the presence of depressive symptoms and there was a significant association found between
abnormal thalamic signal change seen on MRI scan and sleep symptomatology. In this project I found that the clinical research forms used to document sleep symptoms were limited and the full breadth of sleep related issues that this population of patients with prion disease had was not being recorded. I therefore performed a retrospective analysis of all available clinical records in patients with prion disease to assess the full spectrum of sleep related symptoms. I constructed the prion disease sleep questionnaire with the aims of use being: to be an effective bedside screening tool that can monitor sleep disturbances and assess efficacy and response of symptomatic medication given to treat sleep symptoms.

In my final project my theory was that cortical DWI signal change found on sCJD patient’s MRI brain scans correlates with spongiform change found on quantitative analysis of brain tissue at autopsy. I found that my hypothesis proved correct. There were significant difference between the two groups of patients included in the study those with and without cortical signal change. The patients with cortical ribboning present on their MRI brain scans had significantly more cortical grey matter spongiosis than those that didn't. In addition to this spongiosis was most evident in the exterior layers of the cortex. There was also a modest correlation identified in the measures of the amount or quantity of pathology present for each histopathological parameter, PrPSc, gliosis and spongiosis.

There were a number of limitations to my study including the heterogeneity of patients included in quantitative imaging project. The relatively small numbers of patients included in the histopathology study. Unfortunately it is difficult to recruit large numbers of patients with prion disease due to the rarity of the condition as well as the rapid disease progression and in comparison to other similar prion studies the subject numbers were reasonable.
Further work and planned publications

My plan is to write three papers on my research findings. The first of these will detail the cross-sectional and longitudinal quantitative imaging findings in patients with prion disease and how they act as predictors of disease progression, emphasising the importance of finding clinical biomarkers that may be used to predict response to therapeutics in future clinical trials. The second paper will focus on the incidence of sleep disturbance in patients with prion disease, how thalamic signal change parallels sleep disturbance and the development of a sleep scale for use in this patient group. The third paper will concentrate on showing how diffusion weighted imaging correlates with spongiform change on quantitative histopathology analysis.

In regard to the quantitative imaging project I think further work should be aimed at assessing if a biomarker of onset could be developed in patients who are asymptomatic. The group of patients that were included in my study was fairly heterogeneous and many of these patients could have been several years away from their disease onset date. It would be helpful to repeat the study in a smaller more homogenous group of patients and assess if there are early markers of disease activity in these groups. If therapeutics become available in the future then it would seem logical that the earlier on the treatment is given the better the prognosis as prion disease can rapidly progress even in patients with the inherited form of the disease.

In patients who are symptomatic with sleep disturbance I think further work should be aimed at collecting more data on the natural history of sleep symptomatology using the prion disease sleep questionnaire. We then have a better idea of the prevalence of sleep difficulties and have a means to test which symptomatic treatments are most effective in this patient group.

In terms of assessing how MRI signal change reflects underlying prion histopathology, repeating the study using quantitative imaging would be helpful.
in being able to identify what exactly MTR and DTI represent on a histopathological level. In addition to this analysing other cortical grey matter regions and the deep grey matter nuclei to assess if DWI signal change also correlates with spongiosis in these regions too.
Acknowledgements

I thank my supervisors Simon Mead and John Thornton for all their help, encouragement and guidance with my PhD. Thank you also to John Collinge who gave me the opportunity to do a PhD as well as provided the funding through the MRC Prion unit. I thank all those who worked with and helped me with the imaging analysis especially Enrico De Vita who taught me how to conduct the quantitative analysis and helped with running the final analysis. To Harpreet Hyare who worked with me on the quantitative imaging and who reported the majority of the scans of patients recruited to the cohort. To both Ivor Simpson who helped with the MAPS analysis and to Zoe Fox for support with statistical analysis.

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I am most grateful for the patients and their families who have been involved in this research, without them this PhD would not have been possible. A special thanks goes to them.

Finally, I both thank and dedicate this thesis to my family. To my ever optimistic and patient husband, who has maintained the belief that I would eventually finish writing this. To my 3 brilliant and supportive daughters, who hold such unwavering respect and belief in their mother. And to my parents and two siblings who have always been there with their constant love and support.
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### 7.0. Appendix

Clinical Research Form used to collect data on examination and cognitive symptoms at time of assessment including questions on sleep disturbance (see section 12)

#### Clinical significant findings

<table>
<thead>
<tr>
<th>Date of examination</th>
<th>Weight (kg)</th>
<th>BP (mmHg)</th>
<th>Temp (°C)</th>
<th>Pulse (/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>day</td>
<td>month</td>
<td>year</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. Record clinically significant examination findings below:

<table>
<thead>
<tr>
<th>Clinical significant findings</th>
<th>Normal</th>
<th>Abnormal</th>
<th>If abnormal, give details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychiatric disturbance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genito-urinary</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musculo-skeletal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eyes/mouth/throat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver/spleen/abdomen</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Neurological examination and assessments

11. Current neurological features on examination

<table>
<thead>
<tr>
<th>Feature</th>
<th>Absent</th>
<th>Present</th>
<th>Unable to assess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive impairment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extrapyramidal signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyramidal signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myoclonus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chorea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other involuntary movements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supranuclear ophthalmoparesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nystagmus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visual field defects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primitive reflexes</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

12. Current cognitive symptoms at time of assessment

<table>
<thead>
<tr>
<th>Task/Ability</th>
<th>None</th>
<th>Rare</th>
<th>Often</th>
<th>Always</th>
<th>Unable to assess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social behaviour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriateness of speech</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appropriateness of emotion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numeracy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reading skills</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appetite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food preferences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obsessivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ability to get dressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressive symptomatology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

13. Rankin Scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Give reason if not done: .........................................................</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No symptoms; 1=No significant disability despite symptoms (able to carry out all usual duties and activities);</td>
</tr>
<tr>
<td>2</td>
<td>2=Slight disability (unable to carry out all previous activities but able to look after own affairs with assistance);</td>
</tr>
<tr>
<td>3</td>
<td>3=Moderate disability (requiring some help, but able to walk without assistance);</td>
</tr>
<tr>
<td>4</td>
<td>4=Moderately severe disability (unable to walk without assistance and unable to attend to own bodily needs without assistance);</td>
</tr>
<tr>
<td>5</td>
<td>5=Severe disability (bedridden, incontinent and requiring constant nursing care and attention)</td>
</tr>
</tbody>
</table>

14. Myoclonus

| Startle Myoclonus | ................. | 0 | 1 | 2 | 3 |
| Spontaneous Myoclonus | .................. | 0 | 1 | 2 | 3 |
| Action/stimulus-sensitive Myoclonus | ................. | 0 | 1 | 2 | 3 |

<table>
<thead>
<tr>
<th>Severity</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has a video of myoclonus been taken?</td>
<td>Yes (if yes, attach Form 14)</td>
<td>No</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
Medical Prion Disease Rating Scale

### National Prion Monitoring Cohort MRC Scale

A longitudinal observational study of all patients diagnosed with or at high risk of developing human prion disease

<table>
<thead>
<tr>
<th>Score Item</th>
<th>Category criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowel function</td>
<td>At least one episode of incontinence in the last 7 days</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Continent for last 7 days</td>
<td>1</td>
</tr>
<tr>
<td>Bladder function</td>
<td>Always incontinent or catheterised</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Continent or occasional accidents</td>
<td>1</td>
</tr>
<tr>
<td>Toilet use</td>
<td>Fully dependent</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Needs some help</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Independent</td>
<td>2</td>
</tr>
<tr>
<td>Bathing</td>
<td>Fully dependent or needs some help</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Independent</td>
<td>1</td>
</tr>
<tr>
<td>Feeding</td>
<td>Unable or NG/PEG/RIG fed (takes nothing by mouth)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Needs help but can swallow (even if unsafe)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Independent</td>
<td>2</td>
</tr>
</tbody>
</table>

Name of Health professional asking the questions:

Name of Caregiver completing questions:

Relationship to patient:
<table>
<thead>
<tr>
<th>Score Item</th>
<th>Category criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfer and mobility</td>
<td>Bedbound, unable to sit</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Can sit without support or mobilise or transfer with help from person/s</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Can transfer or mobilise independently or both (including use of walking aid)</td>
<td>2</td>
</tr>
<tr>
<td>Stairs</td>
<td>Unable</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Needs help</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Independent</td>
<td>2</td>
</tr>
<tr>
<td>Best verbal response</td>
<td>Mute</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Incomprehensible sounds</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Single words</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Sentences but difficulty in finding words, uses incorrect words or is often disoriented / confused</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Normal conversation</td>
<td>4</td>
</tr>
<tr>
<td>Memory and orientation to surroundings</td>
<td>Shows no awareness of surroundings or any evidence of memory</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Evidence of retaining some highly learned material</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(e.g. recognising familiar people) or awareness of surroundings but no evidence of acquiring new material</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Able to retain some new information BUT memory consistently impaired</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Memory normal or some impairment off and on</td>
<td>3</td>
</tr>
<tr>
<td>Judgement and problem solving</td>
<td>Unable to show any judgement or problem-solving</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Able to show some judgement or problem-solving, even if this is severely impaired (e.g. Choice of meals, need for bathroom etc)</td>
<td>1</td>
</tr>
<tr>
<td>Use of tools</td>
<td>Unable to use any tools or objects</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Able to use some tools or objects, with help if necessary. (e.g. TV remote, knife and fork)</td>
<td>1</td>
</tr>
</tbody>
</table>

**TOTAL SCORE** /20

Scales NPC version 3.1, April 2019