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Validation of the 2017 International Creutzfeldt-Jakob Disease Surveillance Network Diagnostic Criteria for Sporadic Creutzfeldt-Jakob Disease

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Contents

Abstract

Sporadic Creutzfeldt-Jakob disease (sCJD) is the commonest human prion disease. sCJD is rapidly-progressive, universally fatal and transmissible. Rapid, accurate in-life diagnosis is imperative for epidemiological surveillance and public health activities, to exclude treatable differentials and facilitate supportive care. In 2017 the International CJD Support Network diagnostic criteria were revised to incorporate i) cortical ribboning on magnetic resonance imaging (MRI) and ii) the real-time quaking-induced conversion (RT-QuIC) assay.

In this thesis the revised criteria were validated using a three-year clinicopathological cohort of all neuropathologically-confirmed sCJD cases from the UK. France, Germany and Italy, with a control group with alternative neuropathological diagnoses. The sensitivity and specificity of criteria was compared with prior criteria. Sub-analyses were performed assessing sCJD cases grouped by prion protein genotype, neuropathological classification, disease duration and age.

The revised criteria were found to be 98.5% sensitive, a 21.5% increase from previous criteria, with no loss of specificity. Revisions have led to increases in case ascertainment, including among cases with limited clinical features and atypically long disease duration. This increase in sensitivity is of great utility for prion disease surveillance.

Lay Summary

Human prion diseases are caused by accumulation of misfolded prion protein in the nervous system. The commonest form is sporadic Creutzfeldt-Jakob disease (sCJD). The cause is unknown, and the disease is incurable and always fatal, typically within several months. All forms of CJD are transmissible, i.e. it is an infectious disease.

Around the world, surveillance programmes operate to monitor cases of CJD, as well as working to prevent transmission, and to deliver effective supportive healthcare. This requires the ability to make a robust and swift diagnosis while affected individuals are still alive. The international surveillance community uses diagnostic criteria to facilitate this. These criteria were most recently expanded in 2017 to include a pattern of changes on magnetic resonance imaging (MRI) of the brain, termed 'cortical ribboning', and a new test on spinal fluid called real-time quaking-induced conversion (RT-QuIC).

Until this study, only one group had explored how well these criteria perform, and this was only in a small, single-nation sample. I undertook a large, international study using all autopsy-confirmed sCJD cases who died between 2017-2019 from the registries of the United Kingdom (UK), France, Germany and Italy, as well as a control group of 'non-cases' who were investigated for potential CJD during life and had alternative diagnoses made at autopsy. I explored how the diagnostic tests perform, including the new ones mentioned above, and how the diagnostic criteria perform when applied in full, compared to the previous ones.

The new diagnostic criteria were greatly more sensitive for diagnosing sCJD and were just as accurate in distinguishing non-cases. I also demonstrated how the criteria perform in a range of special circumstances, including uncommon forms of sCJD, and cases with unusually short or long survival. Overall, this thesis demonstrates that the latest criteria have great accuracy for the diagnosis of sCJD. They will greatly improve surveillance efforts, enhancing public health measures and ultimately improving the diagnosis and care of all individuals affected by this devastating illness.

Declaration of Originality

This is a statement to verify that this thesis is an original work composed by me, Dr Neil Robert John Watson. I designed the studies within, in conjunction with my supervisor, Dr Suvankar Pal. The intellectual content is my own. All written content as well as supportive figures, tables and boxes are my own work, with editorial suggestions by my supervisor panel.

This work has not been submitted for any other degree or professional qualifications.

Publications referenced are my own unless otherwise stated, in which case the reader is provided with an appropriate reference.

Signature:

Date: 4th October 2022

Publications & presentations derived from this thesis

Below is a list of articles published:

1. Watson N *et al* (2022). Validation of revised International Creutzfeldt-Jakob Disease Surveillance Network diagnostic criteria for sporadic Creutzfeldt-Jakob Disease. *JAMA Network Open* 5(1):e2146319

2. Watson N *et al* (2021). The importance of continued international surveillance for Creutzfeldt-Jakob disease. *Nature Reviews Neurology* 17(6): 362-379

Work related to this thesis has been presented at the following conferences:

1. Association of British Neurologists Annual Meeting. Poster presentations. Clinicopathological characteristics impacting on survival in sporadic Creutzfeldt-Jakob disease: insights from an international autopsy-confirmed series. Date: 18th-20th May 2022

2. 7th Congress of the European Academy of Neurology. Oral and poster presentation: Validation of the 2017 diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Date: 20thth June 2021

3. Association of British Neurologists Annual Meeting. Oral and poster presentation: Validation of the 2017 diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Date: 6th May 2021

4. NRS Mental Health ASM 2020: Transforming mental health science. Poster presentation: Validation of the 2017 diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Date: 74th November 2020

5. Scottish Dementia Research Consortium Conference 2020: Unlocking the Mysteries of Data. Poster presentation: Validation of the 2017 diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Date: 7th September 2020

6. Royal College of Physicians and Surgeons of Glasgow: Neurology 2020 conference. Poster presentation: Validation of the 2017 diagnostic criteria for sporadic Creutzfeldt-Jakob disease. Date: 6th March 2020

Aims & Hypotheses

The diagnostic criteria for sporadic Creutzfeldt-Jakob disease (sCJD) were updated in 2017, incorporating cortical ribboning on magnetic resonance imaging (MRI) of the brain and the real-time quaking-induced conversion (RT-QuIC) assay in cerebrospinal fluid. These diagnostic criteria require large-scale validation using a robust cohort and with a sufficient sample size to evaluate their performance in important subgroups, such as sCJD cases stratified by prion protein gene (*PRNP*) codon 129 polymorphism status and overall disease duration. Their performance, in terms of sensitivity and specificity, must be compared to the previous diagnostic criteria to assess the magnitude of change, with resultant effects on case classification. The epidemiological consequences of this, i.e. the increase in cases classified as *probable* sCJD during life, should be quantified to gauge the impact of the criteria on surveillance and recorded national incidence figures.

The aims of this study were:

1. To assess the sensitivity and specificity of the 2017 diagnostic criteria
2. To quantify the impact on in-life case classification as *probable* sCJD
3. To explore the performance of the criteria in important sCJD subgroups

I hypothesised that:

1. The revised criteria are highly sensitive and specific for in-life diagnosis of *probable* sCJD
2. The revision has driven a significant rise in in-life classification as *probable* sCJD
3. Variations in the sensitivity of investigations and diagnostic criteria in aggregate would be present between c129 groups and sCJD subtypes
4. The diagnostic criteria would be less sensitive in individuals with atypical disease duration (both short and long duration) and atypical age (young and elderly individuals)

List of abbreviations

αS, alpha synuclein

Aβ, amyloid beta

AIE, autoimmune encephalitis

ANE, acute necrotizing encephalopathy

ANOVA, analysis of variance

APS, antiphospholipid syndrome

c-hGH, cadaveric hGH

c129, codon 129

CAA, cerebral amyloid angiopathy

CBD, corticobasal degeneration

CI, confidence interval

CJD, Creutzfeldt-Jakob disease

CNRMCJ – Cellule Nationale de Réference des maladies de Creutzfeldt-Jakob

COVID-19, coronavirus disease

CPD, camel prion disease

CSF, cerebrospinal fluid

CWD, chronic wasting disease

DLB, dementia with Lewy bodies

EEG, electroencephalography

EU, European Union

FET, Fisher's exact test

FFI, fatal familial insomnia

FSE, feline spongiform encephalopathy

FTD, frontotemporal dementia

gCJD, genetic Creutzfeldt-Jakob disease

GSS, Gerstmann-Straussler-Scheinker syndrome

hDM, human dura mater

HE, hepatic encephalopathy

hGH, human growth hormone

iCJD, iatrogenic Creutzfeldt-Jakob disease

IHC, immunohistochemistry

IPD, inherited prion disease

ISS, Istituto Superiore di Sanita

ITU, intensive therapy unit

KW, Kruskall Wallis (test)

MBM, meat-and-bone meal

MELAS, mitochondrial encephalomypathy, lactic acidosis, and stroke-like episodes

MM, methionine homozygous codon 129

MRI, magnetic resonance imaging

MSA, multiple systems atrophy

MV, methionine-valine heterozygous codon 129 of the prion protein gene

MWU, Mann Whitney U (test)

NCJDRSU, National CJD Research & Surveillance Unit

ND, neurodegenerative disease

NOS, not otherwise specified

NRZ-TSE, National Reference Centre for Transmissible Spongiform Encephalopathies

OM, olfactory mucosa

p-tau, phosphorylated tau

PD, Parkinson's disease

PMCA, protein misfolding cyclic amplification

PK, Proteinase-K

PRNP, prion protein gene

PrP, prion protein

PrP^{C}, cellular prion protein

PrP^{res}, protease-K resistant bands of prion protein

PrP^{Sc}, disease-associated misfolded prion protein

PSWC, periodic sharp wave complex

RCC, renal cell carcinoma

RT-QuIC, Real-Time Quaking-Induced Conversion

sCJD, sporadic Creutzfeldt-Jakob disease

SOD1, superoxide dismutase 1

SRM, specified risk material

t-tau, total tau

TSE, transmissible spongiform encephalopathy

UK, United Kingdom

USA, United States of America

vCJD, variant Creutzfeldt-Jakob disease

VV, valine homozygous at codon 129 of the prion protein gene

X^2, Chi squared test

Acknowledgements

This thesis was a substantial undertaking over a three-year period and would not have been possible without the contributions of the many individuals thanked all too concisely in this section. I am indebted to them all.

Firstly, this thesis is dedicated to the participating individuals in this study - the 501 cases of sCJD and the control group of 147 non-cases with alternative disorders - without whose invaluable contributions this study would have been impossible. Likewise, to all the patients and their families and friends whom I had the privilege to meet during my two-year experience as a CJD surveillance registrar. Their compassion and resilience amid devastating suffering will stay with me, as will their generosity in contributing to research in the hope that others will benefit. To think of such lofty motives while dealing with immense personal loss is an inspiration to witness.

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Fourthly, to my supervisor panel Dr Pal, Dr Green and Dr Summers, for their tremendous guidance and support throughout a busy but highly rewarding project. I was very fortunate to be mentored by such a responsive and well-informed team, and my journey was far less turbulent than others as a result. Likewise Dr Breen, who provided much guidance as an independent mentor, including around my career development goals beyond this specific project, and continues to do so.

Fifthly, to Dr Pal for his boundless enthusiasm, energy and creativity as a career mentor to me as a neurologist. His support led to me receiving my first-choice position as a neurology registrar in Edinburgh, and a major part of that was the experience I gained under his guidance in relation to CJD and beyond.

Sixthly, to Cat Graham, lead statistician with the Edinburgh Clinical Research Facility. I met with Cat several times throughout the planning and delivery of this study. She is a credit to the University: it is

a rare gift to make statistics easily accessible, and thanks to her talent and enthusiasm this project was much more straightforward to execute than it might have been.

Finally, to the various friends and family members who took an interest in this project, waded through the dense quagmire of prion disease jargon while reading my manuscripts, provided *pro bono* proofreading support and tolerated my mood swings, prolonged isolation bouts and unsolicited rants. I will be forever grateful for their support, and am delighted they found the topic as fascinating as I have.

List of tables, figures and clinical vignettes

Figures, tables and clinical vignettes are numbered according to chapter and their ordering within it. For example, figure 3.4 is chapter 3 figure 4.

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Vignettes

Chapter 5. Impact of Codon 129 and prion protein glycotype

Figures

Tables

Chapter 1. Introduction: Human prion diseases

This **Chapter** provides an overview of human prion diseases, summarising the different forms and their epidemiology, and outlines the importance of prion disease surveillance in the modern era. While the focus of this thesis is on sporadic Creutzfeldt-Jakob disease, the commonest form, this **Chapter** provides context for the wider goals of surveillance and why it remains a public health priority. Accurate in-life diagnosis is essential for these goals to be delivered effectively.

An edited version of this **Chapter** was published in Nature Reviews Neurology[1]:

> Watson, N. *et al.* The importance of ongoing international surveillance for Creutzfeldt–Jakob disease. *Nature Reviews Neurology* **17**, 362-379

Introduction

Creutzfeldt-Jakob disease (CJD) is a devastating and uniformly fatal human prion disease. The disease typically causes a rapidly-progressive neurological disorder characterised by cognitive and motor dysfunction, with survival typically measured in months[1-6]. Despite coordinated international efforts to conduct therapeutic trials, no disease-modifying interventions exist, and treatment is supportive[7-18].

The discovery that prion diseases are associated with the conversion of host-encoded cellular prion protein (PrP^{C}) to a misfolded form (PrP^{Sc}) by post-translational modification, independently of nucleic acid, became known as the *protein-only hypothesis*, with Stanley Prusiner receiving a Nobel Prize in 1997 for the characterization of a novel infectious agent[19-21]. Prion diseases are transmissible and multiple epidemics affecting humans and animals have emerged globally over the last 50 years[22-31]. A hallmark of prion disease transmission is the potential for prolonged incubation phases lasting several years, sometimes decades[32-36].

CJD is categorised into sporadic, inherited and acquired (comprising iatrogenic CJD (iCJD), variant CJD (vCJD), and Kuru) subtypes[1]. All forms are transmissible, posing serious public health challenges[25,33,37-39]. As a consequence of these, international surveillance has been operational for several decades[40-54]. Surveillance programmes characterised global epidemics of iCJD and vCJD in addition to greatly enhancing knowledge of sporadic and inherited forms. Rates of primary vCJD infection have dramatically declined since the global epidemic, which peaked in the year 2000, with eight cases identified globally since 2012[1,27,34,55,56] (**figure 1.1**).

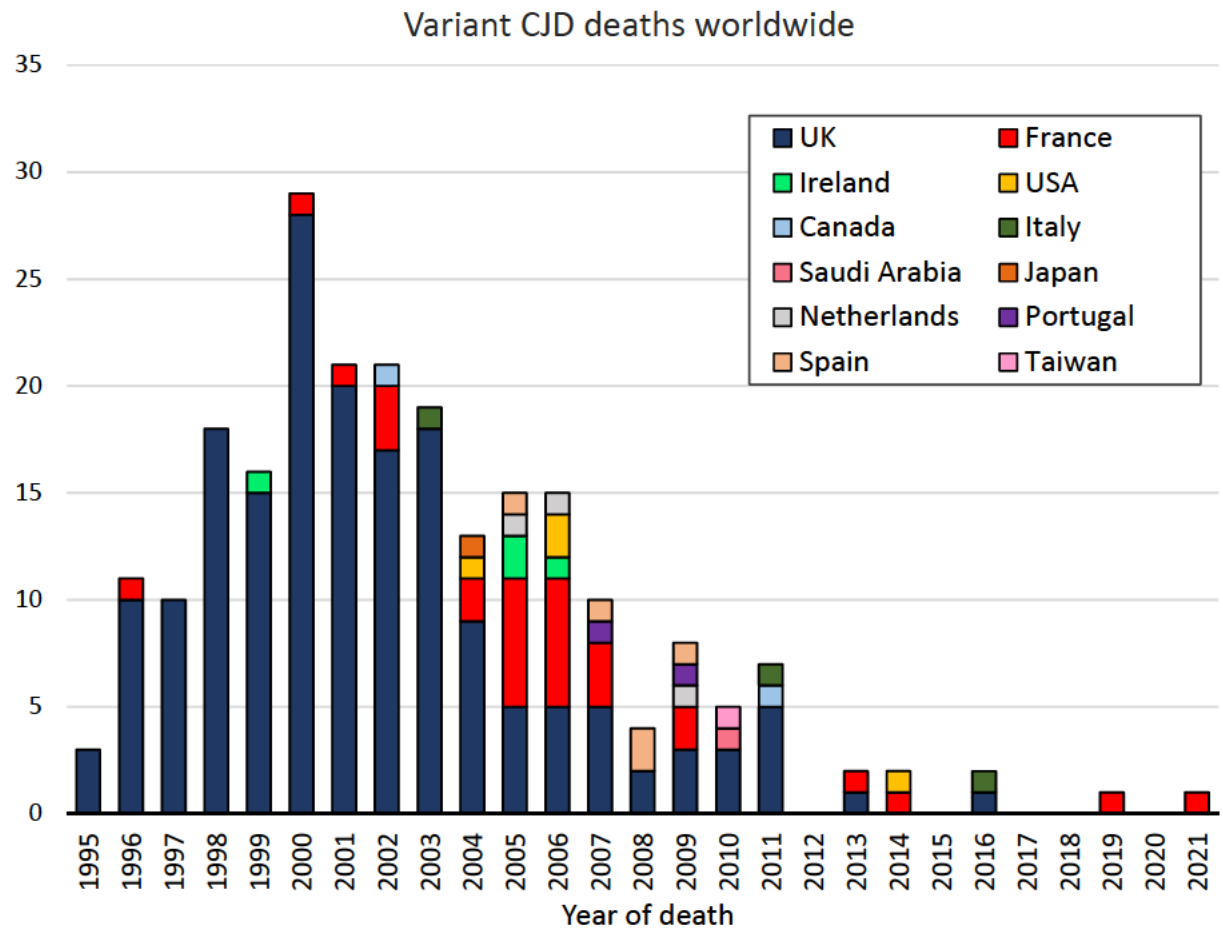

Figure 1.1. The global burden of variant CJD

Global variant CJD deaths are shown. The UK was the epicentre of the epidemic, followed by France.

Abbreviations. UK, United Kingdom. USA, United States of America.

As vCJD promoted the development and enhancement of CJD surveillance programmes, a question emerges over whether CJD surveillance is still necessary following the waning of the epidemic. In this introduction **Chapter** I discuss the importance of ongoing international surveillance. I provide an overview of prion disease surveillance and epidemiology, review the evidence for the potential for further cases of vCJD to arise with extensive incubation, asymptomatic carriage of vCJD-associated prion protein, secondary transmission of vCJD and other forms of CJD, occupational risks, and emerging prion diseases in animals with zoonotic potential. I also discuss the importance of surveillance programmes from the perspective of crucial research into this devastating disease. Finally, I provide a suggestion for a model surveillance programme and suggestions for how international surveillance should progress in the 21st century. The focus of this thesis is on CJD diagnosis: effective capacity for highly sensitive and specific in-life diagnosis is a foundational element of modern CJD surveillance.

Human prion protein

Human prion protein (PrP) is encoded by the prion protein gene (*PRNP*) on chromosome 20. The normal, non-disease associated form is termed cellular prion protein (PrP^{C}) (**figure 1.2**). Its function is incompletely understood. PrP^{C} may have roles in synaptic functioning as well as immunoregulation and resistance to apoptosis[57]. PrP^{C} is a 253 amino acid protein which is bound to the outer layer of the neuronal membrane by a glycophosphatidylinositol (GPI) anchor[58,59]. The structure is predominantly alpha helical[57]. The protein has N- and C-terminal domains; in the latter, two glycosylation sites exist.

The disease-associated form is termed PrP^{Sc} (the ^{Sc} is in reference to Scrapie, a prion disease affecting sheep and goats). While the primary amino acid structure does not differ from PrP^{C}, PrP^{Sc} has a predominantly beta sheet structure, in contrast to PrP^{C}[59,60]. Unlike PrP^{C}, PrP^{Sc} is resistant to digestion by proteinase K (PK). PrP^{Sc} interaction with PrP^{C} induces the auto-catalytic conversion of PrP^{C} to PrP^{Sc} (i.e. protein-induced misfolding) and the mechanism underlying this is incompletely understood, with PrP^{Sc} possibly acting as a template for the process, yielding a chain reaction leading to accumulation of PrP^{Sc} aggregates and progressive, irreversible neurodegeneration[57,59]. This process may be initiated in a sporadic fashion (e.g. by a spontaneous PrP^{C} misfolding event or somatic *PRNP* mutation)[21], by an inherited mutation in *PRNP*[61], or via acquired exposure to PrP^{Sc} from another organism (including from other species) through the diet or medical interventions[1,21,25,62].

PrP^{Sc} is classified according to a standard schema[63]. After treatment with PK, partially-digested fragments of PrP^{Sc}, termed PrP^{res} (denoting protease resistance), yield signature motility patterns on western blot, providing a 'molecular signature' for the disease[63]; the molecular weight of the unglycosylated band determines classification as type 1 (21kDa) or 2 (19kDa) PrP^{res} and the relative proportions of mono- and diglycosylated bands lead to subtyping A (predominant monoglycosylated band), B (diglycosylated) or A/B (equivalent ratio of both)[64] (**figure 1.3**). Different PrP^{res} types are seen in a variety of human prion diseases[64].

Genetic and biochemical characterisation of human prion diseases

A polymorphism at codon 129 (c129) of the *PRNP* significantly influences susceptibility towards, and clinical features of, human prion diseases[6,25,55,65]. Over 90% of East Asians are homozygous for methionine (MM genotype), whilst in populations of European descent, the proportion is approximately 40%, with ~50% heterozygous for methionine and valine (MV), and ~10% homozygous for valine (VV)[2,66,67].

PrP^{res} is classified biochemically into types 1 and 2[63,68], and further subtyped A, B or A/B as above[64]. Different PrP^{res} types are seen in a variety of human prion diseases[64] (**figure 1.3**). Types 1A and 2A are seen in various subtypes of sCJD, while type 2B is seen in variant CJD (vCJD)[69]. Cases of CJD

are classified based on the combination of c129 genotype and PrP isotype, providing biological correlates for clinical manifestations of disease[6,68] .

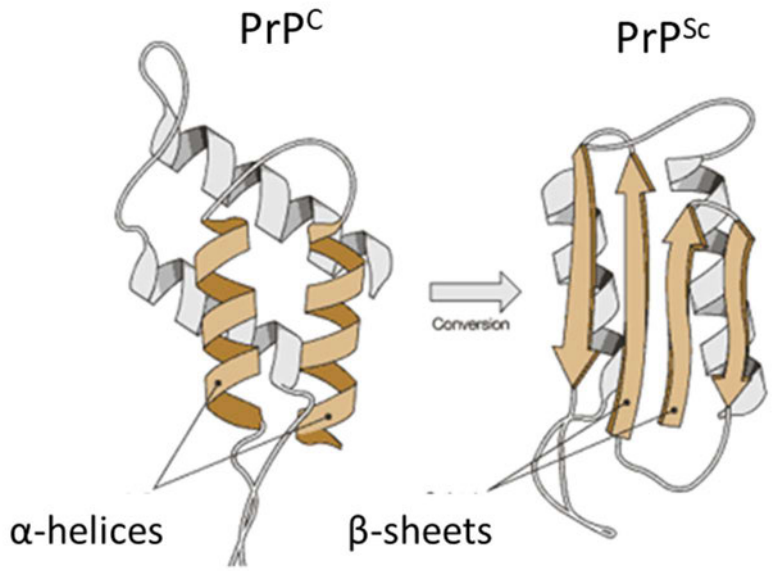

Figure 1.2. Prion protein

Prion protein (PrP) exists in normal states as cellular PrP (PrP^C), and has a predominantly alpha-helical structure. The disease-associated misfolded form associated with all prion diseases is termed PrP^{Sc} and has a predominantly beta sheet structure. PrP^{Sc} forms aggregates which are associated with rapid and irreversible neurodegeneration. PrP^{Sc} also promotes the misfolding of PrP^C, known as protein-induced misfolding. CJD is classified into different forms according to whether this process arises via a sporadic, genetic or acquired aetiology.

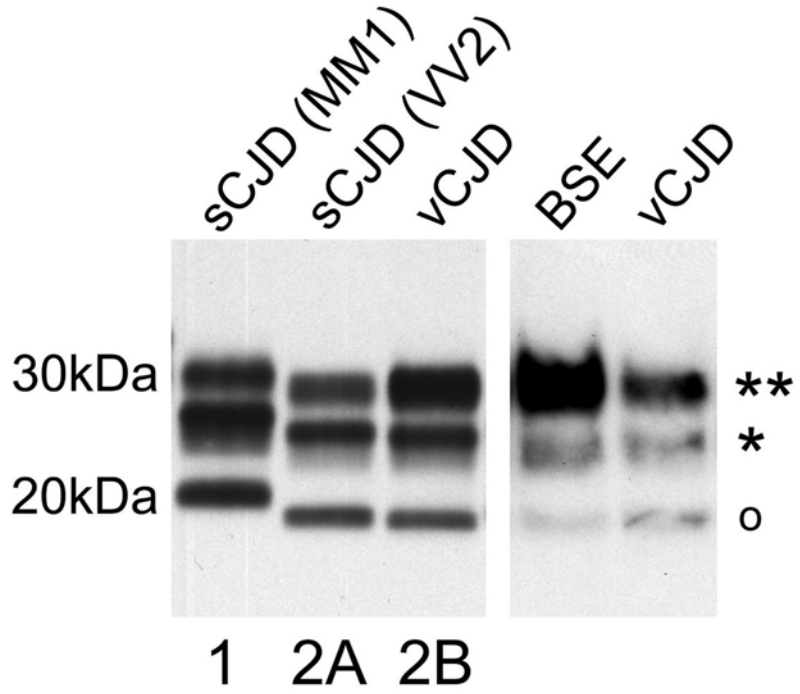

Figure 1.3. Protein biochemistry in CJD

Samples of brain tissue from individuals with Creutzfeldt–Jakob disease (CJD) are treated with proteinase K before western blotting to detect protease-resistant fragments of misfolded prion protein (PrP^{res}). PrP^{res} is classified according to the molecular weight of the unglycosylated fragment, which is 21 kDa in type 1 PrP^{res} and 19 kDa in type 2 PrP^{res}. Type 2 is further classified into type 2A and 2B (variant CJD (vCJD)). Type 2B, present in both vCJD and bovine spongiform encephalopathy (BSE), is characterized by a predominant diglycosylated band (*). Examples are shown from individuals with sporadic CJD (sCJD) MM1, sCJD VV2, vCJD and from a case with BSE.

MM, homozygous for methionine at codon 12 of the gene encoding prion protein; VV, homozygous for valine at codon 129 of the gene encoding prion protein.

Clinical subtypes of human prion diseases

Sporadic CJD

The majority (~85%) of cases of CJD arise sporadically (sporadic CJD, sCJD)[41,46,51,70]. Onset of sporadic CJD (sCJD) is most common between the ages of 60 and 70 years[71], although cases have been identified across a range of age groups[70]. sCJD has been detected in Europe[6,46,49,51,70,72], North America[41], Central America[52], South America[44,47], Africa[73-75], Asia[43,45,76-78] and Australasia[72,79,80] and has a global incidence of 1–2 per million, although reported incidence varies between nations and is influenced by the methods and extent of surveillance performed[81] (**figure 1.4**). Multiple low-income and middle-income countries have reported cases of sCJD[47,73-76,82,83]; as surveillance programmes are absent across much of the world, accurate epidemiological assessment is challenging[2].

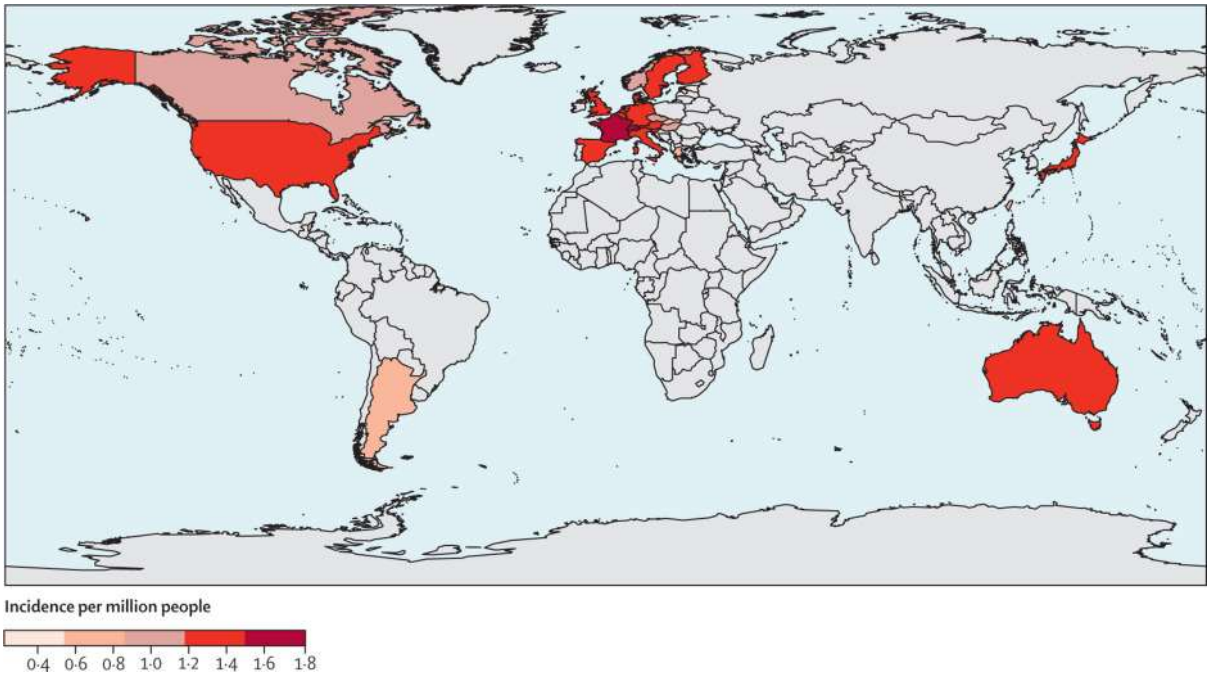

Figure 1.4. Worldwide incidence of sCJD

sCJD has been detected in many nations, but formal estimates of incidence are only available from countries with developed surveillance protrammes. For most of these the incidence is between 1-2 per million people, and this figure has risen over several decades of surveillance activity, most likely owing to a combination of factors including enhanced awareness and recognition of the disease, strengthened surveillance efforts, and the availability of sensitive diagnostic investigations in the modern era.

Image taken from Uttley *et al*, Lancet Infectious Diseases (2020)[81].

The aetiology of sCJD is unknown. The leading hypothesis is of an endogenous origin via a somatic mutation in *PRNP* or alternatively the spontaneous misfolding of PrP^{C} into PrP^{Sc} [84]. Some case-control studies have suggested exogenous risk factors, demonstrating an association with prior surgery[85,86] including non-neurosurgical and non-ophthalmological operations, as well as with blood product transfusion[87]. However, such case–control studies of sCJD have methodological limitations, including the potential for various forms of bias such as recall bias, differences in risk factor reporting (in contrast to healthy controls, most individuals with sCJD are unable to provide a direct history, leading to a reliance on relatives to provide information), and heterogeneity between studies in terms of the time windows of exposure assessed[86,87]. A detailed comparison of 18 studies is provided in a 2012 systematic review by de Pedro Cuesta *et al*[88]. Evidence indicates that c129 genotype has a substantial impact on susceptibility to sCJD: ~70% of individuals with sCJD have the MM genotype[6,89].

sCJD classically presents as a rapidly-progressive dementia with associated motor decline including ataxia, pyramidal, and extrapyramidal features, although presentations can include visual disturbance (most notably in the Heidenhain subtype[90]), neuropsychiatric manifestations, and stroke-like

symptoms[68]. Myoclonus is common. Individuals progress to an akinetic, mute, fully-dependent state. Diagnostic classification follows the International CJD Surveillance Network criteria[91] (**figure 1.5**). Typical clinical features are supported by investigation results including characteristic magnetic resonance imaging (MRI) changes in the basal ganglia and/or cortex (**figure 1.6**) [92,93], cerebrospinal fluid (CSF) biomarkers including 14-3-3 protein[94-96] and the real-time quaking-induced conversion (RT-QuIC), an aggregation assay with almost 100% specificity[97-99], and electroencephalogram (EEG) with characteristic periodic sharp wave complexes[100]. Neuropathology is characterised by vacuolation and spongiform change, neuronal loss, gliosis, and the immunohistochemical detection of PrP^{Sc} [6,101,102]. Median survival is five months from symptom onset[68]. Cases can be categorised by c129 genotype and PrP^{Sc} isotype into six subtypes (MM1, MM2, MV1, MV2, VV1, and VV2) with characteristic clinical and neuropathological phenotypes[6,92] (**figures 1.3 and 1.6**).

Figure 1.5. International CJD Surveillance Network Diagnostic criteria for sporadic Creutzfeldt-Jakob disease (CJD)

1. SPORADIC CJD (from January 2017)

1.1 DEFINITE:

Progressive neurological syndrome **AND**

Neuropathologically **or** immunocytochemically **or** biochemically confirmed

1.2 PROBABLE:

1.2.1 I + 2 of II and typical EEG*

OR

1.2.2 I + 2 of II and typical MRI brain scan**

OR

1.2.3 I + 2 of II and positive 14-3-3

OR

1.2.4 Progressive neurological syndrome and positive RT-QuIC in CSF or other tissues

1.3 POSSIBLE:

I + 2 of II + duration < 2 years

I Rapidly progressive cognitive impairment

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

III Typical EEG

IV High signal in caudate/putamen on MRI brain scan

* Generalised periodic complexes

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

Abbreviations:
CSF, cerebrospinal fluid. DWI, diffusion-weighted imaging. EEG, electroencephalography. FLAIR, fluid-attenuated inversion recovery. MRI, magnetic resonance imaging. RT-QuIC, real-time quaking-induced conversion.

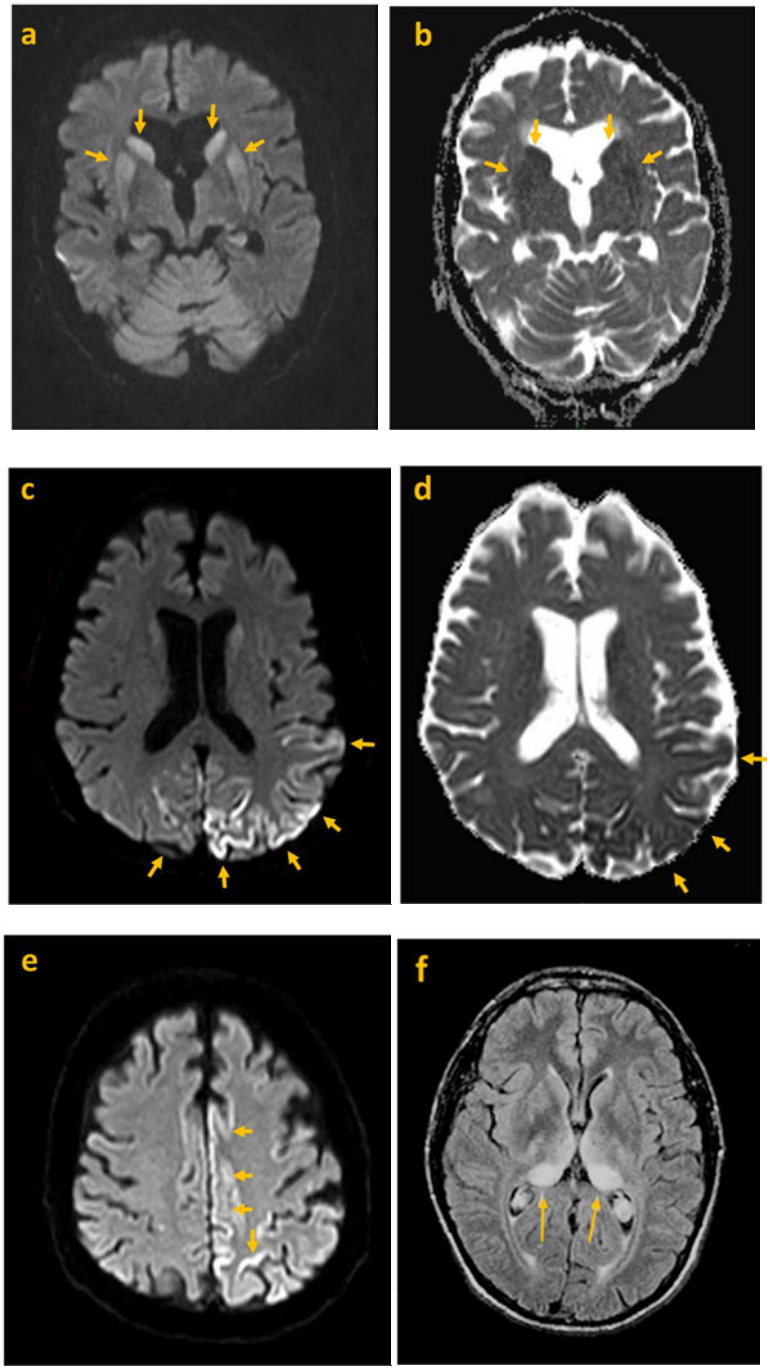

Figure 1.6. MRI in Creutzfeldt-Jakob disease (CJD)

Bilateral basal ganglia hyperintensities (arrowheads) are seen on diffusion weighted imaging (DWI; b=1000) in sporadic Creutzfeldt-Jakob disease (sCJD) (**a**), with corresponding decrease in apparent diffusion coefficient (ADC) values (b). Multifocal cortical ribboning seen a different individual with sCJD (c), with diffusion restriction confirmed on ADC (d); interhemispheric cortical ribboning is shown in the same individual in (e). Bilateral pulvinar hyperintensitities are visible in this fluid attenuated inversion recovery (FLAIR) example from an individual with variant CJD (**e**).

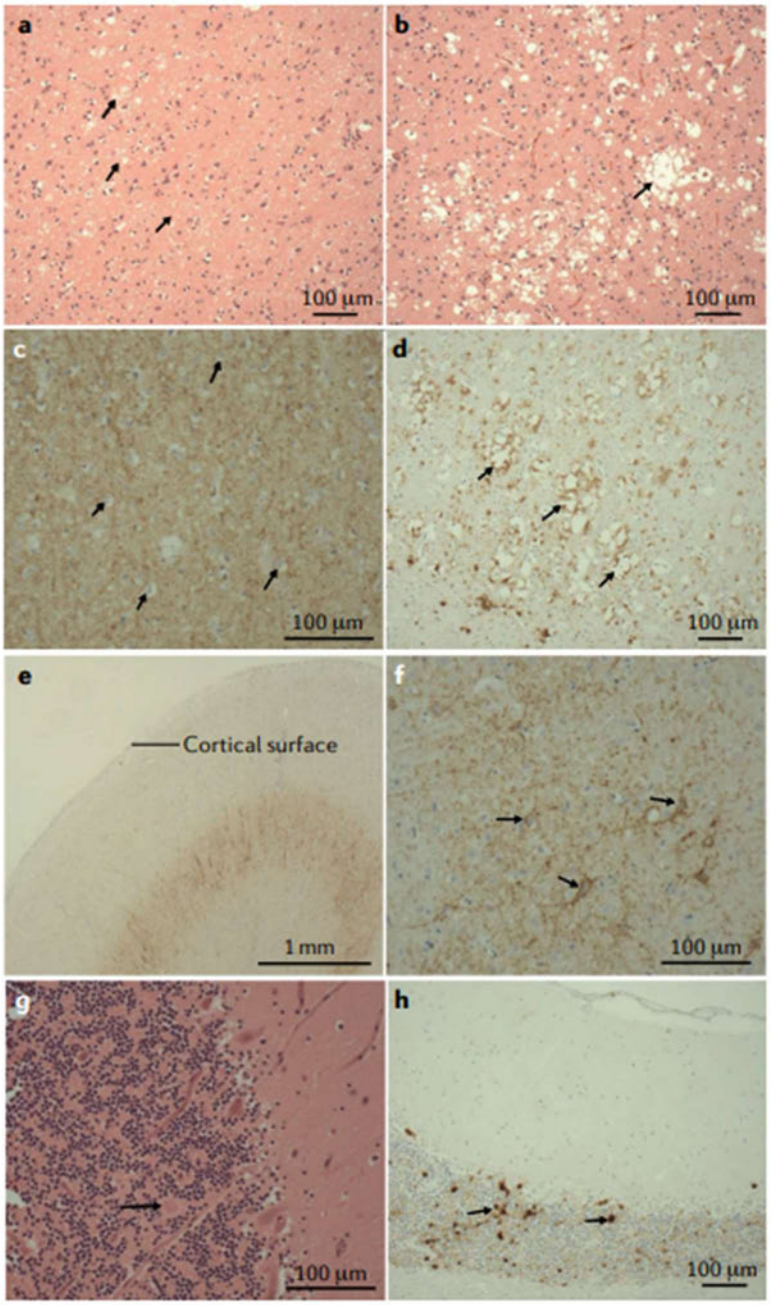

Figure 1.7: Neuropathological features of prion diseases.

Histologically, spongiform change is the characteristic feature of prion disorders. There are different patterns of spongiform change between sporadic, genetic and acquired forms of prion disease, and within sCJD codon 129 polymorphism and protein biochemistry influence the pattern of histological change. Fine cortical vacuolation is common in most prion disorders (**a**: frontal cortex H&E x100, arrows highlighting parenchymal fine vacuoles) and this can be accentuated with the fine vacuoles coalescing to form coarse vacuoles, a feature particularly prominent in sCJD MM2 (**b**: frontal cortex H&E x100, arrow highlighting large coalesced vacuoles).

Immunohistochemistry can be used to highlight abnormal prion protein, the antibody binding the abnormal prion protein being visualised as a brown stain. Patterns of immunohistochemical staining vary between sCJD polymorphisms. MM1 cases show a predominantly fine synaptic pattern (**c**: frontal cortex 12F10 x200, arrows indicating some neuronal nuclei within the cortical parenchyma) whereas in MM2 the staining is more coarse, accentuated around the coalesced vacuoles (**d**: frontal cortex 12F10 x100, representative examples highlighted by arrows). VV2 cases typically show a linear pattern of staining in the deeper cortex (**e**: frontal cortex 12F10 x20), with a perineuronal pattern (f: frontal cortex 12F10 x200). In sCJD MV2 a characteristic finding is of kuru-like plaques particularly in the cerebellar cortex (**g**: cerebellum H&E x400, arrow highlighting a kuru-like plaque within the granule cell layer of the cerebellar cortex). These are easily seen with immunohistochemistry (**h**: cerebellum 12F10 x100, arrows highlighting positively stained plaques).

Inherited prion diseases

In 10–15% of individuals with prion disease[4,61,103], the disease arises secondary to mutations in *PRNP* and is categorised as inherited prion disease (IPD)[61]. Over 50 prion disease-associated *PRNP* mutations have now been described[68,61]. Most of these mutations show autosomal dominant inheritance and high penetrance, although some individuals with IPD do not have a family history of the disease[4,61]. IPD is associated with a longer survival than sCJD, which means that individuals with IPD comprise a substantial proportion of the prevalent population[104] with attendant public health risks relating to transmission. Some cases of IPD can be difficult to distinguish clinically from sCJD and diagnostic *PRNP* genotyping is therefore often helpful[61]. It is currently unclear to what extent asymptomatic *PRNP* mutation carriers pose a public health risk during interventional procedures and/or blood/tissue donation, and risk-reduction measures are in place in many countries for these individuals[37,105].

Considerable phenotypic heterogeneity (for example, in age of onset, disease duration and clinical features) exists between different *PRNP* mutations as well as within families with the same mutation[61]. Clinical features of IPD can also mimic other common neurodegenerative disorders [61,106]; symptoms can resemble Alzheimer's disease[107], Huntington's disease[108-110], frontotemporal dementia[111,112] and spinocerebellar ataxia[109]. Cases associated with a variety of mutations, most commonly E200K, present with features mimicking sCJD, including rapid progression from symptom onset to death within 5 months[4], and given the phenotypical overlap these are termed genetic CJD (gCJD). Gerstmann-Straussler-Scheinker syndrome (GSS), most commonly due to the P102L mutation, causes a progressive ataxia with associated cognitive and sensory abnormalities and typically progresses more slowly than sCJD with death ~5 years from symptom onset[109]. GSS has characteristic neuropathological features (**figure 1.8**). Fatal familial insomnia (FFI) arising due to the D178N mutation with methionine at c129 on the affected allele (D178N-129M) presents with characteristic sleep disorders, autonomic disease, and gait disturbance, and is typically fatal within 2 years of onset[113]. By contrast, individuals with D178N who have valine at c129 on the affected allele (D178-129V) develop different clinical features which some have termed gCJD[114].

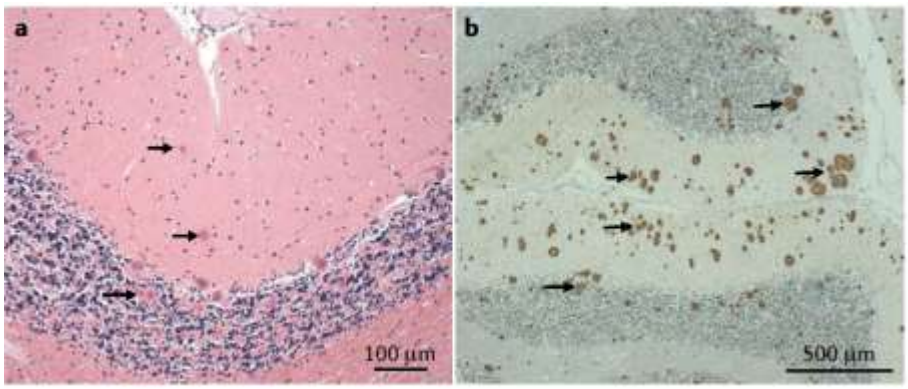

Figure 1.8. Inherited prion diseases: histology

Inherited prion diseases have variable histologic features. Some show unique patterns of pathology, such as the *PRNP* P102L-129M variant of GSS (most common haplotype of GSS) which shows multicentric plaques most numerous in the molecular layer and granule cell layer of the cerebellum (**a**: cerebellum x200 H&E, arrows highlighting plaques; **b**: cerebellum x40 12F10, arrows highlighting representative examples of plaques).

Surveillance systems have greatly enhanced understanding of the international distribution of IPD mutations[4,45,115,116], yielding valuable insights, including into possible founder effects, although many mutations are encountered across a wide range of ethnic groups. The E200K mutation is the commonest mutation internationally, but is particularly common in Slovakia where it accounts for over 65% of prion disease cases[4], as well as in Jewish people with Libyan ancestry, a population prevalent in Israel[117]. V210I is common in Italy, a nation with a relatively high rate of IPD[4]. FFI is encountered globally and has been encountered in Europe, Asia, Australia and the USA[2,4,116,118]; it has relatively high proportions in Spain and Germany[113]. T188K is common in China[116] though uncommon elsewhere, including Asian nations such as Japan[45] and Korea[119] where no individuals with the mutation were identified in published IPD case series . Ongoing global surveillance continues to identify novel mutations, often with small clusters within regions, such as a cohort with R208H in Sardinia suggestive of a founder population[120]. V180I and M232R are commonly encountered in Japanese cases[45] (41.2% and 15.3% of *PRNP* variants respectively), although these mutations might increase susceptibility to sCJD rather than being disease-causing mutations[61,121]. Some authors[121] have cited factors including the sCJD-like phenotype and the absence of relevant family history among affected individuals (present in only 2% of individuals with V180I and 0% with M232R[45]) as evidence that V180I and M232R might be polymorphisms rather than pathogenic mutations. IPD has been reported in in South America[122] and India[123]; however, a lack of structured surveillance programmes in these regions means that prevalence remains unclear.

Acquired prion diseases

Fewer than 5% of individuals diagnosed with CJD[19] have one of the acquired prion diseases, which consist of iatrogenic CJD (iCJD), variant CJD (vCJD), and Kuru[23,81].

Kuru

The Kuru epidemic was confined to the Fore people in Papua New Guinea, caused by ritualistic mortuary cannibalism[22,24,36,124]. The Kuru epidemic is thought to have originated in the 1920s, and peaked in the late 1950s[22,36]. The epidemic subsided following prohibition of cannibalism in the mid-1950s[24,36]. However, in some individuals with MV heterozygosity codon 129, the disease did not manifest until several decades after exposure[36]. This extraordinary finding demonstrates the risk of extensive incubation times in acquired prion diseases, of wider relevance to ongoing surveillance.

iCJD

iCJD was first described in 1974 in a patient who had undergone corneal transplantation using tissue obtained from a deceased donor prior to an autopsy revealing sCJD[125]. Subsequent cases were traced to a number of causes. The two principal aetiologies are cadaveric pituitary-derived human growth hormone (c-hGH)[126] and human dura mater (hDM) grafting[25]. Rarer cases have arisen secondary to cadaveric gonadotropins[53] and following exposure to contaminated neurosurgical instruments and intracerebral depth electrodes[68]. Iatrogenic transmission of vCJD through blood products (discussed later in this **Chapter**) is typically considered separately to iCJD due to major differences in disease biology and manifestations; vCJD is the only form of human prion disease which has been demonstrated to have been transmitted via blood products[127].

Despite effective control measures, including a transition to recombinant hormone synthesis in the mid-1980's[25,32,33], the introduction of enhanced disinfection and processing of hDM grafts in 1987[26,62], shifts in neurosurgical practice away from hDM graft usage[128], and sterilisation and quarantine of infected instruments[37,129], individuals with iCJD continue to be reported[26,70], some after exposures over 3-4 decades prior, highlighting the potential for extensive incubation[25,32,33].

The hDM-associated iCJD (dCJD) epidemic began in 1985 and peaked globally in 1997[25], although cases continue to be identified[26]. The epidemic arose largely from a source of production of Lyodura® in Germany and was focused in Japan, where high numbers of hDM-grafting procedures were performed and the largest number of dCJD cases (n=154 as of March 2018[26]) was encountered, although cases were also reported in other Asian nations, Europe, the USA, Australasia and South Africa[25].

c-hGH-associated iCJD (hGH-iCJD) was most frequently encountered in France[46], the UK[70] and USA[130], and less commonly in other European countries, New Zealand, Qatar and Brazil[25]. The epidemic began in 1984 and peaked globally in 1995[25]; individuals with hGH-iCJD with extensive incubation continue to be reported[33]. The majority of cases in France were methionine homozygous at c129, while in the UK valine homozygotes and methionine-valine heterozygotes were more common[25,131]. hGH-iCJD is believed to have originated from preparation of c-hGH from cadaveric sources likely to have had unknown sCJD. Different PrP^{Sc} strains from cadaveric sources may have contributed to differing susceptibility to hGH-iCJD among c129 groups in cases in the UK and France[33].

Important lessons arise from iCJD. The incubation rate varies, being shortest following exposure to contaminated neurosurgical instruments, and longest in cases associated with hDM and cadaveric hormones[25]. There is marked variability in incubation rates even for the same means of exposure. Lastly, c129 genotype influences incubation, with MV heterozygotes tending to manifest longer incubation periods[25]. These insights are relevant to vCJD where there are legitimate concerns of future cases emerging following extensive incubation, as well as having relevance concerning the potential for secondary transmission of all forms of CJD. Clinical manifestations of iCJD are also variable, with peripheral exposures frequently leading to cerebellar-onset presentations and central exposures leading to cognitive-onset manifestations[25]. Lastly, as a general historical point, medical and agricultural practices which unknowingly posed infection risks at the time have subsequently resulted in emergence of prion disease, sometimes many years later. Prompt identification of iCJD cases through surveillance was integral to the implementation of measures which contained the epidemics, illustrating the essential role for surveillance systems in managing novel prion disease epidemics.

vCJD

vCJD is the rarest form of human prion disease, and was first recognised between 1995 and 1996 following identification of a series of 10 cases in the UK with a novel prion disease characterised by atypical demographical, clinical, radiological and pathological features[132,133]. vCJD predominantly presents in the 3rd decade of life, with longer disease duration than sCJD (median 14 months)[134]. Early psychiatric symptoms including withdrawal, anxiety and dysphoria are common prior to development of cognitive impairment, ataxia and movement disorders[5,135]. Thalamic pain affects many in the early stages.[5]. The pulvinar sign on MRI is highly sensitive for vCJD in the appropriate context[136]. 14-3-3 protein, a biomarker with 75-90% sensitivity for sCJD, is only 50% sensitive in vCJD[137], and EEG does not typically show the characteristic periodic sharp wave complexes that are observed in sCJD[68] .

Neuropathology is characterised by florid plaques and extensive type 2B $PrP^{102,138}$ (**figure 1.3 and 1.9**).

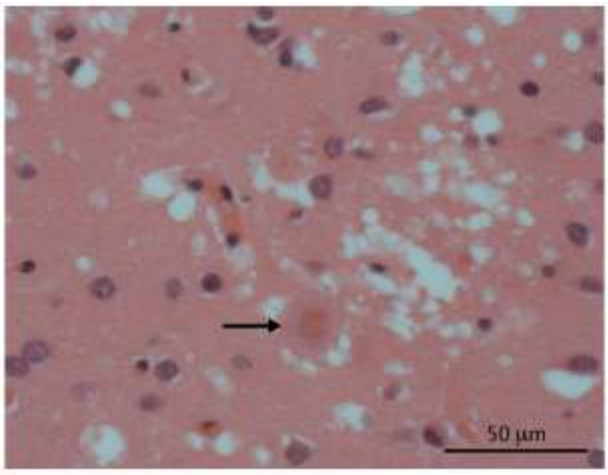

Figure 1.9. Variant CJD: histology

Frontal cortex section from an individual with variant CJD showing a pathognomonic florid plaque (arrow), a fibrillar amyloid plaque surrounded by vacuolation (H&E x400)

Surveillance programmes characterised the vCJD epidemic, which was focused primarily in the UK (n= 178 cases) and France (n= 29 cases). At the time of writing (Autumn 2022) there have been 233 cases identified from 12 nations spanning Europe, the USA, the Middle East and Asia (**figure 1.1**)[27]. vCJD was causally linked to the epizootic of bovine spongiform encephalopathy (BSE), a novel prion disease affecting cattle, , with consumption of contaminated beef products being the primary source of vCJD infection[23,139,140].

The BSE epizootic arose as a result of agricultural feeding practices that involved using ruminant-derived rendered meat-and-bone-meal (MBM) to feed cattle[30]. However, the origin of BSE itself remains unclear. The most popular hypothesis is that material from a scrapie-infected sheep entered the cattle feed supply, with the scrapie agent becoming altered on passage through cattle, rendering it highly infectious[30,141]. Alternative hypotheses, which have garnered less consensus, include the scrapie agent being altered by the rendering process, or the emergence of a novel transmissible spongiform encephalopathy (TSE) in the UK, possibly in cattle, which then entered the ruminant feed chain[30]. The epizootic peaked in 1992 in the UK with 37,280 new cases identified in cattle that year[142]. A host of stringent control measures were adopted[143-147]. In the UK these measures consisted of a ban on feeding ruminant-derived protein to ruminants (1988)[29]; surveillance, reporting and culling of BSE-infected animals (1988)[29]; a ban on feeding specified bovine offal (SBO) to humans (1989)[29]; and a ban on feeding SBO to all farmed animals (1990)[29]. The EU also introduced a ban on feeding mammalian protein to ruminants in 1994. These measures resulted in substantial suppression of BSE over the following years[30,142,148] (**figure 1.10**).

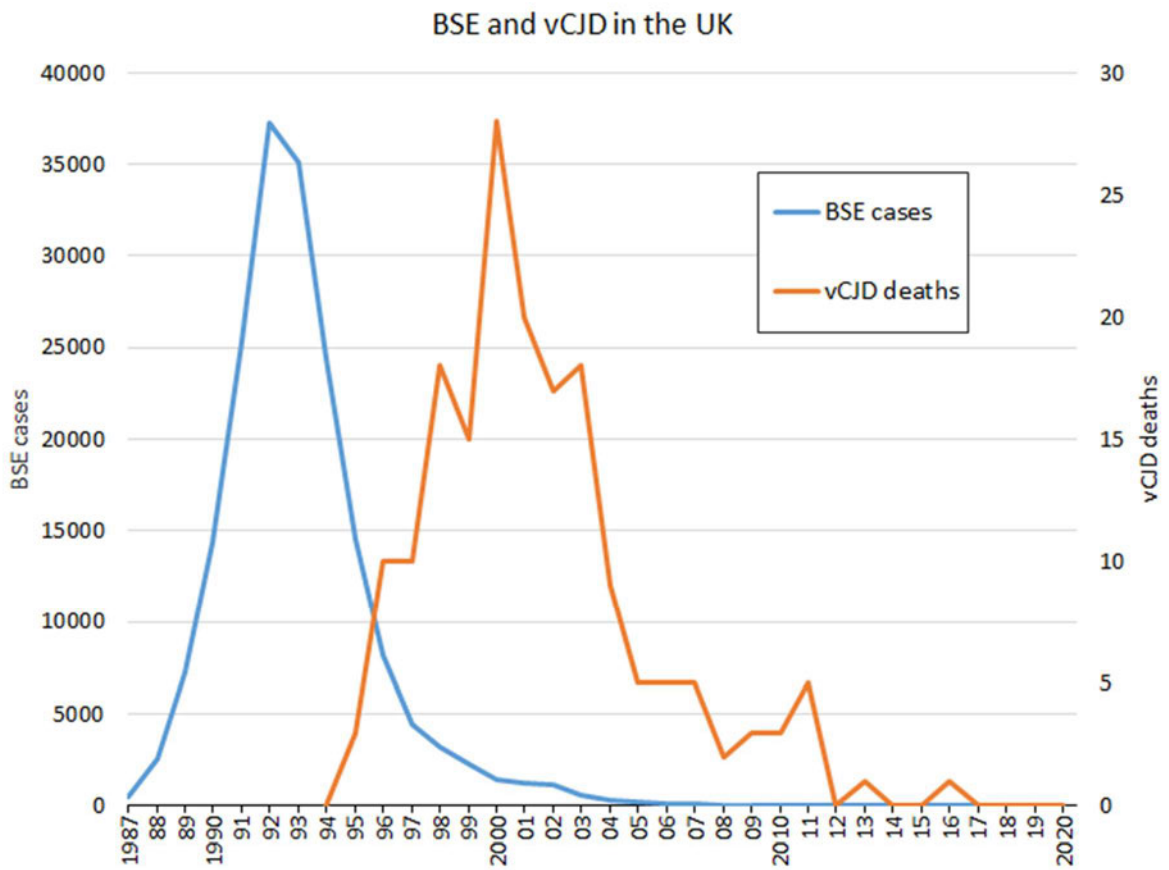

Figure 1.10. BSE & vCJD in the UK.

Effective controls led to the containment of the BSE epizootic in the UK in the 1990s. The vCJD epidemic followed by several years and its trajectory mirrored that of BSE.

Abbreviations. BSE, bovine spongiform encephalopathy. UK, United Kingdom. vCJD, variant Creutzfeldt-Jakob disease.

Following the identification of vCJD in 1996[132], plausibly linked to BSE[149], there was a worldwide ban on British beef exportation[150] along with a ban on feeding mammalian protein to farmed animals (UK 1996[30], subsequently adopted by the EU in 2001[30]), and a ban on human consumption of cattle over 30 months old in the UK[150,151]. The British beef exportation bans were lifted a number of years later (2006 in the EU, 2020 in the USA)[152-155]. In the UK, >184,000 cases of BSE were identified during the epizootic[142] and modelling estimates that the total number of cases was 1–3 million[30], with >4 million cattle slaughtered as part of the containment response[156]. Dietary exposure of the UK population to BSE is likely to have been widespread[157-160], and substantial population exposures occurred in nations importing British beef (both livestock and carcasses), in particular France[161], the largest importer. Smaller domestic BSE epizootics were reported in >20 other countries, posing an additional means of exposure to non-UK citizens[148]. The incidence of BSE is now negligible; occasional cases continue to be reported, but control measures are likely to prevent these animals from entering the human food chain[162,163]. The connection between BSE and vCJD was established through neuropathological studies

that provided evidence that BSE and vCJD are caused by the same strain of prion protein; further supportive evidence arose through epidemiological studies demonstrating that vCJD cases were linked to geographical areas with BSE exposure risk, and the observation of parallel epidemic trajectories several years apart (**figure 1.10)**[149,164-166].

Existing CJD Surveillance programmes[167] were upgraded and new programmes initiated in the early 1990s following concerns over the potential for BSE transmission to humans[46,48,51,168,169]. With identification of the first cases of vCJD in 1996[132,170], many additional nations developed and upgraded CJD surveillance programmes[171,172], leading to international cooperation; for example, via the European CJD Surveillance Network, which is funded by the European Centre for Disease Prevention and Control[169]. International collaboration characterised the global vCJD epidemic[27], which was centred in the UK. France experienced the second highest incidence of vCJD, which was thought to be largely a result of the consumption of beef imported from the UK[161,173]. Spain had the third highest number of vCJD cases (n=5), three of which arose in a region with substantial rates of BSE in farmed cattle[174]. vCJD was also detected in nations that did not import British beef or have substantial rates of BSE, including Japan and the USA, and arose in individuals who had either spent time in the UK or in nations that imported British beef (such as Saudi Arabia)[175]. In 2015 an individual in the USA was found to have vCJD; this individual had not spent time in the UK, and was thought to have been exposed before to emigration to the US, presumably while living in Kuwait or Russia, nations known to have imported British beef[56]. This case demonstrates that vCJD can arise in individuals across a broad geographical area.

CJD in the post-BSE era: ongoing public health concerns

Primary cases of vCJD with extended incubation

Until 2016, all vCJD cases who underwent *PRNP* sequencing were found to be homozygous for methionine (MM status) at c129[134]. A case of vCJD was identified in 2016 with heterozygosity for methionine and valine at codon 129. This case presented at age 35 with personality change, later developing cognitive impairment, ataxia and myoclonus and was described in a paper by Mok *et al*[55], published in 2017. MRI showed abnormal diffusion restriction in basal ganglia, compatible with sCJD; medial thalamic changes were present but pulvinar nuclei were normal. CSF 14-3-3 and RT-QuIC were negative. The total disease duration from symptom onset to death was 16 months. Post-mortem confirmed vCJD. This was the first autopsy-confirmed case with c129 MV genotype reported[55], although an MV heterozygote with clinical and radiological suspicion of vCJD was reported in 2009; this individual did not undergo autopsy[176].

The 2016 case added to growing concerns extrapolated from iCJD[25] and Kuru[36] that prion disease transmission may be associated with extensive incubation in individuals with non-MM c129 genotypes, adding weight to ongoing concerns of a 'second wave' of vCJD cases. Although the exact incubation period of primary vCJD is impossible to calculate given the unknown timing of causative dietary exposure in affected individuals, the UK BSE epizootic was first detected in 1986[177], peaked in 1992 and fell to negligible numbers by the mid 2000s[142]; human exposure to BSE is likely to have been minimal after 1996 following the stringent control measures described above[30,150]. All confirmed cases of vCJD prior to 2016 were seen in MM individuals, with global vCJD deaths peaking in 2000[27]. The 2016 case did not fulfil diagnostic criteria for vCJD during life, but did fulfil criteria for sCJD[55] . Hence, further non-MM cases may present with different demographic and clinical features to the classical vCJD phenotype, as in this case, analogous to the impact of c129 on sCJD phenotypes[6,178-181]. This poses challenges for surveillance. Future cases of vCJD may be difficult to distinguish from sCJD on clinical and imaging grounds, and previously-validated diagnostic criteria[135] (**figure 1.11)** may not accurately detect such cases or discriminate from sCJD.

DEFINITE

1A **and** neuropathological confirmation of vCJD[e].

PROBABLE

1. I **and** 4/5 of II **and** IIIA **and** IIIB
2. I and IV A[d]

POSSIBLE

I **and** 4/5 of II **and** IIIA

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

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

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

IV A Positive tonsil biopsy[d]

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a depression, anxiety, apathy, withdrawal, delusions.

b this includes both frank pain and/or dysaesthesia.

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

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

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

Figure 1.11. Diagnostic criteria for variant CJD (vCJD)

Whilst the 2016 case was significantly younger than typical for sCJD, age may lack discriminatory value for future cases: the paradigm that vCJD patients are typically younger than those with sCJD becomes increasingly flawed given that BSE peaked in 1992 and 22 years have passed since the peak of the first vCJD wave in 2000. If the final year for potential dietary BSE exposure is 1996, as is generally accepted, then by definition the youngest cases of primary vCJD in 2022 would be 26 years old, and must have been exposed early in life at the tail of the period of BSE exposure. The trends in vCJD incidence by birth cohort suggest the highest vCJD risk follows exposure to BSE during youth and teenage years, with lower susceptibility in the young[182]. No individuals born after 1990 have been diagnosed with vCJD in the UK[182]. The highest vCJD incidence was seen in cases born in the 1970-1980 and pre-1970 birth cohorts; these are estimated to have received the largest dietary exposure to BSE[160]. Individuals from these birth cohorts will now be in their 5th and 6th decades of life, substantially older than the individuals encountered in the first vCJD wave, and overlapping increasingly with the age distribution seen in sCJD.

It is worth acknowledging that while the scale of such a second-wave is uncertain, cases with prolonged incubation in Kuru[183] and iCJD[134] are uncommon, possibly reflecting in part the lower attack rate in individuals with less susceptible c129 genotypes (generally MV[25,33,36]). This factor, in combination with extensive incubation rates may lead to a more insidious incidence of new cases than encountered in the most susceptible groups. Newly diagnosed cases of vCJD may similarly arise several decades after initial exposure. Furthermore, prion diseases transmit more readily within species than between them[184,185], reflected in the significantly lower attack rate seen in vCJD than in iCJD and Kuru, the high rates of BSE in cattle versus the low number of human vCJD cases as a proportion of the exposed population, and the detection of several transfusion-mediated vCJD transmission events[39,127].However, even with a low attack rate, there is the potential for substantial further numbers of vCJD cases given the extensive BSE exposure in the UK (i.e. a substantial denominator), as well as exposures arising in other nations through domestic BSE cases and imports of cattle and beef products.

Peripheral tissue distribution of PrP^{Sc} in vCJD

In contrast to sCJD, PrP^{Sc} is widely distributed in the lymphoreticular system[186-188] in vCJD. This is associated with significant public health concerns over iatrogenic transmission through transfusion and procedures. There is evidence supporting the hypothesis that vCJD is acquired through the gut, with gradual spread to the CNS via the lymphoreticular system[189]. A series of immunohistochemical studies have demonstrated PrP^{Sc} deposition in appendicectomy samples from UK subjects (**table 1.1**), estimating a prevalence of 1/4200 in the population [190-192]. These studies raise questions: firstly, whether these individuals represent pre-clinical cases of vCJD, and secondly whether they pose a risk

to public health through secondary transmission via surgical/medical procedures, blood products and organ donation[190-192].

Table 1.1: UK studies of misfolded prion protein (PrP^{Sc}) carriage in resected lymphoreticular tissues

Study	Year	Cohort	Number of positive samples	Estimated prevalence
Appendix-1[190]	2004	Birth cohort 1961-85 Operations 1995-9 14,964 appendectomies 1,739 tonsillectomies	3 appendices	237/million (95% CI 49-692) 1/4,000
Appendix-2[191]	2013	Birth cohort 1941-85 Operations 2000-12 32,441 appendectomies	16 appendices	493/million (95% CI 269-1,596) 1/2,000
Appendix-3[192]	2020	Birth cohort 1: 1891-1965 Operations 1962-79 14,692 appendectomies	2 appendices (earliest removal 1977)	337/million (95% CI 110-787)
		Birth cohort 2: born after 1996 Operations 2000-14 14,824 appendectomies	5 appendices (none from patients born after 2000)	136/million (95% CI 16-492) Combined prevalence:1/4,200

In the initial study[190], published in 2004, Hilton *et al* obtained samples from 14,964 appendicectomies and 1,739 tonsillectomies and detected PrP^{Sc} in three appendices, giving an estimated prevalence of 1 in 4,000. In the second study, written by Gill *et al*[191] and published in 2013, PrP^{Sc} was detected in 16

appendices from a sample of 32,441 specimens, yielding an estimated prevalence of 1 in 2,000. The most recent appendicectomy study[192], published in 2020 by Gill *et al*, aimed to measure the prevalence of PrP^{Sc} in groups not thought to have been exposed to BSE and thus analysed samples from individuals who either had their appendix removed before 1980 (n=14,692), the estimated beginning of the BSE period, or were born after 1996 (n=14,824), the year from which the exposure risk is presumed to have reduced to a minimum. PrP^{Sc} was detectable in the appendices of participants in both groups and the estimated prevalence (1 in 4,200) was not significantly different from estimated in the 2013 study, which assessed a BSE-exposed population[191,192]. Of note, the samples containing PrP^{Sc} were obtained from patients who underwent appendicectomy, or were born, close to the margins of the presumed 'at risk' period, raising concerns that the time-window of exposure to BSE might have commenced in the late 1970's and continued beyond 1996[192], indicating a potentially longer period than previously recognised. BSE may have been in the human food chain from an earlier time point than had been presumed, and likewise, cases may have continued to arise after 1996, and food products prepared pre-1996 may have remained in the food supply.

Some cautionary notes are necessary. Firstly, the confidence intervals on the prevalence estimates are wide, making it difficult to estimate the extent of a possible epidemic relating to ongoing transmission. Secondly, a study of 63,007 tonsil specimens from the UK obtained between 2004 and 2008 did not find PrP^{Sc} in any specimens[193]. Given that tonsil biopsy is highly sensitive and specific in vCJD[194], it seems surprising that it would not be detected in any of these studies; however, it may be that tonsillar involvement is a later stage in subclinical disease development. In addition most individuals undergoing tonsillectomy are young, and samples may be resected before disease has reached this tissue in subclinical vCJD; in this study, 50,254 samples (79.7%) were obtained from individuals born after 1986; the oldest members of this cohort would have been 22 years of age in 2008, the conclusion of the study period, so samples might have been resected before disease the disease reached this tissue.

Finally the presence of PrP^{Sc} in the appendix is of unknown significance; it may be the case that its presence in the appendix reflects dietary exposure, with only a small proportion of those exposed developing vCJD, perhaps influenced by age, gut maturity, c129,and the amount of BSE-contaminated material ingested over time. The factors that influence BSE transmission in humans are not well known and remain a subject of debate, particularly as members of the same households as vCJD patients did not develop the disease despite a common environment and shared meals, with the exception of a mother and son in Spain, both from a region with the highest vCJD incidence in the country and suspected to have had dietary exposure to high-risk material[174]. The transmissibility risk in individuals with appendicular PrP^{Sc} is unknown. As studies were irreversibly anonymised, no subjects were notified as being at-risk of CJD, a status carrying numerous public health implications.

Blood products and transplantation

Three cases of neuropathologically- and biochemically-confirmed vCJD related to transfusion of non-leucodepleted red cells have been identified in the UK[39,127,140,195,196]. Another previously-transfused individual who died of a non-neurological illness was described in 2004 with PrP^{Sc} in the spleen but not in the brain, suggesting subclinical infection via a donor known to have developed vCJD[197]. All blood products were obtained from donors before their disease clinically manifested, and were transfused into recipients prior to the introduction of leucodepletion for all blood products in 1999[39,198]; no transfusion-transmitted cases have been described since this measure. Splenic PrP^{Sc} was identified at autopsy in an individual with haemophilia who was asymptomatic for neurological disease, thought to have been transmitted through pooled plasma products known to have included a vCJD-infected donor[199]. Animal studies demonstrate transmissibility vCJD and BSE through blood product transfusion[200,201]. Spleen inoculum from the aforementioned individual[197] with PrP^{Sc} in spleen but not brain following red-cell transfusion from an affected donor was shown to transmit vCJD in mice, giving further evidence that pre-clinical vCJD cases harbour transmission potential[202].

Epidemiological modelling studies have provided a wide range of estimates of the extent and duration of a secondary transmission epidemic in the UK[203-206]. These addressed the potential impact of variables including incubation time, infectivity, codon 129 genotype, probability of developing subclinical carrier status, and effectiveness of interventions such as leucodepletion and donation restrictions. One study concluded that a self-sustaining secondary epidemic was possible, though biologically implausible[203]. A detailed review of these studies is beyond the scope of this **Chapter**, but it is worth noting that the most recent modelling study (published in 2019) predicted lower transfusion-associated vCJD case numbers than previous models[205]. A particular challenge is estimating the number of sub-clinical infections generated through transfusion, and the risk of ongoing transmission posed by these cases.

International studies matching donor and recipient pairs have not demonstrated transfusion-mediated transmission of other forms of CJD [127,207,208]. Case-control studies have provided conflicting evidence over whether blood products pose an increased risk of CJD[209,210], though an Italian study using a prolonged look-back period suggested an association with transfusion [87]. The prolonged incubation of prion diseases, as well as difficulties in working with records and the potential for various forms of bias, pose challenges for epidemiological studies. Transfusion recipients may die before manifesting CJD. Alternatively the manifestations may be obscured by the comorbid illnesses which necessitated transfusion. Two recipients of UK-derived plasma products died of autopsy confirmed MM1-subtype sCJD[211] . This study raises concerns over plasma as a means of transmitting sCJD, but its findings did not conclusively demonstrate causality, and ongoing observational studies are needed.

Measures to mitigate the risk of blood product-associated CJD[105,212] include leucoreduction, donor bans for those deemed at-risk of CJD and previous transfusion recipients, and restrictions on transfusing plasma to those born after 1996 with UK-derived products; the latter have recently been lifted following a revised risk assessment that demonstrated a low probability of further vCJD deaths arising from plasma exposure[212]. In many nations, people who lived in BSE-exposed regions during the epizootic cannot donate blood, although restrictions in the USA were partially lifted in 2020, partly due to limitations in the blood supply related to COVID-19[105]. There is currently no validated means of testing donors for preclinical vCJD. Such an assay would be invaluable. Protein misfolding cyclic amplification (PMCA) is a highly sensitive and specific diagnostic test for vCJD [213,214] and can identify pre-clinical vCJD in blood[214], although this work needs larger scale replication in order for PMCA to be validated for screening purposes. There are practical limitations, as PMCA amplifies PrP^{Sc} generating an infectious agent with significant biohazard potential[215-217], and results require time to process which may be in excess of the shelf-life of blood products, limiting utility as a screening test. A successful screening test would generate challenges, including the ethics around how to manage the task of informing donors regarding possible pre-clinical vCJD status with uncertain risk of developing this lethal disease.

Potential for laboratory transmission of vCJD

Strict occupational health measures govern all clinical and research activities concerning prion disease tissue samples[218]. Three laboratory workers who had worked with prion disease samples have died from vCJD. In one case, a clear history of a penetrating skin injury from an instrument used to handle BSE material was present 7.5 years prior[34] . This individual had a typical prodromal thalamic pain syndrome and neuropsychiatric features, as well as a typical MRI with pulvinar and dorsomedial thalamic hyperintensities. RT-QuIC was negative, PMCA of plasma and CSF was positive, and post-mortem provided definitive diagnosis of vCJD. Codon 129 genotype was methionine homozygous. The interval between injury and clinical onset was consistent with the incubation period seen in transfusion-transmitted vCJD cases, suggesting that this injury was the vector for disease transmission, as opposed to primary infection through the diet; the latter remains possible, though the overwhelming probability is that this was secondary vCJD transmitted via an occupational accident.

An Italian lab worker who had worked with BSE and vCJD brain material died of vCJD in 2016 (personal communication with Maurizio Pocchiari). By contrast, this subject had no history of accidental injury. There is evidence that scrapie can be transmitted through scarification of the skin, raising concerns for peripheral transmission ofvCJD[34]. A second French individual who had previously worked in a prion research lab developed vCJD[219-221] and died in 2021 (Jean-Philippe Brandel, personal communication).

The potential for occupational exposure in individuals who have handled vCJD and BSE materials remains an important means of transmission in addition to primary and secondary iatrogenic cases.

Peripheral pathogenesis of other forms of CJD and iatrogenic transmission

There is increasing evidence of peripheral pathogenesis in sporadic CJD[222,223]. Studies have now reported PrP^{Sc} in retinal and optic nerve tissues[224,225] with concentrations lower than those found in the brain, and PrP^{Sc} has also been detected in intracranial portions of the vagus nerve, although extracranial portions have not been tested[226]. The advent of amplification techniques including RT-QuIC have allowed demonstration of PrP^{Sc} in peripheral tissues at concentrations below the threshold for detection by the traditional methods of immunohistochemistry and western blot analysis[227,228]. RT-QuIC is now a validated test for diagnosis of sCJD from CSF and olfactory mucosal brushings[97]. Skin has been demonstrated to carry PrP^{Sc} in sporadic and familial CJD cases using RT-QuIC, with levels increasing with disease duration[228]. Whether such levels of PrP^{Sc} are sufficient for transmission via surgical instruments is unclear. There is also evidence that bone marrow contains PrP^{Sc} in sCJD, with transmissibility to transgenic mice expressing human *PRNP*[229], adding to concerns around transfusion-mediated transmission.

Some case-control studies have demonstrated an association with surgery in sCJD patients, including abdominal procedures, although these studies face similar challenges to those used to study blood transfusion, including the need for prolonged look-back periods and the potential for bias[85,86,230]. Peripheral injection of c-hGH has been associated with substantially longer incubation of iCJD (mean 17 years) than neurosurgical transmission (1.4 years for neurosurgical instruments, 1.3 years for stereotactic EEG needles and 12 years for hDM grafting) [33] , and hence hypothetical iatrogenic cases arising from non-CNS surgical and medical procedures may be associated with decades-long incubation. Compared with blood products, it is more challenging to trace patients who have been exposed to shared instruments. PMCA studies have demonstrated transmissibility of vCJD prions in mice via intracerebral inoculation with steel wires following conventional prion decontamination measures (including the use of sodium hypochlorite, sodium hydroxide and steam sterilization at 134°C) [231], raising concerns over the effectiveness of these procedures in sterilising medical equipment. Infectivity has not been found in urine of sCJD patients [232] but prion protein has been detected in urine from patients with vCJD using PMCA [233]. One study has raised concern about the usage of human gonadotrophins sourced from the urine of donors [234], but there is no evidence that recipients of urine-derived hormones are at increased risk of developing CJD. It is not known whether PrP^{Sc} can be detected in the urine pre-clinically in vCJD cases, and whether these quantities of PrP^{Sc} can be transmitted.

Studies assessing occupational risk have suggested a possible increased incidence of sCJD among healthcare workers compared with the general population[230,235,236], although other studies have produced conflicting results[230,235,237]. A recently-published retrospective cohort study identified an increased incidence among medical professionals in Germany[238], with the risk highest among surgeons, raising concern over the potential for significant occupational exposures with potentially decades-long incubation. Caution is advised in the interpretation of these studies given the potential for methodological bias, for example with heightened recognition of sCJD in medical professionals, the availability of more complete epidemiological survey data on occupation in this group, and the inherent limitations of case–control studies, such as the potential for recall bias, the effects of confounding variables, and the inability to conclusively demonstrate a causal relationship[239], should also be considered.

The rising incidence of sCJD

sCJD has been identified in many geographical regions[81]. In nations with sophisticated surveillance programs including the UK[70], France[81], Germany[51], Australia[80], Canada[240] and the USA[41], the numbers of reported cases of sCJD have increased over the last 30 years[79,81,134,241]. This is likely due to a combination of factors, including better case ascertainment with heightened awareness, recognition of atypical phenotypes, the availability of more sensitive tests[242] and revised diagnostic criteria[91,243], population growth and ageing, and the impact of improved population survival seen due to advances in medicine and public health. It is also theoretically possible that currently unknown exogenous risk factors might also account for some of the observed increase in cases of sCJD. However, with the exception of iatrogenic transmission there is no evidence of person-to-person transmission of sporadic or variant CJD to family members, including vertically[244], to carers or to healthcare workers. Geographical clustering of sCJD has been described[245] but studies do not support the existence of local point-source or person-to-person transmission of the disease. Heightened local surveillance following reported cases may be one factor underlying spatial clustering[246].

The rising incidence underscores the importance of the public health implications described in this **Chapter**. National surveillance allows cooperation with national blood services and public health agencies, necessary for tracing exposed contacts and mitigating risks. Large scale look-back studies are performed in multiple nations in conjunction with surveillance[127,207,208] and are essential in order to identify any possible cases, as well as managing risk in exposed recipients. The rising incidence requires careful longitudinal evaluation in a manner that can only be achieved by systematic surveillance programmes.

Emerging Diseases with zoonotic potential

Chronic Wasting Disease and Camel Prion Disease

Two emerging TSEs that pose uncertain risk to humans, chronic wasting disease (CWD) and camel prion disease (CPD), are important from the perspective of ongoing surveillance.

Chronic wasting disease

CWD of cervids was discovered in a captive deer in Colorado, USA in 1969 and classified as a spongiform encephalopathy on histological examination of brain tissue in 1978[247]. Eight cervid species are susceptible to CWD; the disease was detected in five of these in natural conditions and the remaining three are susceptible to experimental transmission[247]. CWD has now been detected in 26 states in the USA, 3 Canadian provinces, as well as Norway, Finland, Sweden and South Korea, the latter being due to transport of infected live animals[248,249]. A case identified in a wild reindeer in Norway in 2020 highlights the ongoing risk of CWD transmission[250]. In contrast to BSE, CWD emerges in free-ranging cervids, although the impact of animal husbandry, feeding and agricultural practices contributes to disease propagation[248]. The prion protein is easily shed into the environment through various secretions and excretions including saliva[251], urine[252] and faeces[253,254] and can survive in soil for prolonged periods[255,256], resistant to environmental challenges such as freeze-thaw cycles[254,257]. CWD transmitted is horizontally between living animals[251] and through environmental exposure to PrP^{sc}[256]. Carcasses are a vector and new animals entering a previously inhabited field can contract the disease, possibly through consumption of plants growing at the site of carcasses, as well as through the soil[255]. PrP^{Sc} is detectable in the flesh of infected animals[258], raising concern that dietary transmission to humans could be possible.

There have been no proven CWD-associated human prion disease cases. Evidence indicates that CWD can be transmitted via intracerebral inoculation to multiple non-cervid species[259, 260, 261, 262, 263, 264], and via oral or intracerebral exposure to squirrel monkeys[265]. However, evidence indicates that CWD is not transmissible by either route to cynomolgus macaques, which are a primate species genetically closer to humans than squirrel monkeys[266]. Humanised transgenic mice expressing human *PRNP* are resistant to CWD infection, whereas transgenic mice expressing cervid *PRNP* are not[267]. However, in one study, CWD brain isolates were able to induce misfolding of human PrP^{C} in vitro[268]. Transgenic mice expressing human *PRNP* modified to express a sequence overlapping with that of elk at residues 165-175 of prion protein are susceptible to CWD inoculation, shedding light into structural elements of the species barrier[269].

A cohort of people exposed to CWD-contaminated products in 2005 was followed up with no evidence of prion disease or other NDs emerging at the time of publication in 2014[270]. Given the

decades-long incubation times seen in various forms of acquired CJD, surveillance must continue to ensure detection of any possible CWD-associated human prion disease cases. It is unknown how these might manifest, and distinction from other forms of CJD may be challenging. vCJD was first identified when the UK surveillance system detected a novel prion disease with previously-unseen clinical, radiological, biochemical and neuropathological features[132]. Therefore, large-scale national surveillance programmes are necessary to identify novel diseases that might be linked to CWD, as well as to provide registry data for case–control studies on exposure risks, to enable follow-up of exposed individuals through cohort registries, and to facilitate international liaison with veterinary surveillance programmes.

There are valid reasons for concern. Human exposure to CWD is highly likely. A survey found that 67.4% of Americans consumed venison, much of it obtained from the wild[271]. Hunting is a popular pastime (18.5% of respondents). Without large-scale testing, the proportion of animals infected with CWD is unknown; estimates vary widely[272,273] by region, species, and between captive vs wild animals, with one study demonstrating a prevalence of 35.4% among white-tailed deer in Wyoming[274]. At present, validated means of screening slaughtered animals for CWD to ensure safe dietary consumption are not widely employed and current methods are highly time-consuming[248]. Furthermore, prions can adhere to steel surfaces[275-277], and instruments used for slaughter and butchery of cervids are frequently not subjected to validated decontamination measures[248]. Finally, concerns exist over the potential for altered transmissibility after passage through intermediate host species. This has been demonstrated in CWD wherein passage through ferrets extends the range of susceptible host species[278], as well as in transgenic mice expressing human or porcine PrP^{C} that display increased susceptibility to sheep-passaged BSE compared with non-sheep-passaged BSE[279,280]. In summary, although the zoonotic potential of CWD is unclear, the risk of human exposure is substantial.

Camel prion disease

A novel prion disease was detected in three symptomatic dromedary camels in Algeria in 2018, termed camel prion disease (CPD) [281]. The PrP^{res} signature did not match that seen in scrapie or BSE. Several concerns arose from this discovery. The disease was presumed to have arisen naturally, with no MBM used for years, no BSE in local cattle, and no naturally-arising scrapie seen in Algeria. Camels were the first non-ruminant species other than humans to naturally manifest prion disease, extending the spectrum of susceptible animals. PrP^{Sc} was detectable in peripheral lymphoid tissues, raising concern for horizontal transmission. Finally as with CWD, there is a possibility (as yet not confirmed) that the causative agent may undergo alteration on passage through an intermediate host, enhancing transmissibility.

A subsequent case of CPD was identified in 2019 in Tunisia[282]. Concerns now exist over the prevalence of this previously-unrecognised TSE, but recognition of cases will likely increase following heightened awareness[283]. The global dromedary population is in the millions, with large populations in Africa and the middle-East, as well as Australia[284,285]. The potential for human exposure is significant given widespread usage of camels for meat, milk and commerce, though the zoonotic potential is unknown, and transmission studies are necessary to further evaluate this. Significant resource constraints as well as geopolitical instability in regions affected pose major challenges to these efforts, many of which do not currently possess national CJD surveillance programmes.

Wider benefits of surveillance and development goals

Novel diagnostic strategies

The diagnosis of CJD during life has greatly improved over several decades of surveillance thanks to advances in MRI[92] and CSF biomarkers[97,98,286]. The current diagnostic criteria for sCJD were revised in 2017 (**figure 1.5**) to include multifocal cortical ribboning on MRI and a positive RT-QuIC assay. In a provisional, single-centre study these have been shown to be have a sensitivity of 97% and specificity of 99% and to have contributed to rising incidence figures through heightened case ascertainment, enhancing surveillance[91].

The merits of prompt and accurate diagnosis during life are multiple. Firstly the potential for swift public health measures to be enacted, such as quarantining of potentially contaminated blood products and medical instruments. Secondly the cessation of ineffective and potentially harmful empirical therapies such as immunosuppression, and avoidance of life-prolonging therapies which do not enhance quality of life[18] in favour of a transition toward palliative care, with transfer to an appropriate facility. Thirdly, to rapidly rule out the possibility of CJD in cases with a mimicking diagnosis such as autoimmune encephalitis, leading to appropriate treatment of a potentially-reversible condition which may be life-saving[287]. Finally, rapid diagnosis is essential for the recruitment of participating individuals into clinical trials.

Clinical Trials

CJD is rare, and apart from in individuals known to be at-risk due to prior exposures or those with inherited mutations, new cases arise in an unpredictable manner with no geographical focus. There is often a considerable latency to reaching the diagnosis[288], and survival duration following diagnosis is typically short. Several of these factors have limited clinical trials in CJD: sample sizes are small, and the window of time available to enrol patients in studies and to assess the benefit of interventions is

limited. No interventions have been demonstrated to improve outcomes, and few randomised-controlled trials have been conducted[11,12,14-16,289,290]. However, multi-national clinical trials in CJD are feasible[289], and surveillance programmes are instrumental for rapid diagnoses and coordinating enrolment of patients in trials, with scope for multinational collaboration to bolster sample sizes. Rapid diagnosis is particularly essential, since the benefit of therapeutic agents shown to work in preclinical studies may be lost when irreversible neurodegeneration has taken place.

Recommendations for ongoing international CJD surveillance

CJD is likely under-recognised in nations which lack sophisticated surveillance systems. Several reports from low- and middle-income nations cite challenges around CJD diagnosis including financial constraints, lack of testing facilities, underdeveloped healthcare infrastructure, low numbers of neurologists, and regional disparities between rural and urban centres[44,73,82,83,291]. Geopolitical instability, the burden of COVID-19 and other communicable diseases will pose further challenges to ascertainment of CJD in the developing world. Significant public health risks arise as a consequence. Nations with established systems should provide support to those developing programmes. Existing systems range from low-fidelity services, such as review of death certificates (known to have limitations and underestimate CJD incidence[41]), through to high-fidelity systems including mandatory reporting, direct clinical assessment, and integrated specialist neuroradiology, genetic, biochemistry and neuropathology facilities aligned closely to public health services[70,292]. International collaboration enables epidemiological comparison between nations as well as enhanced recognition of atypical forms of prion disease.

A model surveillance system is shown (**figure 1.12**). Clinical assessment of cases can include case record review, liaison between local neurologists and national centres, and direct assessment of cases by national specialists, in-person or through telehealth[293,294] , of particular utility during the COVID-19 pandemic[295]. Surveillance centres are well-placed for integration with biomarker laboratories for rapid diagnostic services as well as research into newer-generation non-invasive biochemical and imaging biomarkers [228,296] for early diagnosis and screening,[297,298], and instrument decontamination testing. [299]. Crucially, therapeutic trials may one day offer hope to people affected by this devastating group of diseases[13].

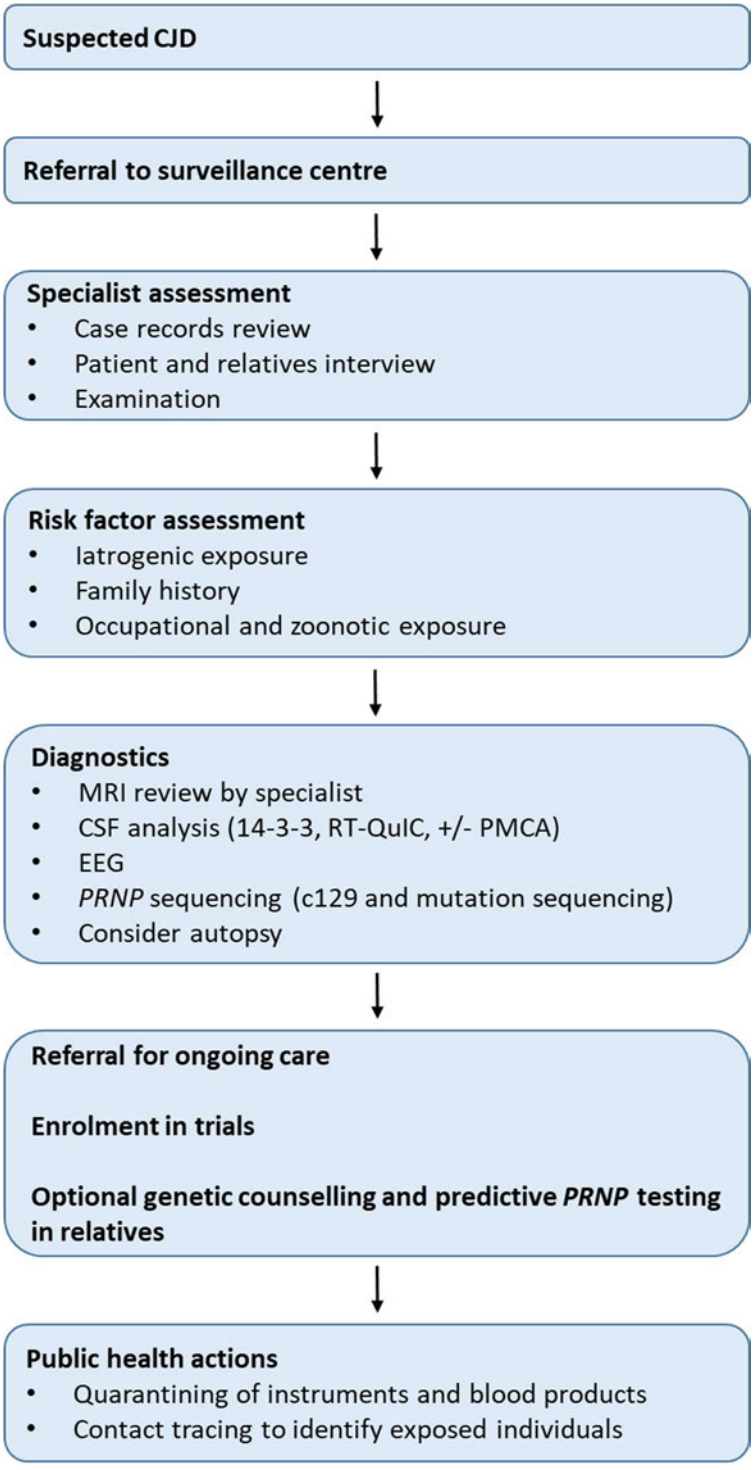


Figure 1.12. A template for modern CJD surveillance

A flowchart depicting a model system for comprehensive national CJD surveillance. This system allows ascertainment of CJD subtypes, screening for important epidemiological risk factors, and identifying and containing risks of transmission to others.

Abbreviations. c129, prion protein gene codon 129. CJD, Creutzfeldt-Jakob disease. CSF, cerebrospinal fluid. EEG, electroencephalogram. MRI, magnetic resonance imaging. PMCA, protein misfolding cyclic amplification. *PRNP*, prion protein gene. RT-QuIC, real-time quaking-induced conversion.

Conclusions

With widespread UK population exposure to BSE over a longer period than may have been assumed, evidence of prevalent carriage of vCJD material in the lymphoreticular systems of healthy individuals, and concerns around secondary transmission through blood products and surgery, vCJD remains a priority for surveillance. Increasing numbers of sporadic and inherited prion disease cases are now recognised globally, and there is evidence for sCJD disease pathogenesis outside of the nervous system, posing potential iatrogenic risks. iCJD cases with extensive incubation are still identified, and the spectrum of IPDs is ever-increasing. Additional concerns arise around potential zoonoses such as CWD and CPD and novel findings suggesting potential transmissibility of other protein-misfolding disorders. Large-scale surveillance with international cooperation remains a priority in order to recognise atypical cases of prion disease in humans as well as to minimise population exposure risks. Finally, national surveillance programmes are uniquely-placed to study this devastating family of diseases, improving diagnosis and symptomatic treatment with the ultimate aim of finding a cure. I advise that prion disease surveillance remains a public health priority, including when other priorities (such as COVID-19 during the period of this study being undertaken) risk taking precedent.

The focus of this thesis is on the in-life diagnosis of sCJD, the commonest form. Rapid and accurate antemortem diagnosis is essential in order for surveillance to be delivered effectively, and improvements in diagnosis lead to enhanced epidemiological assessment and rising case ascertainment. The sensitivity and specificity of the current iteration of the diagnostic criteria for sCJD had not been rigorously evaluated prior to this study. The aims of this study were to validate the current criteria, which I hypothesised would show high sensitivity and specificity. The rising sensitivity would account for a significant increase in case ascertainment, providing compelling evidence that increasing sCJD incidence figures greatly reflect enhanced ability to recognise and appropriately classify cases.

Chapter 2. Diagnosis of human prion diseases

In the century since the original case reports by Creutzfeldt and Jakob[300,301], our understanding of human prion diseases has evolved significantly [22,24,25,140] culminating in its current widespread public health relevance[1]. Over the last five decades, emergence of sensitive and specific neurophysiological, cerebrospinal fluid and imaging biomarkers has resulted in evolution of diagnostic criteria for each human prion disease[91,92,95,103,135,136,243,302-306]These have use in clinical practice as well as classification for surveillance[307].

In this **Chapter** I outline the history of CJD diagnosis and the current approach recommended by the International CJD Surveillance Network for each form.

Contents

- **Diagnostic criteria themes**
- **Sporadic CJD**
- **Inherited prion disease**
- **Iatrogenic CJD**
- **Variant CJD**

Diagnostic criteria: general themes

The International CJD Surveillance Network has produced defined consensus criteria for each form of CJD – sporadic, genetic, iatrogenic and variant[307]. While all forms can be considered to meet a *definite* case definition if neuropathological analysis demonstrates disease, the criteria allow for in-life diagnosis with definitions incorporating clinical and investigation findings. For all forms of CJD these require sufficient clinical features to be present in addition to a positive result in one or more supportive investigations in order for a case to meet criteria definitions as *probable*. CJD is a highly heterogeneous disease and can take on an array of manifestations[6,61,63,101,287], and the criteria reflect these.

The archetypal form of CJD is the commonest, sCJD[68,81]. Manifestations vary tremendously, influenced to a great extent by the individual subtype as defined by the Parchi classification[6]: combinations of c129 genotypes and PrP^{Sc} glycotypes exert a profound influence on nearly all manifestations of the disease. While the majority of individuals with sCJD have MM1 or MV1 glycotypes and develop the 'classic' phenotype, a sizeable minority have other glycotypes including VV2 associated with atypical but characteristic phenotypes and may not display the same symptoms

and signs[308]. The clinical features outlined in the diagnostic criteria are thus quite wide-ranging, allowing for a diverse array of phenotypes. In addition they are quite broad: for example, 'rapidly-progressive cognitive decline' does not specify which cognitive domains must be impaired, rather the focus is the rate of progression. Similarly, other headings such as 'cerebellar features' are not overly prescriptive and permit a variety of potential manifestations. Cases can present in a variety of ways and display features sufficient for diagnosis. The crucial aspect is the variety of manifestations, reflecting the extensive central nervous system (CNS) pathology in CJD[101], as opposed to the requirement for specific individual clinical features.

To be considered a *possible* case, individuals must display rapidly-progressive cognitive decline with two or more additional features (**figure 1.5**)[243]. For classification as a *probable* case investigations must also be supportive; details of these are outlined in this **Chapter**. Criteria used in genetic and iatrogenic CJD stipulate similar clinical requirements as those used for sCJD diagnosis, but a *probable* case requires demonstration of features indicating either an inherited or iatrogenically-acquired aetiology[307].

The clinical presentation of individuals with vCJD differed extensively from sCJD and it was these atypical features which led to its initial recognition by surveillance systems in the UK and subsequently France and other international surveillance programmes[132,133,136,140,149,170,194]. Three characteristic clinical features were prominent: longer disease duration, a neuropsychiatric prodrome, and the frequent presence of noxious cutaneous sensations[5,133,135]. The criteria for vCJD therefore reflect this characteristic phenotype, in contrast to those used in sporadic, genetic and other forms. However, with disease progression, individuals with vCJD generally develop clinical features comparable to the more classic CJD phenotype, including rapidly-progressive cognitive decline and ataxia[5].

Thus, as a general concept, CJD diagnosis in-life requires adequate clinical features, at least one supportive investigation, and in the case of gCJD and iCJD a confirmed genetic or plausible iatrogenic aetiology.

When considering the performance of the diagnostic criteria, a number of key questions need to be addressed:

1. How well do they identify cases (i.e. sensitivity)?
2. How well do they distinguish cases from non-cases (i.e. specificity)?
3. How well do they distinguish from other forms of CJD (noting the main differential for individual prion diseases are other subtypes of prion disease)?
4. How does the sensitivity vary between cases with that form of CJD, and what factors influence this?

This thesis will focus on the criteria used in sCJD, but it is useful to consider the diagnostic approach used in all forms, prior to exploring the current limitations of the sCJD criteria.

Sporadic CJD

Historically the only means of establishing an in-life diagnosis of sCJD was via cerebral biopsy[309], although the classification as sporadic is a more recent definition reflecting epidemiological understanding of the various aetiologies and variants of CJD. Indeed the discovery and classification of prions did not arise until the 1980s. While the characteristic neuropathological features were known in the preceding decades, the causative agent remained a mystery.

EEG

The first investigation which emerged as a means of non-neuropathological testing for prion disease was EEG[302,310,311]. Characteristic abnormalities were described in 1954 by Jones & Nevin[312], and subsequent studies were undertaken to further explore these in CJD[302,310,311,313]. The first formal diagnostic criteria used in prion disease surveillance were developed in 1979 and incorporated characteristic abnormalities in EEG[103] – namely, periodic sharp wave complexes (PSWCs) on a background of generalized slowing. EEG abnormalities in sCJD have subsequently been well-characterised, including their evolution with disease progression. Numerous studies have explored the longitudinal evolution of EEG abnormalities in sCJD, mostly conducted in an era prior to CSF and imaging biomarker development. There is a latency period before PSWCs emerge, and in earlier disease stages regional or diffuse slowing may be seen, and frontal intermittent rhythmic delta activity (FIRDA) is sometimes encountered[314-316]. Periodic activity may be encountered, but not to an extent meeting diagnostic criteria requirements[317]. The latency to PSWC emergence was typically found to be around 12 weeks in published studies[313,318], and correlates to clinical deterioration[317,318]. A study of 2425 EEG recordings from 1728 cases found highest frequency of PSWCs in late stages of disease[304]. However, in advanced, preterminal stages, PSWCs may recede, and areactive traces may be encountered[314,317]. The time to PSWC emergence is influenced by disease duration and sCJD subtype however[6,313], and some subtypes are less closely associated with PSWCs and may only manifest these in late stages (if at all) [6] . Longer disease duration is associated with lower probability of PSWC development[304].

There are variations in sensitivity for the classic EEG pattern in different subtypes of sCJD[6,100,313,314,319,320]. The highest sensitivity is seen in MM1/MV1 subtypes[304], and sensitivity is highest with advanced disease stages[313]. In other subtypes such as VV2 the sensitivity of PSWCs is limited[304], with one study quoting a rate of 17.8%[308]. They were only encountered in 7.1% VV2 and 7.7% MV2 cases and were not found in any MM2 or VV1 cases in the landmark subtyping study by

Parchi *et al* [6]. Heterogeneity of sensitivity between subtypes of sCJD, and poor sensitivity in the early stages of disease limit the diagnostic utility of EEG.

In addition, the specificity of EEG can pose challenges. While the characteristic pattern of changes is said to be between 86-91% specific[100,321], problems can emerge in cases undergoing EEG at early disease stages with limited clinical features, where EEG features can overlap with those seen in focal epilepsy or structural lesions[322,323]. This can confound the diagnosis, and in some cases can result in individuals with sCJD receiving empirical treatment with anticonvulsants[322], sometimes in intensive care environments[324] with attendant risk of morbidity.

14-3-3

Cerebrospinal fluid 14-3-3 emerged as a diagnostic assay for prion diseases in the 1990s[325-328] and was formally included in diagnostic criteria in 1998[329]. The assay is a non-specific marker of neuronal injury, and in addition to sporadic CJD it can be positive in other forms of prion disease[4,137,319,330] as well as other conditions such as stroke, intracerebral haemorrhage, paraneoplastic encephalitis and cerebral malignancies[325,331-333]. This limits its specificity, and the quoted figure in studies varies substantially according to what aetiologies are included among the non-case control groups[334]. Sensitivity varies in the literature[95,243,286,334,335] particularly between sCJD subtypes[304,336] and with rate of disease progression and stage of disease , being highest in those with rapid progression, and advanced disease states[337].

One study explored the sensitivity of 14-3-3 at different disease stages in CSF samples from 833 individuals, dividing total duration into thirds (first, second and third stage), demonstrating high sensitivity throughout all stages with a non-significant trend towards increasing sensitivity[337]. Stratifying by c129 genotype demonstrated significant sensitivity rise in MV individuals alone. In a minority of individuals, serial CSF sampling was performed, with a significant increase in sensitivity on repeat sampling; however, the sensitivity of the first sample was lower than that of the overall cohort, indicating that this subgroup overrepresented individuals with false negative CSF results (hence the requirement for repeat analysis), and analysis demonstrated these individuals were younger, had longer disease duration and more frequently had MV c129 genotype[337].

It should be borne in mind that given the considerable heterogeneity of disease duration in sCJD, it is not feasible or realistic to state a typical time to positive 14-3-3; a short survivor may display this on week 4, whereas a long survivor may only display positivity after many months, or never, and sCJD subtypes will influence both duration and sensitivity[304]. This study instead used calculated stages as proportions of the total duration, and this will have differed considerably among cases. For example, while all individuals reached the third stage after 66.6% of their total duration had elapsed, the time to reach this stage will have differed substantially between short and long survivors.

It is noteworthy that a minority of serial samples (9.5%) actually converted from positive to negative 14-3-3 results[337]. Studies of n=895[304] and n=129[327] cases did not find any significant association between sample timing and 14-3-3 sensitivity, whereas a study of samples from 42 individuals indicated highest concentrations of 14-3-3 in middle stages, reducing in late stages[338]. Another study indicated a non-significant trend to higher sensitivity in early (<6 week) samples than later samples[339].

MRI

In the subsequent two decades after 14-3-3 came into routine usage for surveillance, MRI emerged as a validated diagnostic tool for sCJD diagnosis[92,304,305,340-342]. Two cardinal abnormalities are recognised in sCJD. The basal ganglia (caudate and putamen) display abnormal hyperintensity on T2 and fluid-attenuated inversion recovery (FLAIR) MRI with restricted diffusion on diffusion-weighted imaging (DWI)[92,305,341]. Diagnostic criteria were first adapted in 2010 to incorporate this finding. The other abnormality is abnormal cortical signal on DWI and FLAIR[92,305,343]. This is known as 'cortical ribboning' and is typically patchy, with normal segments separating areas of ribboning. The inclusion of cortical ribboning was one of two adaptations in the 2017 diagnostic criteria[91,243].

The specific MR sequences used influence the likelihood of detecting characteristic MR abnormalities. DWI has superior sensitivity to FLAIR for characteristic hyperintensity patterns in sCJD, both in basal ganglia and cortical regions[68,92,93,344-348]. DWI may be particularly effective in the identification of cortical hyperintensities[347]. An additional advantage of DWI is the speed of acquisition of images. These can reduce motion artefact which is a common problem in individuals with CJD, a group which can experience behavioural agitation as well as stimulus-sensitive myoclonus, degrading image quality[347].

Notably, the International CJD Surveillance Network criteria do not formally stipulate the requirement for a decrease in ADC values in regions with DWI hyperintensity, i.e. restricted diffusion[307]; other criteria such as the UCSF 2017 criteria[68] require corresponding ADC hypointensity in affected regions, and one study evaluating a cohort of 171 neuropathologically-confirmed sCJD cases identified true diffusion restriction (i.e. co-occuring ADC map hypointensities) in 92% of cases[349]. The authors of the UCSF criteria cite the specific requirement for corresponding ADC reduction as a means of preventing the risk of artefactual hyperintensities on DWI being interpreted as false-positive markers of sCJD[68]. However, another study only assessed corresponding ADC values for subcortical grey matter regions with DWI abnormalities seen, citing difficulties in visual recognition of cortical ADC values[341].

With serial imaging and progression of disease, the distribution of affected regions increases, and initially negative investigations may evolve to fulfil criteria for a positive result[345,350-353]. Authors have indicated that this may reflect progressively widespread spongiform change, with restriction of water diffusion in vacuoles. Notably, some reports have indicated DWI hyperintensities can eventually

diminish with advanced disease[351,352], and the underlying cause for this is unclear; this apparent decrease in late-stage sCJD has not been widely studied however, and this may reflect limited availability of data, with the high sensitivity of MRI at earlier disease stages making late-stage MRI unnecessary in most individuals (as well as being inappropriate in those in an end-of-life stage).

Other technical considerations influencing sensitivity of MRI include the field strength and b values used. In published studies, where MRI field strength is stated it is often 1.5T[346,349]. Some authors recommend b2000 sequences[68], with evidence supporting superior sensitivity over b1000 values[354] and one study indicating superior sensitivity using b3000 values compared to b1000[355].

It should be noted that given the nature of CJD surveillance, with sCJD cases emerging in a sporadic pattern and often being referred to surveillance centres after local investigation raises suspicion of the disease, most published studies include data from locally performed MRIs rather than employing standardised imaging protocols (which would require a prospective design), and will differ in terms of sequences obtained and technical considerations such as field strength and b-values. Some quoted sensitivity values were obtained using older technologies than those currently in use. Further, according to my reading of the literature, many studies do not formally state technical factors such as these in relation to MRI in their methodology sections.

Neither pattern of MR abnormalities is fully specific for sCJD[243]. Basal ganglia hyperintensities can be encountered in hypoxic and metabolic brain injuries and certain encephalitides, while cortical signal hyperintensities can be seen in seizures, strokes, hypoxic brain injuries and mitochondrial diseases[243,343].

RT-QuIC

The most recent development in prion disease diagnosis has been the emergence of assays which amplify misfolded prion proteins. The archetypal investigation is the real-time quaking-induced conversion assay (RT-QuIC)[97]. Along with cortical ribboning the RT-QuIC assay was incorporated into the 2017 diagnostic criteria[91,243]. This assay involves a recombinant form of PrP^{C} which can be induced to misfold in the presence of PrP^{Sc} in tissue obtained from the individual undergoing testing, leading to a characteristic reaction[97]. A positive result is indicated by a characteristic pattern of thioflavin T fluorescence above a specific threshold. RT-QuIC has been developed and validated in both CSF and olfactory mucosal tissue[99,242,243,306,356-359].

The RT-QuIC assay has many advantages over conventional biomarkers. The most striking is its near-100% specificity[97,358]: almost no false-positive results have been reported in the literature to date, and only four positive results have been reported in individuals with neuropathological exclusion of prion disease[97,243]. As a consequence of this unique characteristic, the criteria now consider any individual with a progressive neurological syndrome and positive RT-QuIC as having *probable* sCJD[307]. Other

advantages of RT-QuIC include its high sensitivity which appears to be the case in all subtypes of sCJD, and its apparent lack of variation with disease duration or rate of progression[360,361]. The average time to positive RT-QuIC is not known in sCJD. Two studies assessing the influence of timing of CSF sampling on RT-QuIC performance did not find any difference on outcomes at different stages[360,361]. In the NCJDRSU experience of samples taken from 986 cases of definite or probable sCJD, the timing of CSF sampling (measured either in months or as a proportion of the total disease duration from symptom onset to death) does not influence the likelihood of a positive assay (Alison Green, personal communication). Interestingly however, one study identified changing RT-QuIC results in serial samples from 12 individuals, with 8 converting from negative to positive and 4 from positive to negative, indicating that disease progression may have differing effects on RT-QuIC outcomes in some individuals[242], although the change in outcomes in serial CSF samples has not been widely studied.

The diagnosis of other forms of CJD

The core focus of this thesis is the performance of the latest sCJD diagnostic criteria. However, it is worth exploring the criteria used in the other forms of CJD, all of which are differential diagnoses for sCJD, before exploring the sCJD criteria in greater depth, including the limitations and unknown elements.

Inherited prion disease (IPD)

As outlined in **Chapter 1**, inherited prion disease (IPD) accounts for 10-15% of all cases of human prion diseases. Those with CJD-like phenotypes are commonly termed genetic CJD (gCJD), while other characteristic phenotypes exist such as fatal familial insomnia (FFI) and Gerstmann-Straussler-Scheinker (GSS) disease[109,113,114]. IPD has been linked to an ever-increasing number of pathogenic mutations and can present with a diverse array of phenotypes, in some cases mimicking other neurological disorders such as frontotemporal dementia and spinocerebellar ataxias[4,61]. The diagnostic criteria resemble those used in sCJD, but are less prescriptive in clinical features, allowing for any progressive neuropsychiatric syndrome with evidence in support of a genetic aetiology[307] (**figure 2.1**). Many mutations are highly penetrant and inherited in an autosomal dominant manner[61], and hence identification of a pathogenic mutation in the presence of compatible phenotype is diagnostic. In addition, individuals can be diagnosed if they have a clinically-compatible phenotype and are a first-degree relative of a case.

The outcomes of diagnostic investigations vary substantially between forms of IPD with different mutations. E200K closely resembles MM1 sCJD in clinical phenotype and the outcomes of investigations are similar (including RT-QuIC), underscoring the importance of *PRNP* sequencing to

exclude the possibility of a genetic aetiology[61] (**vignette 2.1**). In contrast, fatal familial FFI and GSS have characteristic phenotypes[109,113,114]; the sensitivity of investigations such as 14-3-3 may be substantially lower than in sCJD, while the sensitivity of RT-QuIC remains a subject for ongoing study and may be substantially lower than sCJD[362,363]. The overall sensitivity of RT-QuIC in separate IPD subtypes is incompletely understood, and given the rarity of some individual mutations it will take widespread international collaboration to fully evaluate this.

gCJD is an important differential diagnosis for sCJD and should be considered when evaluating individuals with suspected prion disease. Diagnostic 'clues' suggesting genetic aetiology include a positive (i.e. confirmed) or suggestive (i.e., affected individuals with a CJD-like phenotype) family history, younger age of onset[4,61], and in some cases prolonged disease duration[4]. These features are not entirely reliable however; for example, a large-scale European study identified the absence of a positive family history in 47% of individuals with inherited prion disease[4]. As above, FFI and GSS have characteristic phenotypes, but E200K and other mutations can overlap closely with sCJD in terms of phenotype and investigation outcomes, making distinction challenging. Testing for genetic testing for mutations in *PRNP* is the definitive way to differentiate between sporadic and genetic forms. The NCJDRSU approach is to offer testing in all assessed individuals with CJD.

Figure 2.1. Diagnostic criteria for genetic CJD

3.1 **DEFINITE**

3.1.1 Definite TSE + definite or probable TSE in 1st degree relative

3.1.2 Definite TSE with a pathogenic PRNP mutation (see box)

3.2 **PROBABLE**

3.2.1 Progressive neuropsychiatric disorder + definite or probable TSE in 1st degree relative

3.2.2 Progressive neuropsychiatric disorder + pathogenic PRNP mutation (see box)

- **PRNP MUTATIONS ASSOCIATED WITH <u>GSS</u> NEUROPATHOLOGICAL PHENOTYPE**

  P102L, P105L, A117V, G131V, F198S, D202N, Q212P, Q217R, M232T, 192 bpi

- **PRNP MUTATIONS ASSOCIATED WITH <u>CJD</u> NEUROPATHOLOGICAL PHENOTYPE**

  D178N-129V, V180I, V180I+M232R, T183A, T188A, E196K, E200K, V203I, R208H, V210I, E211Q, M232R, 96 bpi, 120 bpi, 144 bpi, 168 bpi, 48 bp*del*

- **PRNP MUTATIONS ASSOCIATED WITH <u>FFI</u> NEUROPATHOLOGICAL PHENOTYPE** D178N-129M

- **PRNP MUTATION ASSOCIATED WITH VASCULAR PRP AMYLOID**

  Y145s

- **PRNP MUTATIONS ASSOCIATED WITH PROVEN BUT UNCLASSIFIED PRION DISEASE**

  H187R, 216 bpi,

- **MUTATIONS ASSOCIATED WITH NEURO-PSYCHIATRIC DISORDER BUT NOT PROVEN PRION DISEASE** I138M, G142S, Q160S, T188K, M232R, 24 bpi, 48 bpi, 48 bpi + nucleotide substitution in other octapeptides

(additional list of mutations appended)

ADDITIONAL LIST OF MUTATIONS

- **PRNP MUTATIONS WITHOUT CLINICAL AND NEUROPATHOLOGICAL DATA**

  T188R, P238S

- **PRNP POLYMORPHISMS WITH ESTABLISHED INFLUENCE ON PHENOTYPE**

  M129V

- **PRNP POLYMORPHISMS WITH SUGGESTED INFLUENCE ON PHENOTYPE**

  N171S, E219K, 24 bp deletion

- **PRNP POLYMORPHISMS WITHOUT ESTABLISHED INFLUENCE ON PHENOTYPE**

  P68P, A117A, G124G, V161V, N173N, H177H, T188T, D202D, Q212Q, R228R, S230S

Vignette 2.1. E200K genetic CJD

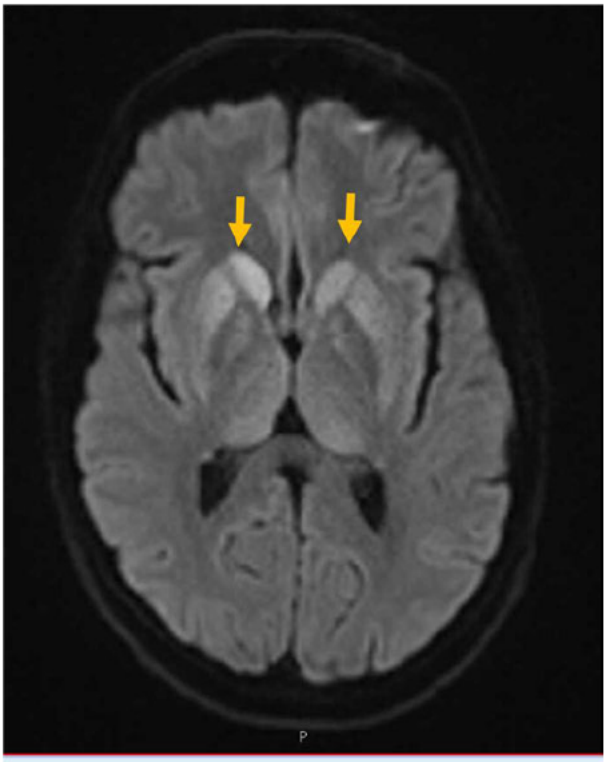
A 58 year old man developed developed rapid-onset gait ataxia with falls. His wife noted his behaviour changing: he became less interactive and displayed loss of procedural knowledge. His short- and long-term memory deteriorated.

One of his parents had previously died from E200K gCJD. Two of this parent's siblings had died of dementia of unknown aetiology prior to this parent's CJD diagnosis. The patient had previously declined predictive testing following the parent's diagnosis.

An MRI demonstrated basal ganglia changes suggestive of CJD (b=1000 DWI example shown below). He was admitted to hospital owing to progressive disability and was referred to the NCJDRSU.

CSF testing revealed positive 14-3-3, S100b (2.05ng/mL) and RT-QuIC assays. Genetic testing confirmed the E200K mutation.

The case illustrates the similar phenotype and investigation profile to sCJD seen in E200K, the commonest cause of gCJD in Europe. Even in the absence of a family history it is useful to offer genetic testing to patients suspected to have sCJD.



Iatrogenic CJD

An overview of the epidemiology of iCJD is provided in the **Chapter 1**. iCJD has been transmitted via numerous mechanisms (**table 2.1**) and shares the spectrum of features identified in sCJD[364].

Interestingly the mechanism of exposure has been reported to influence the phenotype, with peripheral exposures to cadaveric hormones being associated with cerebellar phenotypes, while central exposures (neurosurgical instruments and grafting) were associated with more 'classical' CJD phenotypes[25].

Table 2.1. Iatrogenic CJD: mechanisms of exposure

Surgical and procedural exposure
Dura mater grafting[26,365]
Neurosurgical instruments[366,367]
Stereotactic EEG needles[25]
Corneal transplantation[125]
Cadaveric hormones
Growth hormone[33,126,368]
Gonadotrophin[53,369,370]

Abbreviations. EEG, electroencephalography.

The diagnostic criteria stipulate the same features as sCJD but require an antecedent history of a relevant iatrogenic exposure[307] (**figure 2.2**). The latter consist of treatment with c-hGH[126], gonadotropin or dura mater grafts[26], a corneal transplant obtained from a donor with prion disease[125], or prior exposure to neurosurgical instruments used previously in an individual with prion disease. In addition, any individual with a progressive cerebellar syndrome and prior c-hGH treatment fulfils *probable* iCJD definition.

iCJD is a differential diagnostic possibility in all cases of suspected CJD. The NCJDRSU approach is to ask specific questions about relevant iatrogenic exposures to confirm the diagnosis (see **table 2.1**). Clinical features overlap with sCJD. However, in the case of cadaveric hormones these were reported to overlap more with atypical forms of sCJD, with prominent cerebellar phenotypes at presentation, and with dementia typically not a presenting feature[25,33,126,369-371]. Painful sensations were sometimes encountered[33], which may overlap with the vCJD phenotype. In contrast, DM-iCJD was commonly associated with phenotypes similar to typical sCJD[25], although a minority of Japanese cases displayed atypical phenotypes, for example with slower progression[364]. Factors underlying the phenotypic features in iCJD are not fully understood, and may include the means of infiltration into the CNS (direct vs peripheral), including the site of graft placement[372], as well as the strain of the inoculating agent[373], and the influence of predominant c129 genotypes among affected individuals, including in nations such as Japan where most individuals are MM homozygous.

Much of the global iCJD epidemic preceded the modern diagnostic investigations available in the modern era, and which are the focus of this thesis. It is therefore uncertain how these cases would differ from sCJD using the current algorithm. A sizeable minority of DM-iCJD cases did not display characteristic EEG abnormalities during illness; in a Japanese study, 60% of DM-iCJD cases displayed PSWCs, while a UK study demonstated PSWCs in only 42.9% of cases . 14-3-3 was found to be 85% sensitive and MRI 73% sensitive in DM-iCJD[364]. A 2015 study of c-hGH-iCJD demonstrated similar MRI findings to those seen in sCJD in the majority of individuals (90% had basal ganglia hyperintensities, while cortical hyperintensities were common in cingulate [88.2%] and frontal [82.4%] regions), while only 42.9% of individuals displayed positive CSF 14-3-3[33]. iCJD is less commonly identified in the modern era and hence the performance of RT-QuIC is not fully known, but NCJDRSU data indicate sensitivity of ~67% in c-hGH-iCJD[97].

2.1 **DEFINITE**

Definite CJD with a recognised iatrogenic risk factor (see box)

2.2 **PROBABLE**

2.2.1 Progressive predominant cerebellar syndrome in human pituitary hormone recipients

2.2.2 Probable CJD with recognised iatrogenic risk factor (see box)

2.3 **POSSIBLE**

Possible CJD with a recognised risk factor

RELEVANT EXPOSURE RISKS FOR THE CLASSIFICATION AS IATROGENIC CJD

The relevance of any exposure to disease causation must take into account the timing of the exposure in relation to disease onset

- Treatment with human pituitary growth hormone, human pituitary gonadotrophin or human dura mater graft.
- Corneal graft in which the corneal donor has been classified as definite or probable human prion disease.
- Exposure to neurosurgical instruments previously used in a case of definite or probable human prion disease.

Figure 2.2 Diagnostic criteria for iatrogenic CJD

Variant CJD

Recognition of vCJD is a primary motivation of international CJD surveillance[1], and hence distinction of cases from sCJD is a core objective. vCJD is associated with a characteristic phenotype[5] and the diagnostic criteria reflect this[135,307]. Cases must have a progressive neuropsychiatric disorder. The criteria also stipulate disease duration >6 months, distinguishing cases from the majority of those with sCJD[3], and the absence of a mutation associated with gCJD or a prior exposure to a risk factor for iCJD. In addition, there must not be evidence of an alternative diagnosis to account for symptoms. The clinical features necessary for diagnosis include a typical phenotype with early psychiatric features and noxious cutaneous sensory symptoms in addition to dementia, ataxia and movement disorders.

It is worth highlighting that the clinical features characterising vCJD, namely a longer duration, a neuropsychiatric prodrome, and in some cases noxious cutaneous sensations, may be encountered in other, commoner forms of CJD, and the phenotype alone is not fully specific[6,61]. For example, the VV1 subtype of sCJD, associated with younger onset, a neuropsychiatric profile and longer survival may clinically overlap with vCJD[6,181]. Similarly, individuals with FFI may display prominent neuropsychiatric disorders, prolonged disease duration and have younger age[374,375]. Thus, additional features must be present to support a *probable* vCJD diagnosis.

The pulvinar sign on MRI is highly sensitive and specific for vCJD[136,376] and thus is included as a diagnostic investigation (see **figure 1.6)**. Pulvinar hyperintensity can be identified in other aetiologies, including sCJD[305,377], although not usually without additional basal ganglia or cortical hyperintensities[343]. However, a formal definition of the pulvinar sign stipulates bilateral and symmetrical high signal in the pulvinar nuclei compared to other basal ganglia nuclei and cortex[136,376]. Thalamic hyperinensities may also be encountered in Wernicke's encephalopathy[378] and in Fabry's disease[379] (typically on T1 as opposed to the sequences displaying abnormality in prion diseases). In addition, the typical EEG changes of sCJD are not frequent in vCJD[135], and the absence of a typical EEG pattern seen in sCJD during the early stages of potential vCJD is a supportive feature. This feature is limited in utility given that the typical pattern is not seen in early cases of sCJD either, but it represents one element of a broader criteria and should be considered in the wider clinical context.

A unique feature of vCJD as outlined in **Chapter 1** is widespread lymphoreticular disease distribution. In addition to posing numerous public health risks, this aspect has utility in diagnosis, and tonsil biopsy was developed as a diagnostic investigation early in the vCJD epidemic[186]. Cases with clinically compatible features and a positive tonsil biopsy can be classified as having *probable* vCJD. The investigation has utility where MRI is not supportive (or possible).

PMCA, discussed in **Chapter 1**, has not yet been formally validated as an assay for use in surveillance and is not included in diagnostic criteria. However, the last neuropathologically-

confirmed vCJD case reported (acquired through occupational exposure) displayed a positive assay using blood and CSF[34], and CSF saved from the most recent primary case (and only confirmed MV case) also tested positive[380]. The CSF assay has been demonstrated to have 97-100% sensitivity and 100% specificity for vCJD[380,381]. PMCA technology emerged following the decline in the initial vCJD epidemic and was never included in criteria, but should further cases be identified in surveillance PMCA may become a crucial part of the diagnostic criteria.

In comparison, the RT-QuIC assay does not appear to be sensitive for vCJD. This lack of sensitivity is itself a useful feature for modern surveillance, where a negative RT-QuIC assay in a case of CJD can indicate potential vCJD, and cases can be evaluated for that aetiology; in the absence of other diagnostic test features, post-mortem can be prioritised. The two most recently published vCJD cases were both negative for RT-QuIC[34,55]. Similarly, when vCJD exists as a possible concern, a positive assay provides compelling evidence for an alternative form of CJD, and indicates vCJD is less likely.

While the diagnostic criteria for vCJD appear to be highly sensitive and specific, they were developed and validated during the primary wave of vCJD, which consisted exclusively of MM cases[42]. The most recent primary case was MV genotype[55], and the imaging features in this case more closely resembled sCJD. It is unknown how potential future cases of non-MM vCJD might present, and it is conceivable that, in line with what is seen in sCJD[6], different genotypes may be associated with different manifestations, including on diagnostic investigations, making diagnosis by conventional criteria challenging, as well as differentiation from other forms of prion disease.

Assessment of the current diagnostic criteria in sCJD

The sCJD diagnostic criteria revision in 2017 took place following discussions within the International CJD Surveillance Network which considered the evolving literature in the years following the previous evolution in 2010. Prior to this thesis, no large-scale validation study had been undertaken to assess the real-world performance of the criteria. A single-centre study had been published by Hermann *et al*, indicating 97% sensitivity and 99% specificity[91]. This study was a useful initial demonstration, but was ultimately limited in its scope and employed a relatively small sample in addition to a biased control cohort used for specificity calculation, as evidenced by the low specificity of 14-3-3 (27%) [91]. The study was also unable to explore important subgroups for which the criteria may perform less favourably.

sCJD is rare, and in order to recruit adequate case numbers for such validation studies it is essential to use surveillance systems' registries, which are comprehensive and contain data on all cases from each individual nation as opposed to other approaches which might risk selection bias (e.g. recruiting from cases seen in a tertiary referral centre or outpatient clinic). Multi-national collaboration is optimal;

individual nations would otherwise have to recruit over a long study period, and for a study assessing modern diagnostic tools this is not an option, given that some such as RT-QuIC have only entered use in the last 10 years and have only been widely used in a shorter interval. A large sample is also essential in order to deliver adequate statistical power to assess important subgroups.

Prior to this thesis it was not known how the 2017 diagnostic criteria performed in individual sCJD subtypes. The emphasis on displaying adequate clinical features, with RPCD as an essential precondition, could conceivably have led to inadequate classification of subtypes such as VV2 which may present with ataxia and preserved cognition[308], or those with prominent neuropsychiatric presentations. Likewise, the requirement for individuals to display multiple neurological features might result in other subtypes which do not tend to display as many motor features (e.g. MM2) being under-classified[179]. In addition to clinical features, the outcomes of cardinal diagnostic investigations might vary by subtype, as was already known for the conventional investigations (EEG, 14-3-3, MRI with basal ganglia hyperintensity), further compromising sensitivity for particular subtypes. Emerging data had suggested that RT-QuIC may lack sensitivity for uncommon subtypes such as MM2[97], and the impact of this variation on the performance of the aggregate criteria was unknown.

Additional factors warranting evaluation are the effects of atypical disease duration and atypical age of onset. It is not known how the revised criteria compared to the previous iteration in terms of ability to appropriately classify cases which are outliers in relation to these aspects: those with very rapid progression or prolonged survival, and those with young- or older-onset disease. It is conceivable that those with those with slow progression and long survival might be less likely to display appropriate clinical features during their assessment (for example, myoclonus is a late-emerging sign), and might be less likely to display a positive 14-3-3 or EEG, both of which become more sensitive with advancing disease stages and are more sensitive among shorter survivors[304]. Hence, in-life classification may be challenging until advanced disease stages, posing numerous difficulties for surveillance and clinical management of individuals.

Similarly, young-onset cases might display differences in clinical phenotypes, and investigation sensitivities may vary with age[382]. The incidence of sCJD peaks in the 7th and 8th decades of life, with lower rates in younger and older adults[42]. This pattern has persisted despite increases seen in all age ranges with expanded surveillance and improved diagnostic tools. The extent to which this age-dependent incidence reflects biological factors versus under-ascertainment in individuals with atypical age is not known. To address whether the latter is a contributing factor, it would be worth evaluating the performance of the 2017 diagnostic criteria between age groups, as well as that of the previous criteria in order to gauge the impact of the revisions and individual investigations on classification of different age groups of sCJD cases.

The diagnostic criteria now allow individuals with any progressive neurological syndrome to be classified as *probable* sCJD in the presence of a positive RT-QuIC assay. This is due to the outstanding specificity reported in the literature in the years prior to the revision[98,99,306,360,361]. As a consequence, individuals with isolated clinical features, for example ataxia or dysphasia, can be considered to have *probable* sCJD if the assay is positive. The effects of this on in-life classification of such clinically-limited cases warrant exploration, including the impact on rates of diagnosis compared to prior criteria: a subset of individuals with CJD would conceivably now be re-classified as *probable* sCJD during life, as was seen in the initial study by Hermann *et al*[91].

In contrast to RT-QuIC, cortical ribboning is not 100% specific, and the specificity of both cortical ribboning and *probable* sCJD diagnosis among non-cases with appropriate clinical features and cortical ribboning warrant evaluation, as such cases would have the potential for in-life misdiagnosis as sCJD, an error with many potentially hazardous implications. In addition, the aetiologies of non-cases with cortical ribboning also were important to explore to further understand the potential consequences of the revision on real-world diagnosis, including whether a specific aetiology was at risk of misdiagnosis as sCJD. There are significant implications following a diagnosis of prion disease and potentially serious consequences can follow misclassification of a mimic, most gravely if an inappropriate decision to withdraw active care is made in a potentially treatable condition. Appropriately diagnosing non-cases is also important for surveillance classification, public health activities (including not incorrectly labelling contacts as at-risk of CJD), as well as for robust clinical trial delivery.

Finally, as described above, the potential for phenotypical overlap between forms of CJD raises questions over the performance of the diagnostic criteria in relation to their ability to discriminate between subtypes. In particular, the potential for similar manifestations in both sporadic and variant CJD cases might pose challenges for diagnostic criteria and limit their utility for clinicians assessing potential cases, given the importance of accurate subtyping.

Core aims of the thesis

The diagnostic criteria require large-scale validation using a robust cohort and with a sufficient sample size to evaluate their performance in the aforementioned subgroups. Their performance must be compared to the previous iteration of the diagnostic criteria to assess the magnitude of change in case classification, including the change in specificity given the broader set of criteria for *probable* case definition. The epidemiological consequences of this, i.e. the increase in cases classified as *probable* sCJD during life, should be quantified to estimate the impact of the criteria on surveillance and recorded national incidence figures through heightened case ascertainment (i.e. a rise in

sensitivity), and any potential for non-cases being misclassified as sCJD during life (i.e. a decrease in specificity).

This thesis was designed to evaluate these questions, as will be outlined in **Chapter 3**. The aims of this study were:

1. To assess the sensitivity and specificity of the 2017 diagnostic criteria
2. To quantify the impact on in-life case classification as *probable* sCJD
3. To explore the performance of the criteria in important sCJD subgroups

I hypothesised that:

1. The revised criteria are highly sensitive and specific for in-life diagnosis of *probable* sCJD
2. The revision has driven a significant rise in in-life classification as *probable* sCJD
3. Variations in the sensitivity of investigations and diagnostic criteria in aggregate would be present between c129 groups and sCJD subtypes
4. The diagnostic criteria would be less sensitive in individuals with atypical disease duration (both short and long) and atypical age (young and elderly individuals)

The study objectives, hypotheses and methods are described in **Chapter 3**.

Chapter 3: Evaluation of the 2017 International CJD Surveillance Network diagnostic criteria for sporadic CJD: aims, hypotheses and methods

This **Chapter** describes the aims and hypotheses for this thesis, and provides a detailed description of the methods used for the study, including data analysis.

- **Aims and hypotheses**
- **Cohort selection**
- **Demographic and clinical features and investigation results**
- **Case classification during life**
- **Data harmonisation**
- **Statistical analysis plan**
- **Missing data**
- **Surveillance centre methodology**
- **Clinical case vignettes**

Aims & hypotheses

The study was designed to assess the performance of the revised 2017 International CJD Surveillance Network diagnostic criteria (**figure 1.5**), primarily assessing its sensitivity and specificity. I included cases of sCJD with neuropathological confirmation of disease, i.e. the 'gold standard' for diagnosis: such cases are defined as having *definite* sCJD by the criteria. I sought to assess these cases' in-life classification according to the criteria, evaluating the sensitivity of *possible* and *probable* sCJD classifications, as well as that of the individual investigations which comprise the diagnostic criteria.

The same approach was applied to a cohort of non-cases with neuropathological confirmation of alternative diseases and exclusion of prion disease (i.e. the 'gold standard' for excluding prion disease), to quantify the specificity of the diagnostic criteria during life, as well as the specificity of the individual diagnostic investigations.

The secondary aim of the study was to quantify the impact of the criteria revisions on in-life case classification in comparison to the previous criteria: this has epidemiological relevance, with national surveillance systems commonly including cases with *probable* or *definite* sCJD in official annual incidence figures[40], while those with *possible* or other classifications are not included.

Additional aims of the study were to assess the performance of the criteria in important subgroups, specifically different c129 genotypes, combined c129 and PrP glycotype groupings (i.e. sCJD

subtypes according to the Parchi classification[6,63]), and in individuals with atypical disease duration and age.

Finally, I sought to assess the characteristics of specific subgroups of cases defined by investigation outcomes relevant to the revised criteria, namely those with negative RT-QuIC and those with isolated cortical ribboning. These individuals pose novel challenges in modern surveillance, and their characteristics warranted exploration to aid surveillance and clinical diagnostic efforts.

I hypothesised that:

1. The revised criteria are highly sensitive and specific for in-life diagnosis of *probable* sCJD
2. The revision has driven a significant rise in in-life classification as *probable* sCJD
3. Variations in the sensitivity of investigations and diagnostic criteria in aggregate would be present between *PRNP* c129 groups and sCJD subtypes
4. The diagnostic criteria would be less sensitive in individuals with atypical disease duration (both short and long) and atypical age (young and elderly individuals)

Cohort selection

To recruit adequate numbers of individuals, I collaborated with members of four European surveillance units to perform an international study using data from the national surveillance systems of the United Kingdom (UK), France, Germany, and Italy. These nations are the most populous among those performing surveillance in Europe, and in addition have highly-developed systems which have been operational over decades and have extensive experience in prion disease surveillance and research. Their systems operate by broadly similar methods (outlined in this **Chapter**), and together with their large combined populations would allow for a robust sample derived from similar and comprehensive systems, maximising case ascertainment and quality data capture.

I defined the period of interest as the first of January 2017 until the 31st of December 2019, as this represented the three-year period after the introduction of the revised criteria. I sought to obtain data from all cases of sCJD with neuropathological confirmation of the disease, i.e *definite* sCJD, by autopsy or biopsy, anticipating the vast majority of individuals would be in the former group; biopsy is not commonly performed in suspected prion disease for reasons including the availability of highly-sensitive and less invasive ante-mortem investigations[383]. I included all cases with final diagnoses of *definite* sCJD; exclusion of inherited prion disease by *PRNP* sequencing was desirable but not mandatory for inclusion, as not all cases undergo genetic sequencing.

A similar approach was used to obtain the cohort of non-cases used as a control group: I obtained data on individuals assessed during life for potential or suspected CJD who received an alternative diagnosis on neuropathology, either by autopsy or biopsy (using morphology, immunohistochemistry and, in most cases, western blot analysis). Efforts were made to obtain final tissue diagnoses in these cases. Where this was possible, I classified non-cases into aetiological categories:

- Neurodegenerative disorders (ND) included Alzheimer's disease (AD), dementia with Lewy bodies (DLB), tauopathy, and TDP-43 associated disorders
- Vascular diseases included cerebrovascular disease and vascular dementia, cerebral vasculitis, and cerebral amyloid angiopathy (CAA)
- Cerebral insults comprised various forms of acquired brain damage such as hypoglycaemic or anoxic brain injuries, status epilepticus, and metabolic disorders such as hepatic encephalopathy
- Inflammatory disorders included autoimmune encephalitides
- Infectious disorders included viral encephalitis, central nervous system (CNS) abscesses, and human immunodeficiency virus (HIV)-associated conditions
- Diagnoses in the remaining non-cases were classified as miscellaneous (any other tissue-confirmed diagnosis) or otherwise as non-diagnostic examinations

In some non-cases, more than one pathological finding was present, and I sought to quantify the presence of these; both in terms of co-pathology within the same category (for example, co-present AD and Lewy body pathology) and when more than one category was present (for example, AD and CAA).

Finally, I did not include any individuals with forms of prion diseases other than sCJD in the control group: the objective of the study was to assess the performance of the criteria in detection of sCJD and distinction from non-prion aetiologies, rather than to differentiate between forms of human prion disease.

Demographic and clinical features and investigation results

I extracted information regarding demographic features: sex, age at tissue diagnosis, and dates of disease onset (estimated retrospectively from clinical assessments ascertaining the approximate onset of symptoms) and tissue sampling, with the latter used to calculate disease duration in deceased individuals undergoing autopsy; duration estimates were not included among individuals undergoing biopsy unless date of subsequent death was available. Individuals were stratified into three duration groups - short, typical and long survival - by calculated disease duration being in the 1st, 2nd to 3rd, and 4th quartiles respectively.

I extracted information on clinical features relevant to the diagnostic criteria (**figure 1.5**):

- Rapidly-progressive cognitive decline (RPCD) was defined by new cognitive impairment (affecting various domains such as memory, attention, fluency, executive functioning and visuospatial functioning) and/or dementia emerging within a time period of under two years[384,385]
- Cerebellar features included ataxia (of gait, limbs or trunk), cerebellar-type dysarthria, and nystagmus
- Visual features included any non-ophthalmological causes of visual loss (including field defects and cortical blindness), hallucinations, and higher-order visual disturbance (for example, macro- or micropsia, teleopsia, palinopsia, and disturbance of colour vision)
- Pyramidal features included typical patterns of weakness (upper limb extensors weaker than flexors and vice versa in lower limbs), brisk deep tendon reflexes, spasticity and upgoing plantar reflexes
- Extrapyramidal features included various movement disorders (tremors other than intention-type, chorea, athetosis, ballismus, dystonia, and alien limb), rigidity, and particular gait patterns (for example, Parkinsonian)
- Myoclonus included documented myoclonic jerks affecting limb, trunk, head or neck regions

I collated results of the diagnostic investigations comprising the diagnostic criteria. For the 14-3-3 assay, only results classified as 'positive' were considered positive; 'weak positive' results were considered negative. Likewise, 'equivocal' RT-QuIC results were considered negative for the analysis.

In addition I collated data on *PRNP* c129 genotype and PrP^{Sc} glycotype, using these to group cases into subtypes, including those with dual glycotypes (i.e. co-present types 1 and 2A PrP^{Sc}).

Case classification during life

Individuals' in-life diagnoses were classified by the diagnostic criteria. Those with adequate clinical features were classified as *possible* sCJD. Of these, any with at least one supportive investigation were classified as *probable* sCJD. In addition, any individual with progressive neurological disease was classified as having *probable* sCJD in the presence of a positive RT-QuIC assay. Finally, the diagnostic category in those not fulfilling the above definitions was classified as *unclear* – this group included individuals with a positive investigation (other than RT-QuIC) but insufficient clinical features to fulfil a *probable* diagnosis.

Data Collection and Harmonisation

Analysis required homogeneity of data as far as possible. This required translation of variable categories (such as demographic and investigation result headings in data extraction spreadsheets) and qualitative entries (such as findings from neuropathology reports) from their original non-English,

performed by me with guidance from Angeline Denouel, Peter Hermann and Anna Ladogana (on behalf of the French, German and Italian centres respectively). Results were categorised into positive and negative categories as described above. Data collection for UK individuals was kindly facilitated by Jan MacKenzie, UK NCJDRSU coordinator, and for other nations was performed when I visited their centres (Italy 9/12/2019, Germany 13/01/2020, France 23/01/2020), with additional data transferred digitally following the initial visits, including some results not available at the time (for example, neuropathology report data, whether unavailable during my visits or with examinations not yet finalised), likewise where subsequent activity altered outcomes, as in some cases where MRI findings were revised for certain individuals after expert analysis, or where data errors were identified by participating centres for correction. I performed all the data processing and harmonisation, kindly supported by the above individuals whenever queries arose.

Data collection ceased in August 2020 to permit final analysis. Preliminary analyses were performed and presented to centres in the months prior to this to allow targeted collection of outstanding data, for example on neuropathology reports.

Statistical analysis plan I was guided in the planning and delivery of this study by Cat Graham, lead statistician with the Edinburgh Clinical Research Facility, and am grateful for her input over several meetings in 2020 and 2021 when drafting the study design as well as when presenting initial and final results.

i. Sensitivity and specificity

The study was designed to quantify the sensitivity and specificity of the diagnostic criteria during life using a cohort of sCJD cases and a control group of non-cases with alternative diagnoses. The primary analysis was comparing these two groups. The majority of this analysis concerned binary categorical variables, largely positive/negative classification by criteria or diagnostic investigations.

For a given diagnostic investigation, sensitivity is defined as the proportion (commonly expressed as a percentage) of individuals with the condition of interest in whom a positive diagnostic investigation outcome is present – in simplified terms, 'how many individuals with the disease test positive?'[386]. The converse of this is specificity: the proportion (or percentage) or individuals without the condition who appropriately test negative. In some situations this population is composed of healthy controls; in the study this group was a cohort with conditions suspected to be potential CJD during life but with neuropathological exclusion of prion disease and confirmation of an alternative condition.

Individuals were classified by the diagnostic criteria firstly according to clinical features, allowing quantification of sensitivity and specificity of a *possible sCJD* diagnosis. Following this, individuals

were classified by the presence or absence of supportive diagnostic investigations to quantify sensitivity and specificity of a *probable sCJD* diagnosis.

Additional analyses were performed to quantify the sensitivity and specificity of separate investigations in the entire cohort. This was performed irrespective of the presence/absence of clinical features and the outcomes of other investigations (i.e. the sensitivity/specificity of a given investigation taken in isolation).

Subgroups of sCJD cases were generated as follows:

- *PRNP* c129 genotypes (MM, MV and VV)
- Parchi classification (MM1/2, MV1/2, VV1/2, and those with dual PrP glycotypes)
- Age (young and old defined as <1 and >1 standard deviations below and among the mean age respectively)
- Duration (quartile 1, quartiles 2-3, quartile 4)

The sensitivity for diagnostic criteria in aggregate (*possible* and *probable* sCJD classification) and for individual investigations was calculated performed across each of these subgroups. I presented all categorical data as percentages with 95% confidence intervals calculated using the exact binomial method in R software.

ii. Demographic analyses

Demographics between cases and controls, as well as within subgroups, were compared. Age was demonstrated to be approximately normally distributed within the cohort, and age data was presented as mean age in years with standard deviations. Duration was positively skewed: the majority of cases and non-cases were deceased within several months of onset and a minority of individuals in both groups surviving for longer periods. Thus, duration was not normally distributed, and data were presented using median duration measured in days with interquartile ranges.

Age comparison between cases and non-cases was performed using Student's independent samples t-test, while duration comparison was performed using the Mann Whitney U test. When comparing sCJD subgroups, as there were more than two subgroups the analysis of variance (ANOVA) was used for age (with post-hoc analysis for multiple comparisons using Tukey's test) and the Kurskall Wallis test (with post-hoc analysis using Dunn's test and Bonferroni correction factors for multiple comparisons) for duration.

Biological sex data were categorical. Proportions were presented as percentages for cases and non-cases, as well as all subgroups. Comparisons between groups were performed using X^2 tests, or Fisher's exact tests when small numbers were present in subgroups.

As there were nine groups of sCJD subtypes defined according to the Parchi classification schema when including cases with mixed PrP glycotypes, I restricted analysis of these to descriptive methods and did not perform hypothesis testing, as for many groups individual sample sizes were small (in some, n<5), limiting statistical power for such analyses.

iii. Quantification of case re-classification by revised criteria

I compared the revised and previous diagnostic criteria in terms of proportions of individuals classified as having *probable* sCJD during life in a binary fashion (*probable* vs *not probable*, the latter comprising *possible* and *unclear* groups) for cases and non-cases. Individuals were classified by both criteria and the proportions were compared to quantify the change. In practice, no individual could be 'down-graded' in classification by the revised criteria from *probable* to *not-probable*, as by definition any individual fulfilling a *probable* diagnosis by the previous criteria would continue to do so via the revised criteria (the presence of negative RT-QuIC and/or cortical ribboning would not 'disqualify' them); hence the change in proportions represented individuals 'upgraded' to *probable sCJD*.

McNemar's test was used to quantify whether the changes in *probable* classification among cases and non-cases were statistically significant.

Missing Data

It was anticipated that this study would encounter missing data in various forms.

Firstly, individuals undergoing investigation for CJD do not always receive the full complement of investigations. For example, individuals may have contraindications and/or barriers to lumbar puncture (such as intolerance, excessive agitation, anatomical challenges such as degenerative spinal disease or obesity, unacceptably high risks of bleeding complications, and a perception that invasive testing is inappropriate due to advanced or end-of-life status)[387] or MRI (for example, pacemaker devices, claustrophobia or inability to lie still). Assay-specific considerations may preclude testing for some CJD-specific biomarkers, for example RT-QuIC in the presence of heavily blood-stained CSF[97]. Access to some diagnostic investigations may be limited in certain centres, for example EEG in district general hospitals, and MRI in intubated and ventilated patients. Lastly, in individuals with an evident diagnosis supported by a diagnostic investigation (for example a positive MRI), additional supplementary investigations may be considered unnecessary, particularly if invasive, costly, or with results unlikely to be available until a later time point, posing unnecessary delays to diagnosis and decision-making around management, which can be time-critical in a CJD patient with a prognosis limited to a short number of weeks.

Secondly, the study was predicted to experience missing data as a consequence of variations in surveillance methodology between national centres as detailed below. For some nations during the study period, RT-QuIC was performed in the majority of patients with potential CJD, while in others, the role of this novel assay was reserved for complicated or unresolved cases, for example those without other supportive diagnostic investigations. Likewise, in the majority of German cases undergoing autopsy for potential CJD, the national centre of excellence for neuropathological examination in prion disease does not typically perform *PRNP* genotyping and PrP^{Sc} glycotyping via western blot, which was anticipated as a source of missing data.

Thirdly, in a large, multinational study such as this, with each contributing surveillance unit enrolling individuals from sites all across their respective nations, it was anticipated that there would be limitations in the availability of data such as records and investigation results. For example, in some individuals in the UK, surveillance assessments are declined by relatives, resulting in limited clinical information. Among assessed individuals, data collection might have been incomplete, for example due to omissions in sections of assessment questionnaires, or when collateral history from surrogates would be limited (for example where they had limited contact with the assessed individual during the disease). In addition, some regional centres were anticipated to have not responded to requests for clinical information or investigation results raised by national surveillance centres, including for important data such as post-mortem reports in individuals without prion disease. The emergence of COVID-19 during the data collection phase, with widespread resultant disruptions to healthcare infrastructure, was an unanticipated contributing factor to such efforts for data retrieval.

I sought to analyse available data and quantify this as far as possible. Thus, for percentages and proportions in categorical variables such as investigation sensitivity, these were calculated from available data, similarly with mean and median figures for continuous variables. I did not employ methods to impute missing data. Missing data, combined with the rarity of some subgroups, meant that I anticipated that some analyses would be limited in statistical power, for example investigation sensitivity in sCJD subtypes; for these domains I aimed to provide descriptive analyses.

Surveillance centre methodology

Surveillance centres have been developed over 3 decades in response to the BSE epizootic, centred in the UK. Following identification of the first vCJD cases in 1995-1996, systems were upgraded and expanded, and new systems developed in additional nations. While surveillance systems feature broadly overlapping methods and have developed in cooperation, for example as part of the Euro-CJD network, individual nations' systems operate independently and differ in aspects of methodology. A discussion of these is essential to provide context for the presentation of discussion of results from the overall cohort and individual nations.

i. Surveillance in the UK

CJD surveillance existed in the UK[167] prior to the discovery of the BSE epizootic. This discovery fuelled concerns of zoonotic transmission to humans, leading to the development of the UK National CJD Research & Surveillance Unit (NCJDRSU) in 1990 as an upgraded and enhanced surveillance system[1]. The NCJDRSU is based in the University of Edinburgh, currently sited at the Royal Infirmary of Edinburgh, and delivers national surveillance in the UK.

The UK has unique and extensive experiences of CJD. The vCJD epidemic was initially discovered in the UK[132], and the UK experienced the majority of the global epidemic, with 178 cases identified to date[27], the most recent of which died in 2016 and remains the only confirmed case of c129 MV-associated vCJD[55]. Consequently the index of suspicion for potential vCJD is high, particularly among atypical cases, for example young patients, those with prolonged duration, those with atypical phenotypes including psychiatric or painful sensory prodromes, those in whom there may be iatrogenic or occupational exposure to vCJD, and those with negative RT-QuIC, and there are legitimate concerns surrounding the potential for a 'second wave' of vCJD as outlined in **Chapter 1**[1]. The only transfusion-transmitted vCJD cases (n=3) were identified in the UK[127]. Furthermore, 89 cases of iCJD have been identified in the UK since 1970 at the time of writing (August 2022), the majority arising in individuals exposed to c-hGH[42,388].

Reporting of potential or confirmed CJD is not mandatory in the UK. The NCJDRSU receives approximately 33 enquiries per month (monthly average 2017-2020, calculated by me) from a variety of sources[42]. These largely originate from neurologists, but a number of referrals are received from other specialties including psychiatry, medicine of the elderly, and acute/internal medicine. Individuals are referred both from inpatient and outpatient settings. Enquiries comprise individuals with varying indices of suspicion, including some with clinical features and investigation results strongly supportive of prion disease, some with possible prion disease but alternative conditions possible, and some in whom disease is unlikely but testing and a specialist opinion is desired to help definitively exclude prion disease. In some situations individuals are referred following an MRI report raising suspicion of the disease despite the diagnosis not being previously considered based on presenting clinical features.

All enquiries are discussed with a NCJDRSU neurologist. In most patients, MRI brain imaging is recommended and images transferred to the unit for specialist review by unit neurologists and subsequently by an expert neuroradiologist with experience in prion disease imaging (David Summers); this neuroradiologist is blinded to clinical information at the time of imaging review to minimise any introduction of bias. These blinded reports are used as data for research purposes. The distribution of affected regions is recorded for all positive MRI scans. In addition, in individuals with

potential prion disease (i.e. a moderate-to-high pre-test probability), CSF testing is offered. Samples are transported to the NCJDRSU for analysis in the national reference laboratory. Analysis consists of 14-3-3 and S100b proteins and RT-QuIC. 256 samples were processed for suspected CJD in 2020[42]. In situations where CJD is thought unlikely, testing is usually not performed, although CSF can be banked should the clinical picture evolve and the likelihood of CJD increases.

All individuals with a moderate-to-high suspicion of CJD are offered specialist surveillance assessments by NCJDRSU clinicians; the monthly average between 2017-2020 was 11 visits. Assessments are delivered using a structured questionnaire and consist of a detailed epidemiological assessment ascertaining specific risks (including family history and iatrogenic, zoonotic and occupational exposures of relevance) as well as a detailed clinical history and examination, the former commonly given by one or more surrogate sources, typically the patient's next-of-kin. Case notes and diagnostic investigations are reviewed during the visits. During the time period of this study (January 2017-December 2019), all assessments were performed in-person, but with the subsequent COVID-19 pandemic a high number were delivered via telehealth[293].

Genetic testing of *PRNP* is performed in the majority of individuals at the NCJDRSU for ascertainment of codon 129 status. Diagnostic *PRNP* sequencing for pathogenic mutations causing inherited prion disease is performed in individuals who consent (or when capacity is impaired, their next-of-kin consents on their behalf); the NCJDRSU performs this in Scottish cases and for the rest of the UK these are performed by the National Prion Clinic based at University College London.

A minority of individuals undergo autopsy, and the proportion has been falling over several years[42]; the initial procedure is performed in regional neuropathology centres with materials transferred to the NCJDRSU for additional analysis, including western blotting for PrP glycotyping and genetic sequencing of *PRNP* using fresh frozen tissue.

A minority of individuals are diagnosed at post-mortem and were not known to the NCJDRSU during life. In these individuals a 'late' (i.e. posthumous) visit is offered and performed when accepted. The standard clinical and epidemiological assessment questionnaire is completed (albeit without clinical examination), likewise case notes and in-life investigations are reviewed by NCJDRSU clinicians and, where imaging was obtained, the unit neuroradiologist. Depending on the initial autopsy, it may or may not be possible to perform genetic and biochemical PrP testing, which requires fresh frozen tissue to have been obtained and stored.

ii. Surveillance in France

France has experienced the second largest number of vCJD cases (n= 29). The two most recent cases arose in laboratory workers who had worked with prion disease materials. The first had a recorded history of mucocutaneous exposure to BSE material several years prior to symptom onset and had

autopsy-confirmed vCJD[34], while the second case[219] received a *probable* vCJD diagnosis (i.e. autopsy was not performed; Jean-Philippe Brandel, personal communication). In addition, France experienced the highest number of c-hGH-iCJD cases (n=123 as of April 2022; Angeline Denouel, personal communication). The Cellule Nationale de Réference des maladies de Creutzfeldt-Jakob (CNRMCJ) is based in Paris and is led by Jean-Philippe Brandel, a senior neurologist with extensive prion disease experience. Referrals are processed by the unit and medical records and physicians' reports are sent. In contrast to the NJCDRSU, in-person visits are only performed in atypical cases, such as those with suspicion of vCJD, as well as local regional cases.

Biochemical testing is mostly performed by a laboratory in Paris, although nationally five labs perform 14-3-3 testing; besides Paris, the second-largest unit is in Lyon. Unlike the NCJDRSU model, referrers do not need to discuss cases with the unit prior to testing, and consequently a higher number of assays are performed (annual mean of 1812 assays, 2010-2020; Angeline Denouel, personal communication). The RT-QuIC assay is performed in Paris. At the time of my visit to the unit this was only being performed in a select proportion of cases, mostly those with atypical features or a clinically unclear syndrome; for example if a patient had a positive 14-3-3 but a syndrome not suggestive of CJD, RT-QuIC might be used to rule out prion disease if negative. Consequently during the study period only a minority of French individuals were undergoing RT-QuIC testing.

Imaging is reported by regional neuroradiologists, but Professor Jean-Philippe Brandel sometimes reviews imaging, for example in unclear or complex cases. Genetic testing is performed in a centre in Paris. Finally, neuropathological examination is performed in a sixteen centres nation-wide, with additional analysis performed in centres in Paris or Lyon (including western blotting for PrP glycotyping).

iii. Surveillance in Germany

Germany began its formal CJD surveillance programme in 1993[51], but has thus far not identified any cases of vCJD. The National Reference Centre for Transmissible Spongiform Encephalopathies (NRZ-TSE) is based in Göttingen. The NRZ-TSE receives referrals from three sources. Firstly, reporting of potential CJD cases is mandatory in Germany, initially to local health authorities and subsequently to the NRZ-TSE. Secondly, many regional laboratories perform 14-3-3 testing, and positive assay results are referred onto the NRZ-TSE, which accounts for several thousand enquiries annually. Finally, similar to the UK and French systems, the NRZ-TSE receives direct enquiries from treating physicians around Germany concerning cases of suspected CJD, typically around 400 per year.

In a manner similar to the French system, local clinicians complete a specific questionnaire to screen for the potential of prion disease as well as risk factors for iatrogenic and genetic forms. The NRZ-TSE clinicians maintain ongoing contact with treating clinicians and receive formal reports on clinical

progress in cases of suspected CJD. Historically, visits were performed, but in the modern era these are only performed in cases of potential vCJD or other atypical circumstances, for example cases thought to harbour a potential novel TSE.

CSF RT-QuIC testing is performed in any patient with a positive 14-3-3 assay, as well as some cases with negative 14-3-3 in whom clinical suspicion is present for CJD. MRI brain images are reported by regional neuroradiologists, and images are usually reviewed by the unit clinicians, a group with extensive expertise in the imaging features of prion disease[92,93,389]. Genetic testing is performed when affected individuals or their next-of-kin provide consent; Peter Hermann, NRZ-TSE neurologist, quoted an approximate figure of 20% of cases. This allows *PRNP* c129 genotyping, though this is sometimes performed separately; the NRZ-TSE uses this information both for research and surveillance classification and, in contrast to the NJCDRSU, as a diagnostic test, for example in atypical cases with prolonged disease duration, to aid in-life distinction of sCJD subtypes.

Autopsies are performed in 20-30% of cases, initially in regional centres and with supplementary analysis in two sites: Hamburg and Homburg. The former centre performs the majority of these, and the neuropathology service does not perform biochemical PrP glycotyping, instead providing morphological diagnoses of the apparent sCJD subtype (for example if typical features of MM2-C sCJD are present).

iv. Surveillance in Italy

The Italian surveillance unit is based in the Istituto Superiore di Sanita (ISS) in Rome and has been operational since 1993. Three cases of vCJD have been identified to date; one of these was a laboratory worker who previously worked with prion material, and in contrast to the published French case, no historical occupational injury was identified in this individual[34]. No formal report was published in the literature concerning this vCJD case. The unit receives referrals from physicians across Italy, predominantly neurologists. The unit clinicians liaise with referring physicians and complete a questionnaire to evaluate the potential for prion disease and the presence or absence of important risk factors. In-person visits are not performed with the exception of local cases.

CSF biomarker testing, including RT-QuIC analysis, is performed in multiple centres across Italy. Italian prion disease experts have been instrumental in the development of olfactory mucosa RT-QuIC, a technique which is described as less invasive than CSF sampling and can boost sensitivity to near-100%[359]. This technique is employed in a number of cases in Italy and was not widely employed in other contributing nations during the period of this study. MRI brain imaging data is mostly collected from regional reporting, with a minority of images reviewed by the unit.

Genetic testing is offered in most cases and is agreed to in the majority of cases (Anna Ladogana, ISS lead neurologist; personal communication). Autopsies are performed in a relatively high number of individuals (~50%) in contrast to other nations contributing to this study, and are mandatory in any cases of suspected vCJD.

Clinical case vignettes

Numerous cases are described in separate clinical vignette boxes throughout the text. These selected examples are included for illustration of the variety of clinical phenotypes encountered in CJD surveillance as well as challenging situations, including atypical subtypes, genetic forms and CJD mimics. All were included from the UK surveillance cohort, and are either i) individuals I directly assessed, or ii) individuals included within the UK cohort in this thesis.

Conclusion

This study includes a large and comprehensive international cohort of sCJD cases and non-case controls derived from four leading centres of expertise in prion disease surveillance. The primary objective is to validate the diagnostic criteria and explore the impact of revisions on case ascertainment, as well as the impact of important subgroups of sCJD cases. Surveillance methods used between participating centres broadly overlap, though there are important differences of relevance to the analysis which are outlined above.

The results of the study are presented subsequently in **Chapters 4-8**.

Chapter 4. Full international cohort

This **Chapter** presents analysis results from the full international cohort, comprising individuals with sCJD and alternative diagnoses from a three-year period (2017-2019) of national surveillance performed in the UK, France, Germany and Italy (see **Chapter 3** for a description of these and their methods).

Contents

1. Demographics

647 individuals were included in the study from the four participating nations. The Italian centres contributed the largest number (n=263, 40.7% of the cohort), followed by centres in Germany (n=146, 22.6%), France (n=135, 20.9%) and the UK (n=103, 15.9%) respectively.

501 individuals had neuropathologically-confirmed sCJD and were classified as 'cases', comprising 77.4% of the cohort (**table 4.1**). The remaining 146 individuals had alternative, non-prion neuropathological diagnoses; these 'non-cases' comprised 22.6% of the cohort. There was a similar sex distribution between cases and non-cases (male n=253 (50.5%) versus n=74 (50.7%) respectively; P=0.98). Mean age in cases was statistically significantly younger than non-cases [68.9 years (SD 9.5) versus 71.1 years (SD11.6); **P<0.001**]. Median total disease duration was statistically significantly longer in cases than non-cases [118 days (IQR 74.75-222.5) vs 85 (51.5-205.5); **P=0.002**]. All cases were diagnosed via autopsy, while 139 (95.2%) non-cases were diagnosed at autopsy, with the remaining 7 (4.8%) diagnosed via brain biopsy.

Table 4.1. Demographic features, international cohort

Feature	Cases	Non-cases	*P*
Cohort size	501	146	
Male (%)	253 (50.5)	74 (50.7)	0.98
Female (%)	247 (49.3)	72 (49.3)	
Missing (%)	1 (0.2%)	-	
Mean age, years (SD)	68.9 (9.5)	71.1 (11.6)	**<0.001**
Median duration, days (IQR)	118 (74.75-222.25)	85 (51.5-205.5)	**0.002**
Biopsy, n (%)	-	7 (4.8)	
Autopsy, n (%)	501 (100.0)	139 (95.2)	

Abbreviations. IQR, interquartile range. SD, standard deviation.

2. Non-case diagnoses

Neuropathological examination reports providing tissue diagnoses were available in 97 non-cases (66.0% of cohort). For the remainder, efforts to obtain final reports were unsuccessful during the data collection period; all individuals were known to have had CJD excluded via neuropathology however. Diagnoses are summarised in **table 4.2**.

The commonest category was neurodegenerative aetiologies representing 42.3% (n=41) of non-cases, followed by vascular at 16.5% (n=16). The commonest individual diagnosis was Alzheimer's disease (AD) (n=21; 21.6% of cohort). A further 12.4% (n=12) of non-cases had AD co-occurring with additional pathology, 10 (10.3%) with co-morbid dementia with Lewy bodies (DLB), 1 (1%) with multiple system atrophy (MSA) and 1 (1%) with tauopathy. The commonest cerebral insult was anoxic brain injury, accounting for 8 (8.2%) non-cases. Neuropathological examination was non-diagnostic in 8.2% of non-cases, although a prion disease was excluded.

Table 4.2. Neuropathological examination results among non-cases (where reports available)

Neurodegenerative			Vascular			Cerebral insult			Inflammation		
Dx	n	%	Dx	n	%	Dx	n	%	Dx	n	%
Total	41	42.3	Total	16	16.5	Total	15	15.5	Total	8	8.2
AD	21	21.6	Cerebro-vascular	12	12.4	Anoxia	8	8.2	CD8+ encephalitis	3	3.1
Dual	12	12.4	Vasculitis	2	2.1	Hypoglycaemia	1	1.0	AIE	2	2.1
AD + DLB	10	10.3	APS	1	1.0	Seizure	1	1.0	Influenza-associated ANE	1	1.0
AD + MSA	1	1.0	CAA	1	1.0	HE + seizure	1	1.0	Behcet's	1	1.0
AD + Tau	1	1.0				Anoxia + seizures	1	1.0	Inflammation, NOS	1	1.0
DLB	3	3.1				Subcortical Necrosis	1	1.0			
Tauopathy	2	2.1				Metabolic, NOS	2	2.1			
TDP43	2	2.1									
CBD	1	1.0									

Abbreviations. AD, Alzheimer's disease. AIE, autoimmune encephalitis. ANE, acute necrotizing encephalopathy. APS, antiphospholipid syndrome. CAA, cerebral amyloid angiopathy. CBD, corticobasal degeneration. DLB, dementia with Lewy Bodies. Dx, diagnosis. HE, hepatic encephalopathy. MSA, multiple system atrophy. NOS, not otherwise specified. RCC, renal cell cancer. TDP-43, TAR DNA-binding protein 43.

Table 4.2 (continued).

Infection			Miscellaneous			Non-diagnostic	
Dx	n	%	Dx	n	%	n	%
Total	2	2.1	Total	7	7.2	8	8.2
Abscess	1	1.0	Gliosis	1	1.0		
Toxoplasmosis	1	1.0	Fahr disease	1	1.0		
			Leuko-encephalopathy	1	1.0		
			Metastatic RCC	1	1.0		
			Encephalopathy, NOS	1	1.0		
			Other, NOS	2	2.1		

Abbreviations. Dx, diagnosis. RCC, renal cell cancer. NOS, not otherwise specified

3. Clinical features

Clinical information regarding cardinal neurological features used in the diagnostic criteria for sCJD was available in 487 (97.2%) cases and 127 (87.0%) non-cases (**table 4.3**). All cardinal clinical features were more frequently reported in cases than non-cases. Rapidly-progressive cognitive decline (RPCD) was the commonest symptom, encountered in 479 (98.4% [95% CI, 96.8%-99.3%]) cases and 112 (88.2% [95% CI, 81.3%-93.2%]) non-cases (**P<0.001**). Myoclonus was reported in 334 (68.6% [95% CI, 64.3%-72.7%]) cases and 52 (40.9% [32.3%-50.0%]) non-cases (**P<0.001**). Visual features were the least frequently reported feature in non-cases (n=11, 8.7% [95% CI, 4.4%-15.0%]) compared with cases (n=235, 48.3% [95% CI, 43.7%-52.8%]; **P<0.001).** Pyramidal features were the least frequently identified feature in cases (n=215, 44.1% [95% CI, 39.7%-48.7%) and were uncommon in non-cases (n=33, 26.0% [95% CI, 18.6%-34.5%]; **P<0.001**).

Table 4.3. Clinical features

	Cases (n=487)		Non-cases (n=127)		
Feature	**n**	**% [95% CI]**	**n**	**% [95% CI]**	***P***
RPCD	479	98.4 [96.8-99.3]	112	88.2 [81.3-93.2]	**<0.001**
Myoclonus	334	68.6 [64.3-72.7]	52	40.9 [32.3-50.0]	**<0.001**
Visual	235	48.3 [43.7-52.8]	11	8.7 [4.4-15.0]	**<0.001**
Cerebellar	360	73.9 [69.8-77.8]	34	26.8 [19.3-35.4]	**<0.001**
Pyramidal	215	44.1 [39.7-48.7]	33	26.0 [18.6-34.5]	**<0.001**
Extrapyramidal	247	50.7 [46.2-55.3]	43	33.9 [25.7-42.8]	**0.001**
Akinetic Mutism	217	44.6 [40.1-49.1]	30	23.6 [16.5-32.0]	**<0.001**

Figures calculated using individuals for whom clinical data were available.
F.E.T. used for RPCD

Abbreviations. 95% CI, 95% confidence interval. FET, Fisher's exact test. RPCD, rapidly-progressive cognitive decline.

4. Case classification

Classification by diagnostic criteria was possible in 488 sCJD cases (97.4% of cohort) in whom data was available regarding clinical features and one or more relevant investigation (MRI brain, CSF RT-QuIC, or EEG) had been completed ('any' analysis) (**tables 4.4 & 4.5, figure 4.1**). 378 (77.5% [95% CI, 73.3%-81.1%]) cases met criteria definitions of *probable* sCJD using the prior criteria, rising to 450 (92.2% [89.5%-94.4%]) cases using revised criteria, a 14.7% increase; thus the sensitivity of *probable* sCJD diagnosis using prior and revised (2017) criteria was 77.5% and 92.2% respectively (**P<0.001**). Applying prior criteria, 42 cases (8.6% [95% CI 6.3%-11.5%]) fulfilled classification as

possible sCJD and 68 (13.9% [95% CI, 11.0%-17.3%]) were classified as *unclear*; these numbers decreased to 10 (2.1% [95% CI, 1.0%-3.7%]; 6.6% decrease) and 28 (5.7% [95% CI, 3.9%-8.2%]; 8.2% decrease) respectively using revised criteria. 17 (3.5%) cases were reclassified from *possible* to *probable* sCJD due to cortical ribboning on MRI, 5 (1.0%) by RT-QuIC, and 10 (2.1%) by both. 40 (8.2%) cases were reclassified from *unclear* to *probable* sCJD by RT-QuIC (**table 4.6**).

Table 4.4. Classification by diagnostic criteria

	Prior		Revised		
Classification	**n**	**% [95% CI]**	**n**	**% [95% CI]**	**Change (%)**
Any investigation					
Cases (n=488)					
Probable	378	77.5 [73.5-81.1]	450	92.2 [89.5-94.4]	14.7
Possible	42	8.6 [6.3-11.5]	10	2.1 [1.0-3.7]	-6.5
Unclear	68	13.9 [11.0-17.3]	28	5.7 [3.9-8.2]	-8.2
Non-cases (n=125)					
Probable	23	18.4 [12.0-26.3]	24	19.2 [12.7-27.2]	0.8
Possible	26	20.8 [14.1-29.0]	25	20.0 [13.4-28.1]	-0.8
Unclear	76	60.8 [51.7-69.4]	76	60.8 [51.7-69.4]	0.0
All investigations					
Cases (n=223)					
Probable	170	76.2 [70.1-81.7]	218	97.8 [94.9-99.3]	21.5
Possible	14	6.3 [3.5-10.3]	1	0.5 [0.0-2.5]	-5.8
Unclear	39	17.5 [12.7-23.1]	4	1.8 [0.5-4.5]	-15.7
Non-cases (n=52)					
Probable	16	30.8 [18.7-45.1]	17	32.7 [20.3-47.1]	1.9
Possible	11	21.2 [11.1-34.7]	10	19.2 [9.6-32.5]	-1.9
Unclear	25	48.1 [34.0-62.4]	25	48.1 [34.0-62.4]	0.0

Abbreviations. CI, confidence interval.

Table 4.5. Sensitivity & specificity of diagnostic criteria

	Sensitivity		Specificity	
	Positive/total	% [95% CI]	Negative/total	% [95% CI]
Diagnostic criteira				
Probable sCJD				
Any investigation				
Revised	450/488	92.2 [89.5-94.4]	101/125	80.8 [72.8-87.3]
Prior	378/488	77.5 [73.5-81.1]	102/125	81.6 [73.7-88.0]
All investigations				
Revised	218/223	97.8 [94.9-99.3]	35/52	67.3 [52.9-79.7]
Prior	170/223	76.2 [70.1-81.7]	36/52	69.2 [54.9-81.3]

Table 4.6. Cases reclassified as probable sCJD shown by prior classification

	Possible (n=32)		Unclear (n=40)	
Investigation	n	%	n	%
Cortical ribboning	17	3.5	-	
RT-QuIC	5	1.0	40	8.2
Both	10	2.1	-	

Abbreviations. RT-QuIC, real-time quaking-induced conversion. sCJD, sporadic Creutzfeldt-Jakob disease.

Of the 28 cases who remained classified as *unclear* by revised criteria due to a clinically incompatible or limited phenotype, 27 (96.4%) had at least one positive/supportive investigation. 7 (25.0%) had a positive EEG, 15 (53.6%) had positive MRI (5 with isolated basal ganglia hyperintensities, 8 with isolated cortical ribboning, and 2 with both), and 11 (39.3%) had positive 14-3-3. In 2 (7.1%) individuals, EEG, MRI and 14-3-3 were all positive.

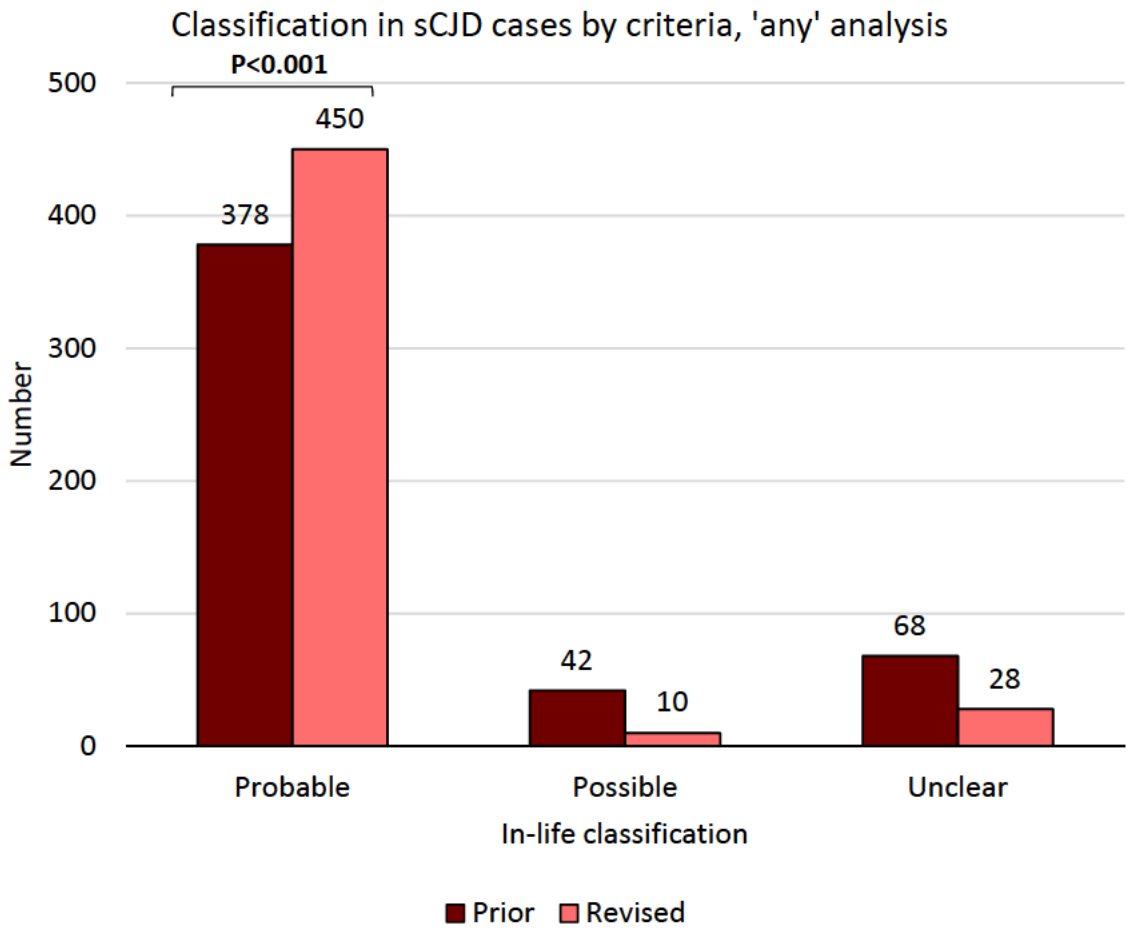


Figure 4.1. Classification in sCJD cases by criteria, 'any' analysis.

In-life classification of cases by prior and revised diagnostic criteria is shown for sCJD cases undergoing any combination of investigations. There was a significant increase in the number of cases fulfilling *probable* case definitions.

To exclude the possibility that some of the above individuals in the 'any' analysis not fulfilling *probable* classification might have done so if they had undergone the full complement of investigations, a sub-analysis ('all' analysis) was performed of the 223 cases (44.5% of cohort) with clinical information available who underwent all (MRI, CSF, and EEG) investigations (**tables 4.4 & 4.5**, **figure 4.2**). Using this approach, 170 (76.2% [95% CI, 70.1%-81.7%]) of cases fulfilled *probable* sCJD classification by prior criteria, rising 21.5% to 218 (97.8% [95% CI, 94.9%-99.3%]) with revised criteria (**P<0.001**). When all clinical features were available and cases had undergone all investigations, only 1 (0.5% [95% CI, 0.0%-2.5%]; 5.8% decrease) case remained classified as *possible* sCJD, and 4 (1.8% [95% CI, 0.5%-4.5%]; 15.7% decrease) remained classified *unclear* (**table 4.7**).

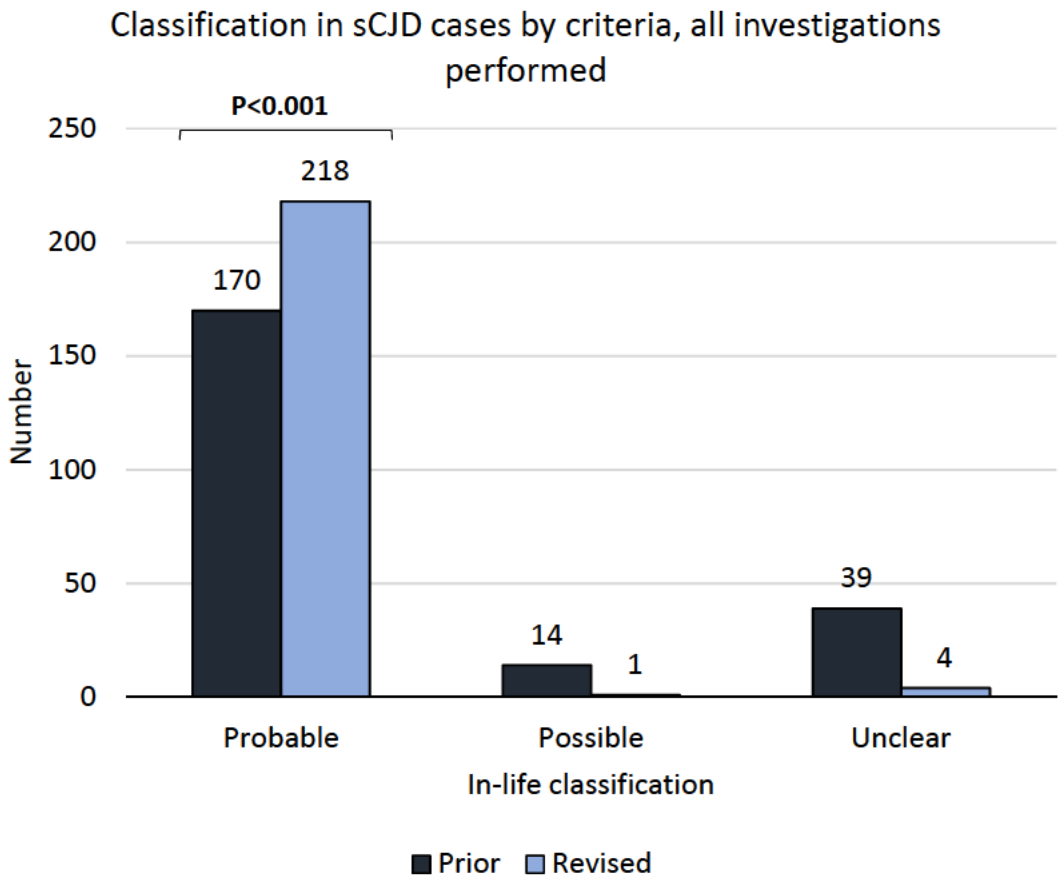


Figure 4.2. Classification in sCJD cases by criteria, 'all' analysis

In-life classification of cases undergoing the full panel of investigations. There was a significant increase in the number of cases fulfilling *probable* case definitions.

Table 4.7. sCJD cases not fulfilling *probable* classification when all investigations performed

	Case 1	Case 2	Case 3	Case 4	Case 5
Nation	Germany	Germany	Italy	UK	Italy
Age (years)	57.5	82.7	76	53	85
Sex	M	M	F	F	M
Duration (days)	954	196	487	911	71
Clinical features	RPCD, cerebellar	RPCD, cerebellar	RPCD, visual, cerebellar	RPCD, pyramidal	RPCD, myoclonus, pyramidal
MRI	Cortical ribboning	Negative	Negative	Basal ganglia	Negative
14-3-3	Positive	Positive	Positive	Negative	Negative
RT-QuIC	Negative	Negative	Negative	Negative	Negative
EEG	Negative	Negative	Negative	Negative	Negative
c129	-	-	-	MM	-
PrP^{Sc}	-	-	Type 1	Type 2	Type 2
Classification	*Unclear*	*Unclear*	*Unclear*	*Unclear*	*Possible*

Abbreviations. EEG, electroencephalography. MRI, magnetic resonance imaging. PrP^{Sc}, prion protein glycotype. RPCD, rapidly-progressive cognitive decline. RT-QuIC, real-time quaking-induced conversion. sCJD, sporadic Creutzfeldt-Jakob disease. UK, United Kingdom.

In non-cases, data were available for classification in 125 (85.6%) of individuals for a similar 'any' analysis (**tables 4.4 & 4.5, figure 4.3**). There was little change in case classification using prior and revised criteria; the number classified as *probable* sCJD rose from 23 (18.4% [95% CI, 12.0%-26.3%]) to 24 (19.2% [95% CI, 12.7%-27.2%]; 0.8% increase), with only one individual being re-classified from *possible* to *probable* sCJD. This individual was within the Italian cohort and was re-classified due to the presence of cortical ribboning on MRI; data on the underlying neuropathological diagnosis was not available during the study. Thus the specificity of *probable* sCJD diagnosis was 80.8% (95% CI, 72.8%-87.3%) with revised criteria, with a 0.8% decrease from 81.6% (95% CI, 73.7%-88.0%) on prior criteria; this decrease was not statistically significant (P>0.99).

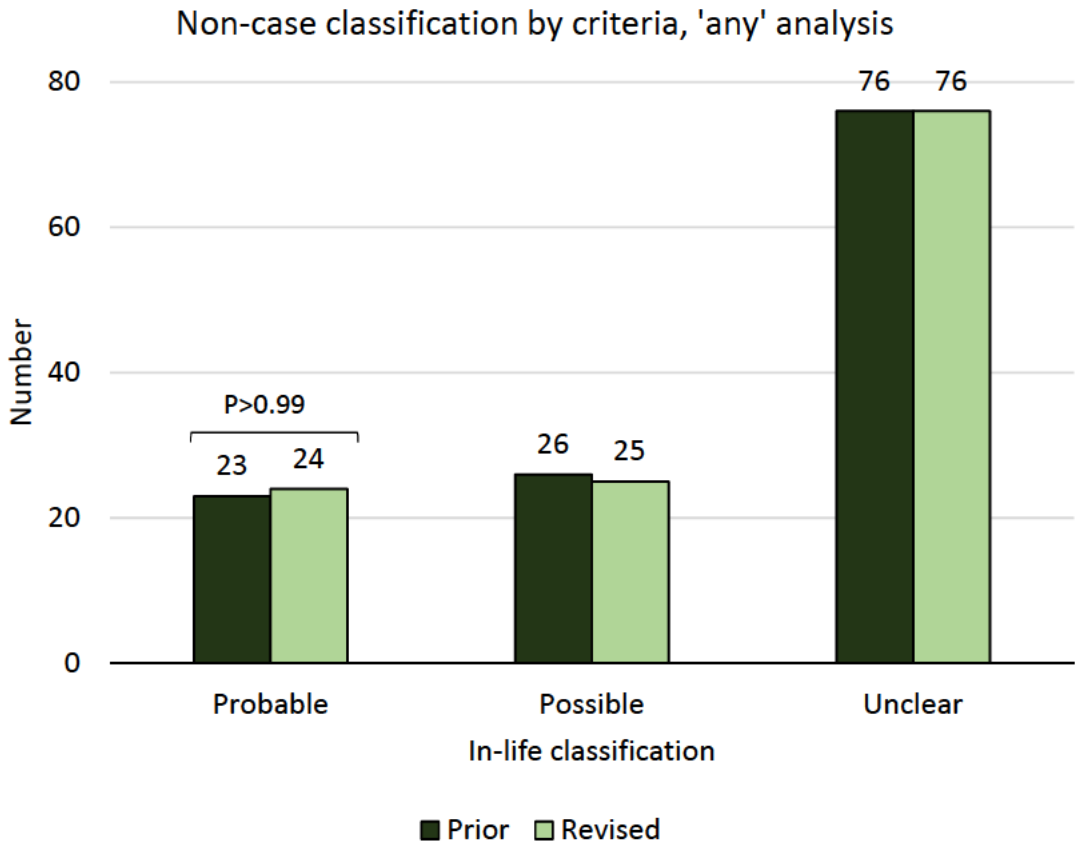


Figure 4.3. Non-case classification by criteria, 'any' analysis

In-life classification of non-cases undergoing any combination of investigations. There was no significant change in the number fulfilling *probable* case definitions.

Neuropathological data were available in 16 (66.7%) non-cases fulfilling criteria for *probable* sCJD; diagnoses were Alzheimer's disease (AD) (7, 43.8%; 1 had additional dementia with Lewy body

(DLB) pathology), anoxic injury (3, 15.8%), CD8+ encephalitis (2, 12.5%), cerebrovascular disease (1, 6.3%), DLB (1, 6.3%), cerebral abscess (1, 6.3%) and influenza-associated necrotizing encephalopathy (1, 6.3%).

Analogous to the 'all' analysis approach used in cases, 52 non-cases had clinical data available and had completed the full panel of investigations ((**tables 4.4 & 4.5, figure 4.4)**. In this 'all' analysis subgroup, 16 (30.8% [95% CI, 18.7%-45.1%] met prior criteria definitions for *probable* sCJD; this increased by 1 to 17 (32.7% [95% CI, 20.3%-47.1%]) with revised criteria. Thus the specificity for *probable* sCJD diagnosis was 67.3% (95% CI, 52.9%-79.7%) with revised criteria compared to 69.2% (54.9%-81.3%) using prior criteria. This 1.9% decrease was not statistically significant (P>0.99).

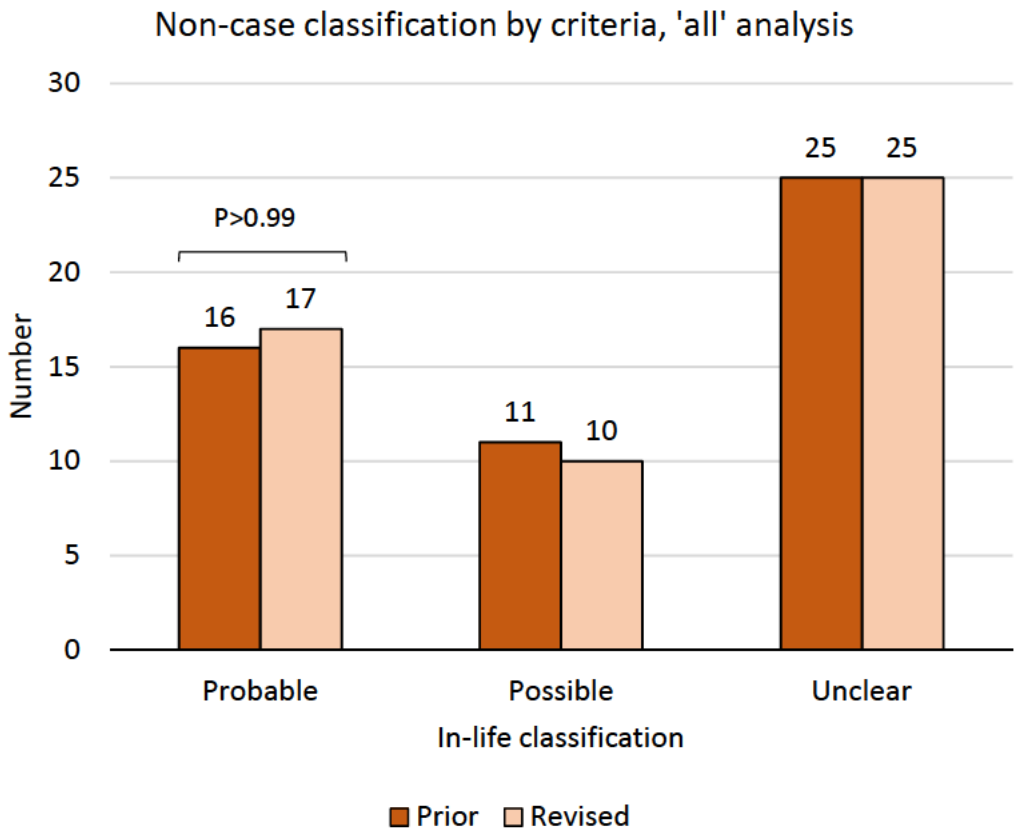


Figure 4.4. Non-case classification by criteria, 'all' analysis

In-life classification of non-cases undergoing the full panel of investigations. There was no significant change in the number fulfilling *probable* case definitions.

5. Sensitivity and specificity

5.1. Clinical features

Of the 487 cases with detailed clinical information available, 420 (86.2%) had sufficient clinical features present to fulfil the *possible* sCJD case definition (i.e. rapidly-progressive dementia plus two or more additional features), thus the sensitivity of clinical features was 86.2% [95% CI, 82.9%-89.2%]). In the 125 non-cases with information available, 49 (39.2%) had sufficient clinical features present, hence the specificity of clinical features was 60.8% [95% CI, 51.7-69.4%].

5.2. Diagnostic investigations

5.2.1. CSF assays

Sensitivity and specificity were calculated for diagnostic investigations (**table 4.8**). CSF RT-QuIC was the most sensitive investigation (251 of 274, 91.6% [95% CI, 87.7%-94.6%]), as well as the most specific (77 of 77, 100% [95% CI, 96.2%-100%]), with no positive results identified in non-cases. 453 cases had 14-3-3 examination, with a sensitivity of 72.0% (95% CI, 67.6%-76.0%). The specificity was lower, with 67 of 123 non-cases testing negative (54.5%; 95% CI, 45.2%-63.5%)

Table 4.8. Sensitivity and specificity of investigations

	Sensitivity		Specificity	
Investigation	Positive/total	% [95% CI]	Negative/total	% [95% CI]
EEG	207/448	46.2 [41.5-50.9]	104/118	88.1 [80.9-93.4]
MRI (all)	395/455	86.8 [83.4-89.8]	91/111	82.0 [73.6-88.6]
CR & BG	181/455	39.8 [35.3-44.4]	107/111	96.4 [91.0-99.0]
CR alone	128/455	28.1 [24.0-32.5]	100/111	90.1 [83.0-94.9]
BG alone	86/455	18.9 [15.4-22.8]	106/111	95.5 [89.8-98.5]
CR (any)	309/455	67.9 [63.4-72.2]	96/111	86.5 [78.7-92.2]
BG (any)	267/455	58.7 [54.0-63.2]	102/111	91.9 [85.2-96.2]
14-3-3	326/453	72.0 [67.6-76.0]	56/123	45.5 [36.5-54.8]
RT-QuIC	251/275	91.6 [87.7-94.6]	77/77	100.0 [96.2-100]

Sensitivity defined as positive outcome/total for cases. Specificity defined as negative outcome/total for non-cases. Brackets show percentages
Abbreviations. BG, basal ganglia. CR, cortical ribboning.

Among the 56 (45.5%) non-cases with positive 14-3-3, neuropathological data were available in 41 (73.2%); aetiologies were neurodegenerative (11, 26.8%), cerebral insults (10, 24.4%), vascular (7, 17.1%), inflammatory (6, 14.6%), cerebral abscess (1, 2.4%), Fahr disease (1, 2.4%), leukoencephalopathy (1, 2.4%), cerebral metastases (1, 2.4%), non-specific encephalopathy (1) and non-diagnostic autopsies (2, 4.8%).

5.2.2. MRI

455 cases and 111 non-cases underwent MRI brain; sensitivity was 86.8% (95% CI, 83.4%-89.8%) and specificity 88.0% (95% CI, 73.6-88.6%). Sensitivity of multifocal cortical ribboning was 67.8% (95% CI, 63.4%-72.2%) and specificity 86.5% (95% CI, 78.7%-92.2%). Sensitivity of basal ganglia hyperintensity was 58.3% (95% CI, 54%-63.2%) and specificity 91.9% (95% CI, 85.2%-96.2%). Co-occurrence of cortical ribboning and basal ganglia hyperintensity was seen in 39.8% of cases (95% CI, 35.3%-44.4%), and 3.6% of non-cases (i.e. specificity 96.4%; 95% CI, 91.0%-99.0%). Isolated cortical ribboning was seen in 28.1% of cases (95% CI, 24.0%-32.5%) and 9.9% of non-cases (i.e. specificity 90.1%; 95% CI, 83.0%-94.9%), while isolated basal ganglia hyperintensity was seen in 18.9% of cases (95% CI, 15.4%-22.8%) and 4.5% non-cases (specificity 95.5%; 95% CI, 89.8%-98.5%).

Among non-cases with cortical ribboning, autopsy results were available in 10 (66.7%); diagnoses were AD (5, 50.0%; 2 had dual pathology: 1 had co-occurring DLB, 1 had a tauopathy. Seizures were present in 3), autoimmune encephalitis (1, 10.0%), hepatic encephalopathy with seizures (1, 10.0%), antiphospholipid syndrome (1, 10.0%), non-specific encephalopathy (1, 10.0%) and non-diagnostic autopsy (1, 10.0%).

In 9 (8.1%) non-cases with basal ganglia hyperintensities, autopsy data were available in 5 (55.6%); diagnoses were AD (1, 20.0%), dual AD and DLB (1, 20.0%), DLB (1, 20.0%) and non-diagnostic autopsies (2, 40.0%).

5.2.3. EEG

EEG was performed in 448 cases and was positive in 207 (sensitivity 46.2%; 95% CI, 41.5%-50.9%). This was the least sensitive diagnostic investigation. 118 non-cases underwent EEG, which was negative in 104 non-cases (specificity 88.1%; 95% CI, 80.9%-93.4%).

In the 14 (11.9%) non-cases with positive EEG findings, autopsy data were available in 7 (50.0%) individuals; diagnoses were AD (2, 11.8%), antiphospholipid syndrome (APS) (1, 14.3%), hypoglycaemic encephalopathy (1, 14.3%), anoxic brain injury and status epilepticus (1, 14.3%), tauopathy (1, 14.3%) and non-diagnostic autopsy (1, 14.3%).

5.2.4. Investigation combinations

The number of diagnostic investigations performed in cases and non-groups was quantified (**table 4.9, figure 4.5**). In both groups the majority of individuals underwent at least three investigations (438 [87.4%] cases and 106 [72.6%] non-cases). Partial investigation (≤3 investigations) was commoner than undergoing all investigations in both groups (55.1% of cases, 64.4% of non-cases). The most frequent scenario in cases was to have undergone the full panel of MRI, CSF RT-QuIC, CSF 14-3-3, and EEG (n=225, 44.9% of cohort), followed by three (n=213, 42.5%), two (n=35, 7.0%), one (n=21, 4.2%) and least frequently no investigations (n=7, 1.4%). In non-cases the most frequent scenario was to have undergone three investigations (n=54, 37.0%) followed by the full panel (n=52, 35.6%), then two (n=22, 15.1%), one (n=15, 10.3%) and least frequently no investigations (n=3, 2.1%).

Specific combinations of investigations undergone are shown in **table 4.9.** In individuals who underwent three investigations, the commonest combination in both groups was to have undergone EEG, MRI brain and CSF 14-3-3 (166 [77.9%] cases vs 32 [59.3%] non-cases). Among those who underwent only one investigation this was most commonly MRI brain in cases (n=14, 66.7% of subgroup), while equal numbers of non-cases underwent MRI alone (n=6, 40.0%) and 14-3-3 alone (n=6, 40.0%). No individuals in either group underwent RT-QuIC alone.

Table 4.9. Combinations of diagnostic investigations performed

	Investigation performed				Cases		Non-cases	
No.	EEG	MRI	14-3-3	RT-QuIC	No.	%	No.	%
0					7	1.4	3	2.1
1	Yes				2	0.4	3	2.1
		Yes			14	2.8	6	4.1
			Yes		5	1.0	6	4.1
				Yes	0	0.0	0	0.0
2	Yes	Yes			18	3.6	9	6.2
	Yes		Yes		10	2.0	9	6.2
	Yes			Yes	1	0.2	0	0.0
		Yes	Yes		5	1.0	1	0.7
		Yes		Yes	0	0.0	0	0.0
			Yes	Yes	1	0.2	3	2.1
3	Yes	Yes	Yes		166	33.1	32	21.9
	Yes	Yes		Yes	6	1.2	2	1.4
	Yes		Yes	Yes	20	4.0	11	7.5
		Yes	Yes	Yes	21	4.2	9	6.2
4	Yes	Yes	Yes	Yes	225	44.9	52	35.6

Left-most column indicates the number of investigations performed.

Abbreviations. EEG, electroencephalography. MRI, magnetic resonance imaging. RT-QuIC, real-time quaking-induced conversion.

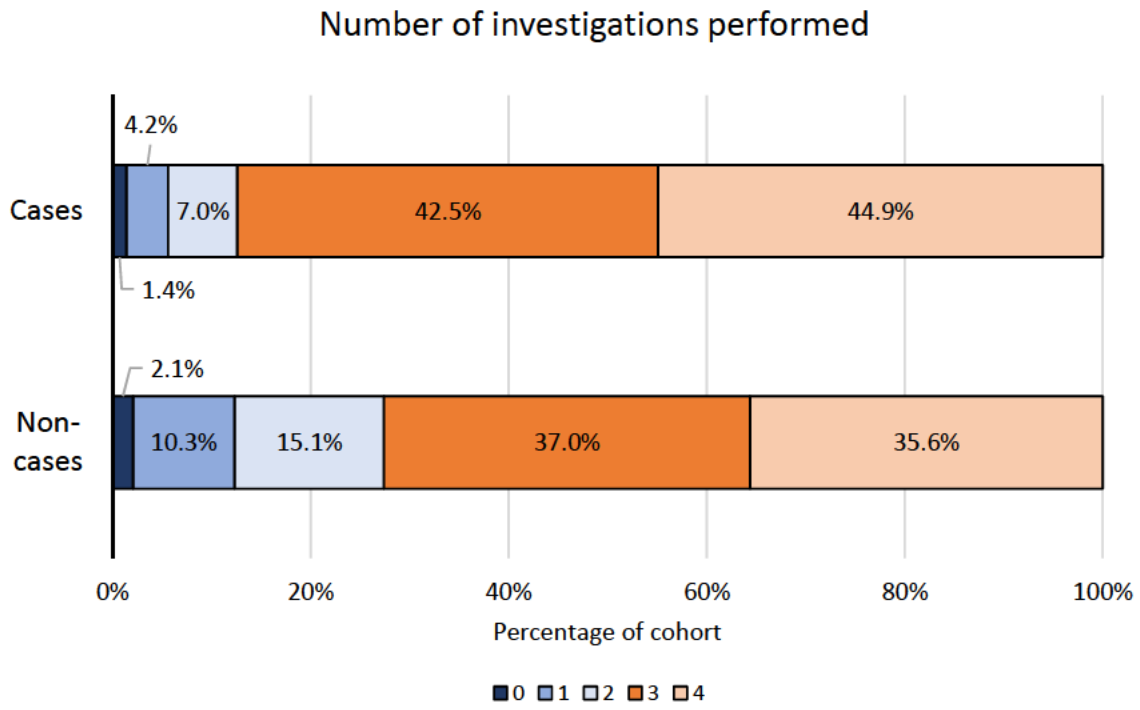


Figure 4.5. Number of CJD-related diagnostic investigations performed, cases and non-cases.

Cases of definite sCJD and non-cases with alternative disorders confirmed on neuropathological examination are shown according to the number of diagnostic investigations for sCJD they underwent during life. The commonest situation for cases was for all the investigations to be performed, while in non-cases the commonest situation was to have undergone three investigations.

I evaluated the sensitivity and specificity of combinations of two and then three investigations (**table 4.10**). Sensitivity was calculated from cases with ≥1 positive investigation, while specificity was calculated from non-cases with all investigations negative. MRI with RT-QuIC was the most sensitive investigation pairing (248 of 252 [98.4%; 95% CI, 96.0%-99.6%]), while EEG with RT-QuIC was the most specific (both negative in 58 of 63 non-cases [92.1%; 95% CI, 82.4%-97.4%]). For groups of three investigations, MRI with both 14-3-3 and RT-QuIC performed was the most sensitive combination (244 of 246 [99.2%; 95% CI, 97.1%-99.9%]), while the most specific combination of three investigations was EEG with MRI and RT-QuIC (45 of 54 [83.3%; 95% CI, 70.7%-92.1%]). No combination of investigations which included 14-3-3 achieved specificity >50% (**table 4.10**).

Table 4.10. Sensitivity & specificity of investigation combinations

	Sensitivity			Specificity		
		%	95% CI		%	95% CI
Two investigations						
EEG						
+ MRI	388/415	93.5	90.7-95.7	62/84	73.8	63.1-82.8
+ 14-3-3	351/421	83.4	79.5-86.8	48/104	46.2	36.3-56.2
+ RT-QuIC	239/252	94.8	91.3-97.2	58/63	92.1	82.4-97.4
MRI						
+ 14-3-3	407/417	97.6	95.6-98.8	41/94	43.6	33.4-54.2
+ RT-QuIC	248/252	98.4	96.0-99.6	53/61	86.9	75.8-94.2
14-3-3						
+ RT-QuIC	259/267	97.0	94.2-98.7	34/75	45.3	33.8-57.3
Three investigations						
EEG						
+ MRI + 14-3-3	386/391	98.7	97.0-99.6	34/84	40.5	29.9-51.8
+ MRI + RT-QuIC	228/231	98.7	96.3-99.7	45/54	83.3	70.7-92.1
+ 14-3-3 + RT-QuIC	240/245	98.0	97.1-99.9	27/63	42.9	30.5-56.0
MRI						
+ 14-3-3 + RT-QuIC	244/246	99.2	97.1-99.9	22/61	36.1	24.2-49.4

Sensitivity defined as the proportion of cases with any positive result.
Specificity calculated using non-cases with exclusively negative results.

Abbreviations. CI, confidence interval. EEG, electroencephalography. MRI, magnetic resonance imaging. RT-QuIC, real-time quaking-induced conversion.

Discussion

- **Summary**
- **Sensitivity of diagnostic criteria**
- **MRI**
- **RT-QuIC**
- **14-3-3**
- **EEG**

Summary

Results in this **Chapter** evaluate the sensitivity and specificity of the 2017 diagnostic criteria for sporadic CJD in a large clinicopathological series of cases and non-case controls. The primary purpose of this study was to assess the sensitivity and specificity of the diagnostic criteria. Initial analysis of the revised 2017 diagnostic criteria for sCJD conducted by the NRZ-TSE in Germany reported a sensitivity of 97% and a specificity of 99%, but the study was limited to a small, single-centre cohort and was unable to assess performance across important subgroups[91]. This thesis study has expanded on this initial study, demonstrating high sensitivity and specificity. The sensitivity of revised criteria has increased substantially, both in situations where individuals are only partially-investigated, a common scenario in surveillance (and the situation in the majority of cases and non-cases in the study; see **figure 4.5**), as well as in individuals undergoing the full panel of investigations in the criteria. Specificity did not significantly decrease in either group. It is worth discussing both dimensions in turn, considering the dual uses of diagnostic criteria for both case classification for national surveillance and for clinicians assessing patients in the diagnostic workup for potential CJD.

The sensitivity of any diagnostic tool, be it a clinical sign, individual investigation or compound scoring system such as diagnostic criteria, quantifies its ability to appropriately detect individuals with a disease of interest, avoiding 'missed diagnoses'[386]. The excellent sensitivity (97.%) of the revised diagnostic criteria in the study is a major advantage for surveillance systems faced with the task of identifying cases of sCJD on a population level. In systems such as the UK NCJDRSU where CSF biomarkers are only available from a central laboratory, many cases will be identified and referred on the basis of MRI reporting, in addition to a clinically-suggestive syndrome. Taking these findings in aggregate it is unlikely that cases of sCJD would be missed by rigorously-applied surveillance systems, assuming appropriate selection of individuals for testing (see below). Likewise, assuming clinicians are aware of the possibility of sCJD and its manifestations, they are well-equipped by the current criteria to make this diagnosis ante-mortem, supported by national expert centres. This study demonstrates that even partial investigation is generally highly-sensitive, and quantifies this for particular combinations of investigations, such as MRI and RT-QuIC (98.4%).

In contrast, the specificity of a tool quantifies its ability to appropriately identify individuals who do not have a disease of interest with a 'true' negative outcome, avoiding 'mis-diagnoses'[386]. From the perspective of delivering both national surveillance and the diagnosis of individual patients this is crucial: epidemiological classification would be jeopardised by inadequate ability to differentiate between cases and non-cases, leading to inaccurate incidence and mortality figures, and costly public health interventions such as recall of blood products, contact tracing and quarantining of instruments. The psychological consequences to individuals incorrectly termed at-risk of prion disease would also be substantial; this is a status carrying significant uncertainty in relation to the probability of

developing a rapidly-progressive and incurable disease. Furthermore, sCJD and other prion diseases remain incurable, in contrast to a number of mimicking diagnoses which may respond to interventions[288,390]. Inappropriate classification as *probable* sCJD by surveillance centres and assessing clinicians might lead to an erroneous course of supportive/palliative management for a life-threatening aetiology which may otherwise be treatable.

The specificity of the revised diagnostic criteria was 80.8%. The criteria were somewhat less specific when investigations were applied in full (67.3%), indicating a higher propensity to 'false' positive investigation results and potential misclassification when a higher number of investigations are performed. However, this assumes that clinicians would simply apply the criteria in a rigid fashion, rather than considering the overall clinical context and the importance of negative investigation results as well as investigations suggesting an alternative condition. It is worth considering the importance of expert assessment in cases of potential CJD to demonstrate the optimal usage of criteria.

sCJD is a rare disorder, and local clinicians working in regional centres may not have much individual experience with the condition. The availability of clinical expertise is one of the benefits of having a national surveillance centre (as outlined in **Chapter 1)**. This is analogous to the benefits of expert neuroradiology reporting, known to improve recognition of characteristic abnormalities on MRI[391]. While reference articles exist to guide clinicians assessing potential CJD cases and allow distinction from mimics[287], there is much to be gained from dialogue with surveillance specialist neurologists. This includes being aware of some potential pitfalls, such as applying the diagnostic criteria in a rigid manner, and overlooking markers of an alternative disorder.

For example, in an individual who had undergone numerous investigations of which only one yielded a positive outcome (e.g. an MRI with basal ganglia changes along with a negative RT-QuIC and 14-3-3), caution would be advised prior to making the diagnosis of *probable* sCJD given the sensitivity of the other investigations; it would raise questions over whether the MRI was in fact the investigation with an incorrect outcome. The criteria also do not take into account other additional features suggesting an alternative diagnosis, for example the presence of characteristic clinical or investigation features of CJD-mimicking disorders. Features that might alert clinicians to alternative diagnoses in these reported cases would include the presence of seizures[392], steroid responsiveness[392], lack of compatible changes on MRI[360] or resolution of cortical hyperintensities on serial imaging[392], the presence of a characteristic auto-antibody[393,394], or nuclear/functional imaging studies consistent with alternative neurodegenerative disorders[356]. Modification of the criteria to include the caveat, 'lack of clinical or investigation evidence supporting an alternative disorder' would enhance specificity, as was used in the study by Hermann *et al*[91]. I was not able to assess the impact of such modification in this series. Given the large proportion of non-cases with cerebral insults such as hypoxic brain injuries, which tend to feature an evident preceding trigger such as a cardiac arrest or severe

hypotensive episode[395], it is likely that consideration of these additional factors would dramatically reduce the chances of misclassification.

Features of the international cohort

This study included a large sample recruited by using an international approach over a 3-year time period. The rarity of sCJD, along with the small numbers of autopsy-confirmed cases in the modern era, necessitated this approach. This sample is substantially larger than the study by Hermann *et al*[91]. The case cohort in this thesis study had a typical age at death in addition to an equal sex balance, in line with established literature on sCJD[2]. The median disease duration of 118 days is perhaps slightly shorter than described figures, typically in the order of 4-5 months[3]. The distribution of *PRNP* c129 genotypes and sCJD subtypes defined by the Parchi classification system is discussed in **Chapter 5.**

The non-case cohort contained a variety of tissue-confirmed non-prion aetiologies, the distribution of which resembled a large series published by the US National Prion Disease Pathology Surveillance Center (NPDPSC), in which AD and vascular disease were the commonest aetiologies[390]. Similar distributions of non-case aetiologies have been reported in Greek and Spanish studies of individuals with rapidly-progressive dementia[396,397]. Inflammatory aetiologies accounted for 8.2% of the non-case cohort, similar to other studies[390,396,397], although in other centres such as the tertiary rapidly-progressive dementia service in the University of California San Francisco (UCSF) the frequency is higher, at 13%[384] which may reflect selection biases and nuances of regional ambulatory/outpatient versus national, including inpatient- and community-based, comprehensive services. It may be the case that many of these individuals do not proceed to autopsy or biopsy, with diagnoses being apparent through other means, and thus the cohort may have selected away from these aetiologies. Non-cases in this study had significantly shorter disease duration than cases in the series (median 85 days), indicating rapid progression from onset to death.

Whilst all non-cases included in the study were known to have had CJD excluded on neuropathology, a noteworthy proportion did not have formal diagnoses available. This was despite efforts to retrieve autopsy reports (as is detailed in **Chapter 8** the majority of these non-cases were in the Italian cohort). Further detail on tissue diagnoses would have enhanced characterisation of the cohort as well as in relation to non-case aetiologies associated with positive investigation outcomes.

MRI

The inclusion of cortical ribboning on MRI brain was one of two changes in the revised criteria. MRI is widely accessible and a highly useful initial diagnostic investigation when assessing individuals with potential sCJD, and is a frequent source of referral to surveillance centres for biomarker testing

and clinical assessment. MRI was the most frequently performed investigation in cases (n=455, 90.8%) and the third-most frequent in non-cases (n=111, 76.0%).

MRI sensitivity (86.8%) was lower than typically reported figures (generally >92%)[341,349]. MRI reporting by neuroradiologists with expertise in human prion diseases improves sensitivity[398]. Methodological variations between centres may have reduced sensitivity, for example with local reporting by non-expert radiologists. These variations are explored in **Chapter 8**.

Other factors that might have impacted on sensitivity include technological considerations discussed in **Chapter 2** such as field strength and b-values, which may not have been uniform given that the cohort included cases from four nations, and MRI sequences will have been obtained using regional scanners in each nation. Likewise, there may have been variations in sequences obtained (some individuals will likely not have undergone DWI, the most sensitive modality). Information on technical considerations and the imaging modalities obtained was not available in this study, which is a limitation of the analysis. However, as a cohort derived from nations with advanced healthcare systems and undergoing workup in the modern era, it is likely that most individuals who underwent MRI received imaging using sophisticated techniques, so it is unlikely that the identified sensitivity reflected widespread use of less sensitive modalities.

Similarly, I did not have data on individual MRI reports, and could not comment on whether DWI abnormalities were hyperintensities alone or true diffusion restriction (i.e. associated with reduced ADC values) as discussed in **Chapter 2**. While the criteria do not formally state the requirement for confirmation via ADC, this can reduce the potential for misclassification (for example due to artefact), and it would be valuable to know the sensitivity of DWI defined using the presence of true diffusion restriction, likewise how many false-positive MRI results reflected diffusion restriction or hyperintensities alone, allowing comparison of specificity values.

As discussed in **Chapter 2**, characteristic abnormalities on MRI develop over time in sCJD. It is a limitation of the study that I did not assess longitudinal evolution of MRI abnormalities, or subdivide cases according to the timing of imaging. This was not the focus of the study, and other groups have explored this, but it is possible that the seemingly lower sensitivity in part reflected imaging at earlier stages; however, there is no reason to suspect that this arose on a systematic scale. Likewise, the cohort was not disproportionately composed of individuals with atypically long survival or atypical subtypes (which might have biased results producing lower MRI sensitivity).

The most frequent individual outcome on MRI was co-occurrence of cortical ribboning and basal ganglia hyperintensity (39.8%). 28.1% of cases had MRI sequences demonstrating isolated cortical ribboning (**vignette 4.1**). This frequency is similar to that identified in a 2008 study of 55 cases by Meissner *et al*, in which 33% of the cohort had isolated cortical ribboning[389]. The 28.1% of cases with isolated cortical ribboning in this thesis study would not have previously been classified as having

diagnostic MRIs, and in the absence of other supportive features and investigations would not have achieved classification as *probable* sCJD. The characteristics of this subgroup are explored in **Chapter 7**. The expansion of the diagnostic criteria to include these individuals is a strength: 17 (3.5%) of cases were reclassified from *possible* to *probable* sCJD by cortical ribboning in isolation, and another 10 (2.1%) by both cortical ribboning and RT-QuIC.

The specificity of isolated cortical ribboning was 90.1%, and 86.5% for cortical ribboning +/- basal ganglia hyperintensity. Cortical ribboning co-occurring with basal ganglia hyperintensities was 96.4% specific. It is perhaps intuitive that co-occurrence of cortical and basal ganglia abnormalities is 6.3% more specific than isolated cortical ribboning, as the former implies more extensive disease distribution would be less likely to appear in non-cases, although certain aetiologies such as hypoxia[399] and osmotic demyelination syndrome (ODS)[400] may affect both basal ganglia and cortex – though both such examples would usually have a clear preceding history. The Zerr *et al* 2009 study[92] explored the diagnostic accuracy of both cortical and basal ganglia abnormalities, but did not quantify this for individuals with these abnormalities co-occurring.

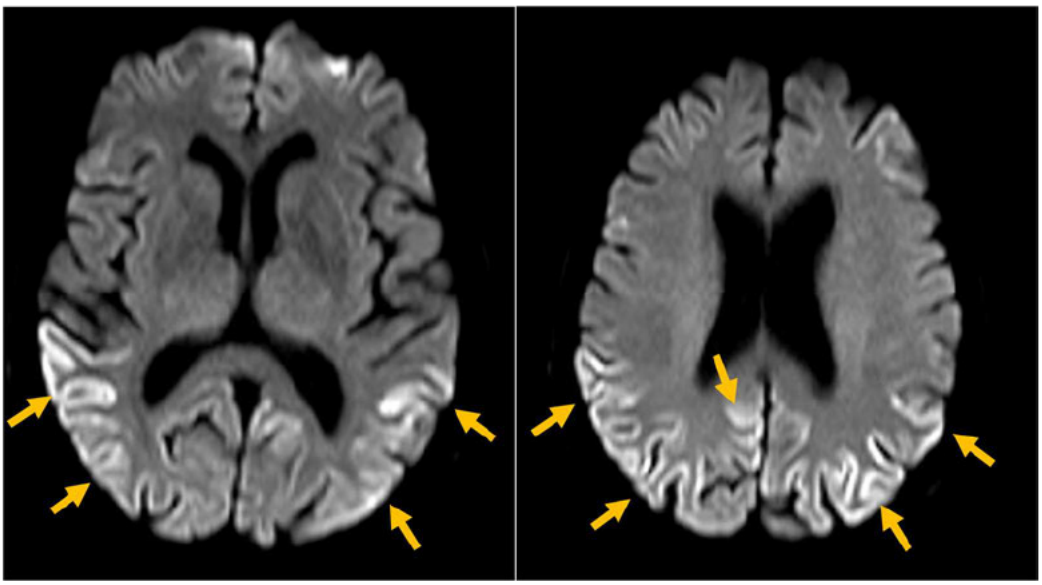
Vignette 4.1. Isolated cortical ribboning

A 63 year-old woman developed acute-onset expressive dysphasia. In the following weeks she developed disorientation, impaired short-term memory, and disturbance of behaviour and gait.

On examination she was disorientated, and had mixed expressive and receptive dysphasia. Left leg reflexes were brisk. She had dyspraxia, and some unsteadiness of gait.

DWI (b=1000) MRI demonstrated multifocal cortical ribboning in parietal, occipital and interhemispheric cortex. CSF 14-3-3 and RT-QuIC were positive. EEG had diffuse periodic sharp wave complexes.

The patient died 102 days post-onset. Post-mortem demonstrated MM1 sCJD.



This thesis study provides valuable clinico-pathological correlation for the spectrum of non-cases which may display sCJD-like abnormalities on MRI, particularly cortical ribboning (see **table 4.11** for a list of causative non-prion aetiologies**)**. Cortical diffusion restriction can emerge in a variety of conditions[401]. It is worth exploring the aetiologies featuring this manifestation as well as the additional features which may aid clinicians in distinction from sCJD.

In this study, 15 non-cases displayed cortical ribboning in ≥2 areas. AD was the commonest aetiology: of the 5 non-cases with AD, 3 had seizures, which can be seen in AD[402] and can produce cortical DWI abnormalities[403,404] (**vignette 4.2)**. This is the likely explanation. Seizures are uncommon in CJD[6,314], and a history of recent seizures can provide both an explanation for cortical ribboning and evidence for an alternative diagnosis[287]. However, the remaining 2 non-cases with AD did not have documented seizures; AD patients may therefore display cortical ribboning in the absence of epilepsy. Alternatively, seizures may have been present but been subclinical or non-convulsive, unremembered by patients, or unwitnessed by surrogates providing a case history.

The non-case with multifocal cortical ribboning and underlying AIE had limbic encephalitis (LE). This condition is an important treatable differential diagnosis for sCJD[384] and can present with comparable manifestations[405,406]. I did not have data on the distribution of cortical changes, but these would likely have been seen in the characteristic regions, i.e. the medial temporal lobes[405]. sCJD cases may display limbic region cortical ribboning, but seldom in isolation; isolated limbic involvement has been reported as a marker for non-prion disorders, particularly AIE[341], and some diagnostic criteria for AIE incorporate MRI abnormalities restricted to these regions[405]. Other features indicating AIE include CSF with raised white cell counts and protein levels[405], not seen in sCJD.

Hepatic encephalopathy (HE) with seizures, as identified in the non-case cohort, has been reported to feature cortical ribboning[407]. Features of hepatic failure (such as jaundice, asterixis, coagulopathy and hyperammonaemia) should alert clinicians to this potentially-treatable aetiology[408]. However, patients may simply present with fulminant confusion and coma, and clinicians should consider this disorder when assessing patients for potential sCJD.

The individual with cortical ribboning and underlying APS is noteworthy. APS can have an array of neurological manifestations. Arterial and venous strokes are frequent[409], both of which can produce cortical diffusion restriction[341]. Seizures can also be seen[409]. Seizures may be evident from clinical assessment, while strokes tend to cause sudden onset of negative deficits, and in the case of arterial strokes these conform to artery-supplied territories[410]; these features would support distinction from sCJD. The associated swelling and subcortical involvement would not be in keeping with sCJD[341,401]. In addition, the distribution of cortical ribboning may point away from sCJD if it involves the primary motor or sensory strips (i.e. the perirolandic area), areas typically spared in sCJD[343]. APS is treatable

and may present with dementia or psychiatric disturbance[409] and should be considered in the differential diagnosis for prion disease.

Table 4.11. Differential diagnosis for CJD-related MRI abnormalities

Basal ganglia hyperintensity
Metabolic
Hypoxic brain injury[343]
Hypoglycaemia[343]
Osmotic demyelination[411]
Toxins[412]
Inflammatory
Autoimmune encephalitis[287]
Infectious
Viral encephalitis[343]
Infarction[413]
Cortical hyperintensity
Metabolic
Hypoxic brain injury[343,401]
Hypoglycaemia[411]
Osmotic demyelination[400,414]
Hyperammonaemia[407]
MELAS[401,415]
Inflammatory
Autoimmune encephalitis[343]
Infectious
Viral encephalitis[401]
Seizures[401]
Vascular
Arterial infarction[401]
Venous infarction[401]
Artefact[401]

Abbreviations. MELAS, metabolic encephalopathy with lactic acidosis and stroke-like episodes.

In the experience of the UK NCJDRSU, seizures are a common cause of cortical diffusion restriction prompting consideration of CJD, yet are an uncommon feature in CJD. As above, a suggestive clinical history is a valuable clue, but there are additional imaging features which can aid distinction. Diffusion restriction is often continuous following a seizure, rather than patchy as in sCJD, and cortical swelling and subcortical involvement are often present[401] (**vignette 4.2**). Follow-up imaging after seizure cessation shows resolution of changes after 2-4 weeks[416]; in contrast, imaging abnormalities in sCJD progress alongside clinical disease[342].

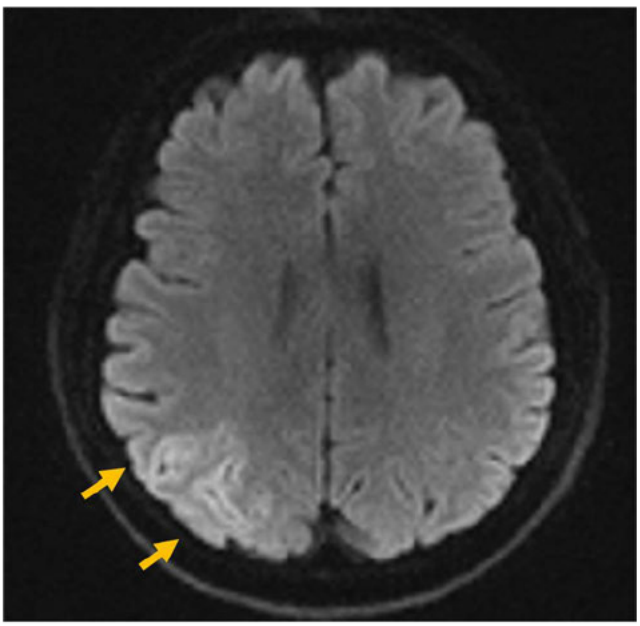
In summary, I recommend judicious clinical review, paying vigilance to the onset of symptoms and the possibility of seizures, as well as considering factors such as the CSF constituents and features on MRI including distribution of changes and associated oedema and white matter involvement. When the situation cannot be resolved, CSF biomarker testing and possibly serial clinical assessment and MRI tend to provide resolution.

Vignette 4.2. **Seizures**

A 46 year-old woman was admitted with three generalized tonic-clonic seizures. She recovered from these, though initially had minor post-ictal visuospatial difficulties which resolved over days.

MRI brain (b=1000 DWI shown) demonstrated changes in the right posterior parietal lobe on DWI and FLAIR: the cortex appeared mildly swollen, with a degree of underlying oedema in the white matter, consistent with post-ictal changes. CSF was acellular with negative 14-3-3 and RT-QuIC.

The patient made a full recovery with no recurrence of seizures. Imaging changes resolved on serial MRI.



Basal ganglia hyperintensities on MRI can be identified in many diseases[417]. In the study, 9 non-cases had basal ganglia hyperintensities present, with 5 having neuropathological data available. 1 had AD, 1 had AD with co-present DLB, 1 had DLB and 2 had non-diagnostic examinations. AD and DLB were not associated with basal ganglia DWI abnormalities in prior studies[341,349], and the results therefore broaden the spectrum of aetiologies worth considering in the differential diagnosis. Other important conditions associated with basal ganglia imaging abnormalities, include Wernicke's encephalopathy, osmotic demyelination syndrome (ODS)[400], stroke[413], cerebral insults due to hypoxia[399] or hypoglycaemia, Leigh disease[418] and toxoplasmosis[417]. Interestingly, while the cohort contained non-cases with several of these aetiologies, none were reported to have displayed basal ganglia hyperintensities.

A challenge in surveillance arises when individuals with limited clinical features display CJD-like features on MRI, as with 46 (9.4%) cases in the study. Such cases may have isolated cortical ribboning (n=23 in the study) or basal ganglia hyperintensities (n=12), or both (n=11). I classified these individuals' diagnostic category as *unclear*. Examples of such cases encountered in my experience of surveillance included cognitively-spared individuals with ataxia or with isolated cortical deficits such as dysphasia (**Vignettes 4.3 & 4.4)**.

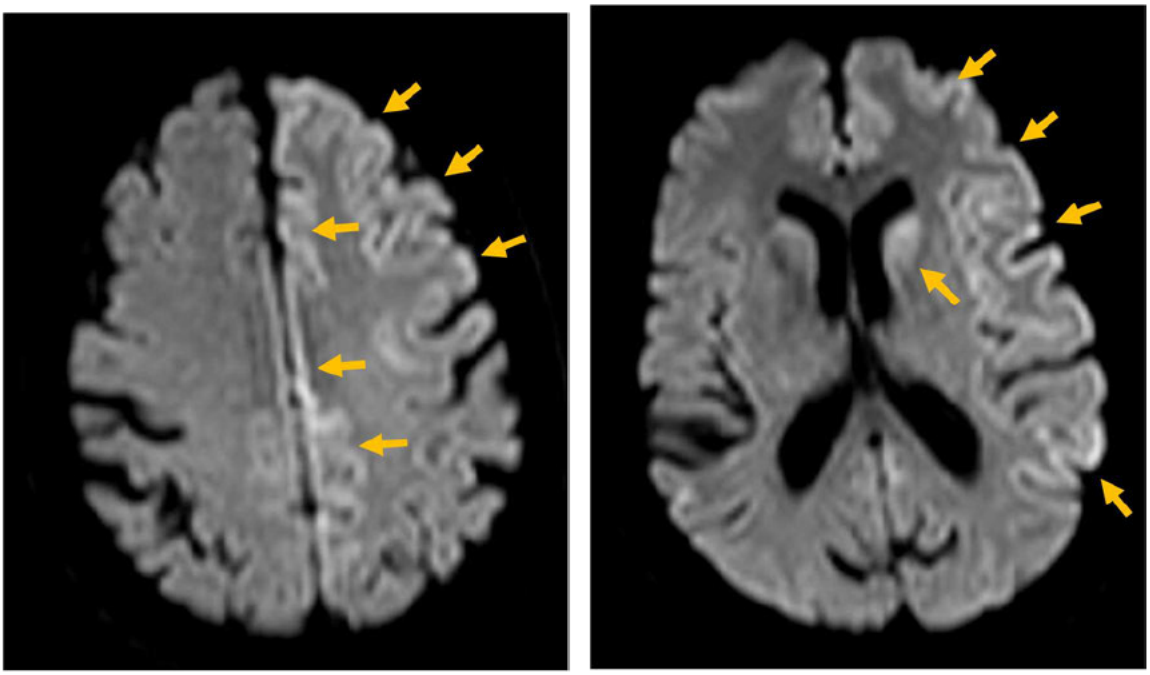
Vignette 4.3. Isolated dysphasia

A 58-year-old man developed acute-onset expressive language difficulties, initially struggling with word-finding, then progressing to severe expressive dysphasia over two weeks. He became anxious and prone to panic attacks. His coordination began to deteriorate. He was admitted to hospital due to ongoing decline. An MRI prompted NCJDRSU referral.

During my review he had severe expressive dysphasia, with speech limited to short, effortful sentences with frequent paraphasias. He displayed intact comprehension for instructions, and intact visuospatial, memory and arithmetical skills. He had mild dyspraxia, and had right-sided intention tremor, dysmetria and dysdiadochokinesis.

MRI ((b=1000 DWI shown) demonstrated left hemispheric cortical ribboning and bilateral basal ganglia hyperintensities (arrowheads). CSF testing for 14-3-3 and RT-QuIC was not possible due to contamination with blood. EEG showed bi-frontal slowing and intermittent sharp waves.

The patient continued to decline over the following months. Total disease duration was 89 days. *PRNP* c129 status was MM. He did not undergo post-mortem, precluding PrP^{Sc} typing.



Vignette 4.4. Ataxic-onset

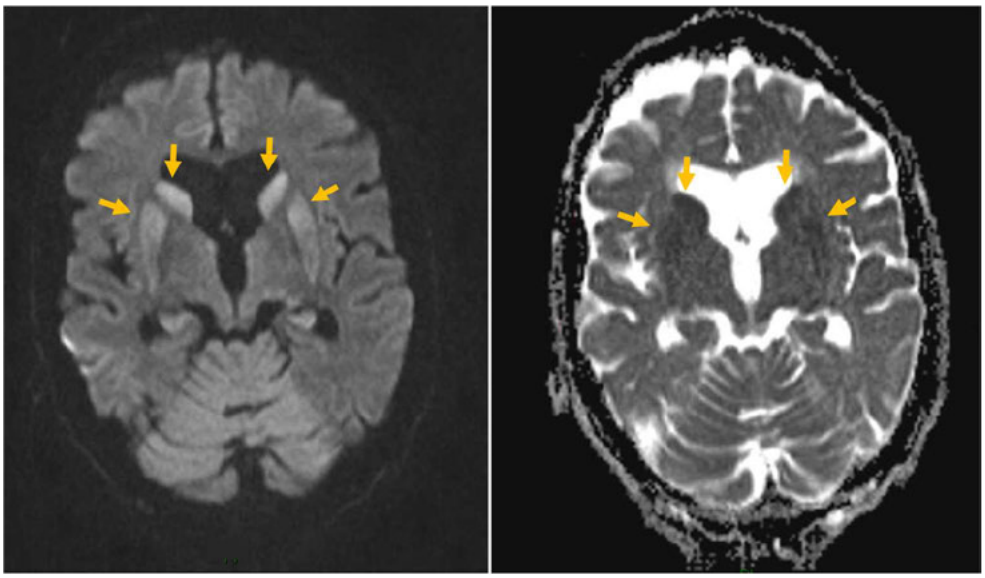
A 74 year-old woman developed gait ataxia with falls over 3 months. She subsequently developed emotional lability and short-term memory impairment. Her upper limb coordination became affected, impacting on functioning.

She saw her general practitioner 4 months after onset and was referred to a dementia clinic. She was hospitalised following a fall while awaiting this. She then displayed rapid deterioration. Her speech deteriorated to short, broken sentences, and she became confused and disorientated. She required hoist transfers due to severe ataxia. She developed myoclonus and visual hallucinations.

At the time of my review, the patient was drowsy but rousable, encephalopathic, and only able to obey very simple commands, displaying perseveration. She had saccadic intrusions to smooth pursuit, dysarthric speech, gross ataxia, and bilateral upper limb intention tremors and myoclonus. The right plantar was upgoing.

MRI showed diffusion restriction in bilateral basal ganglia (images below; b=1000 DWI on left, ADC on right). EEG showed slowing. Insufficient CSF was present to test 14-3-3, but RT-QuIC was positive.

The patient died 297 days after onset. Post-mortem confirmed VV2 sCJD.



There are challenges around establishing the diagnosis at clinically-limited stages given the devastating and incurable prognosis of sCJD. Two strategies can help. Firstly, the RT-QuIC assay, as discussed above, has the advantages of being both highly specific[97] and highly sensitive across multiple stages of disease[360], and should be pursued; a positive outcome reliably enables classification as *probable* sCJD as discussed above. Secondly, serial clinical review after a short interval (typically weeks) detects progression in features enabling re-classification. In the experience of the NCJDRSU, such clinically-limited cases tend to evolve soon after initial assessment, for example with

deterioration in cognitive functioning and mobility, and emergence of myoclonus and pyramidal signs. Despite this, a small minority will progress very slowly or even appear to have arrested progression, posing challenges.

Of the 46 cases with insufficient clinical features and a positive MRI, RT-QuIC led to classification as *probable* sCJD in 31 (6.4% of cohort, and 67.4% of this subgroup). The remaining 15 (32.6%) cases remained classified *unclear*; 4 (0.8%) had negative RT-QuIC assays, and the remaining 11 (2.3%) did not undergo RT-QuIC testing. Thus, while RT-QuIC is highly valuable in such cases, in some it will return a false-negative result, and in others it may not be performed.

MRI does not have the same outstanding specificity as RT-QuIC. If I defined a positive MRI in individuals with clinically-limited features as meeting criteria for *probable* sCJD, the aggregate sensitivity of criteria would have risen from 92.2% (450 of 488) to 95.3% (465 of 488). However, 6 (4.8%) non-cases with insufficient clinical features to enable *probable* classification had a positive MRI. Applying this approach in the series would have lowered the aggregate specificity for *probable* classification from 80.8% (101 of 125) to 76.0% (95 of 125). Thus I do not recommend altering the diagnostic criteria to include the grading of individuals with limited clinical features and a positive MRI as having *probable* sCJD.

In addition to MRI scans with false-positive changes (i.e. true CJD-like changes but due to other aetiologies), a number of MRI sequences reported as suggestive of CJD will in fact display artefactual changes, and in my experience of surveillance this was a common source of referral. This is also important in terms of discussion of the specificity of MRI. Anterior frontal lobe neocortical regions were particularly prone to artefact. The absence of restriction on ADC mapping can discriminate artefactual from pathological cortical signal changes[341] and was frequently of use in resolving such dilemmas. The Zerr *et al* 2009 study indicated 65% specificity of frontal lobe DWI changes, although ADC maps were not examined[92], and it possible that some of these reflected artefactual changes[341]. Zerr *et al* only advocated for temporal, parietal and occipital regions being included in their novel diagnostic criteria owing to superior specificity in these regions. In contrast, the University of California, San Francisco (UCSF) group, led by Dr Michael Geschwind, proposed alternative criteria including frontal lobe involvement when ADC restriction was present[341]. I recommend judicious review of imaging by experts and particular attention to ADC mapping. In this series, a number of MRI sequences were recorded featuring artefactual changes and which were classified as negative.

In the study, MRI sequences without sufficient features were classified as negative. This will have included scans with single affected regions - for example one region of cortical ribboning alone. I did not quantify the numbers of affected regions per scan, nor which specific regions were affected (e.g. temporal cortex in one or both hemispheres). Rather, I opted for a binary *yes/no* classification for both

multifocal cortical ribboning and basal ganglia hyperintensity individually, in keeping with the established criteria I sought to validate.

In addition to the implications for clinically-limited cases, questions arise over the sensitivity and specificity of MRI abnormalities limited to single regions. In the study, MRI sequences without sufficient features were classified as negative, in line with criteria definitions. However, a study by Bizzi *et al* exploring single-region MRI abnormalities using four neuroradiologists found sensitivity 90-95% and specificity 90-100%, with high inter-rater reliability, significantly increased sensitivity from current criteria (69-76%) and no change in specificity[419]. This contrasts from the findings of the 2009 study by Zerr *et al*, in which the specificity of ≥1 region of cortical ribboning on DWI was 74.1%, whereas with ≥2 regions it was 88.9%. Of note, the Bizzi *et al* study involved highly-specialised neuroradiologists, limiting its transferability to the 'real-world' of diagnosis where non-experts may under-report findings[398]. It also featured a highly biased cohort: 75% had prion diseases, in contrast to my experience of surveillance, where approximately 25% of referred individuals had underlying prion disease. The broader world of neurological diagnosis will feature a dramatically lower pre-test probability of CJD.

Bizzi *et al* recommended this approach to enable earlier diagnosis of sCJD and suggest that such individuals are highly likely to progress to multi-focal abnormalities on longitudinal MRI, hence there is little requirement to delay diagnosis until repeat imaging demonstrates evolution. This is an appealing prospect given the challenges around diagnostic latency (averaging two-thirds of the duration of illness) and short survival in sCJD[3,6,288]. However, this approach requires validation against a larger and less biased cohort. Misdiagnosis of patients as *probable* sCJD at an early time point, particularly when the actual diagnoses may be reversible (consider the regional cortical signal change induced by seizures in **vignette 4.2** as an example), would be catastrophic to affected individuals and would jeopardise the validity of surveillance data.

I cannot comment on how the criteria proposed by Bizzi *et al* would perform in this cohort as the study did not quantify individuals with single-region cortical signal changes. These individuals' MRIs would have been classified as negative from the perspective of cortical ribboning. The sensitivity would conceivably have increased using this approach, but the effect on specificity is uncertain. A number of non-cases with single-region cortical ribboning might be inappropriately classified as having *probable* sCJD, for example those with seizures, negatively impacting on specificity.

RT-QuIC

Inclusion of RT-QuIC was one of the major changes in the 2017 diagnostic criteria. As the newest investigation and therefore the least established in participating nations, RT-QuIC was performed the

least frequently of all investigations in cases (n=271, 54.7%) and controls (n=77, 52.7%). The rate of RT-QuIC performance in individual nations varied, and is explored in **Chapter 8**.

The excellent sensitivity (91.6%) and outstanding specificity (100%) of RT-QuIC in this series is consistent with the established literature[98,242,349,356-361,363,420-423]. This underscores its significant utility for surveillance and diagnostic work. The performance of RT-QuIC in specific subgroups such as different *PRNP* c129 genotypes and cases stratified by age or duration is explored in **Chapters 5-6**, as are the characteristics of RT-QuIC negative cases in **Chapter 7**.

RT-QuIC was subjected to multiple international ring trials, with both first- and second-generation assays demonstrating high sensitivity and 100% specificity for sCJD and high concordance between laboratories assessing the same samples in a blinded manner[306,424]. These studies demonstrated its reproducibility, a major advantage to international surveillance, and have allowed harmonization of internationally-used protocols. Some nations have been piloting the use of these to enhance their own surveillance efforts[291]. It should however be noted that there are differences between nations in the availability of RT-QuIC as well as in strategies regarding its use in surveillance (see **Chapter 8** for examples within the participating nations in this study). Furthermore, the use of different generation assays may influence sensitivity and specificity outcomes within centres.

Among the many advantages posed by RT-QuIC are its robust ability for diagnosis in otherwise ambiguous circumstances. A large proportion (40 of 72, 55.6%) of sCJD cases whose classification was upgraded to *probable* by the revised criteria were individuals with limited features classified as *unclear* by prior criteria. The RT-QuIC assay drove this re-classification. In my experience, these cases can be challenging to assess in surveillance work, with a clinical syndrome suggestive of sCJD (for example, rapidly-progressive dementia) and often a suggestive diagnostic investigation, but not meeting criteria definitions for formal classification. The presence of a positive RT-QuIC assay at a clinically-limited stage allows robust diagnosis with confidence. The advantages of this are numerous.

Firstly, patients can be diagnosed at an earlier stage, and with the outstanding specificity of the assay, clinicians can resolve the ongoing diagnostic process and enable a transition to supportive care. This is of great holistic benefit to patients and their networks, given the limited prognosis in sCJD: time is best spent in an appropriate centre equipped to address the needs of patients, for example, minimising noise and factors which can compound agitation, hallucination and myoclonus[17].

Secondly, in my experience, when assessing CJD patients with partially- or fully-preserved cognition, it is possible to involve them directly in discussions, allowing them to advocate for their own priorities and wishes, in contrast to situations where disease progression is more advanced and patients have marked cognitive impairment or are at an end-of-life stage. Whilst it can be challenging for patients and their families to learn of their devastating diagnosis and prognosis, it enables proactive discussions which are ultimately highly rewarding. This was a rare opportunity in my

experience of surveillance, but RT-QuIC was instrumental in facilitating this. By criteria definitions (**figure 1.5)**, individuals with preserved cognition would not fulfil classification as *probable* sCJD in the absence of RT-QuIC. Traditionally, clinicians would have to adopt a 'watch-and-wait' approach with longitudinal review to demonstrate progression in clinical features necessary to achieve a *probable* sCJD diagnosis. This could pose considerable anxiety for affected individuals and their relatives in comparison to achieving a robust, early diagnosis.

Thirdly, recognition of limited or focal-onset sCJD presentations, and the availability of a biomarker with utility in these situations, can improve recognition of sCJD in situations where it might not traditionally be considered in the absence of other deficits, for example dysphasic-onset presentations (**vignette 4.3)**. The ability to appropriately diagnose and classify these individuals enhances surveillance work, prompt public health actions and expands the known phenotypes of recognised cases. The results indicate a greatly enhanced capacity for classification of these cases.

Fourthly, as is explored in **Chapter 9**, there is a major unmet need for clinical trials in sCJD. Challenges in delivering trials include sCJD diagnosis being made at late stages[288], whereas RT-QuIC permits diagnosis in clinically-limited (previously *unclear*) circumstances and, as will be discussed in **Chapter 6**, across groups of sCJD cases with a range of different survival durations and at various stages of disease.

I did not identify any false-positive RT-QuIC assays among the non-case cohort (the test is therefore 100% specific in this series). The majority of other published studies on RT-QuIC have also documented 100% specificity[358]. Nevertheless, a small number of individuals with non-prion diagnoses have been identified and reported in the literature (**table 4.12**). At the time of writing, nine individuals have been reported. These individuals would have posed challenges to this analysis and classification. Only four underwent autopsy.

Table 4.12. Publications featuring individuals with non-prion diagnoses and positive CSF RT-QuIC

Year	Study	Journal	Cases	Diagnosis	Autopsy
2012	McGuire *et al*[360]	Annals of Neurology	1	Vascular dementia and SDH	No
2016	Hayashi *et al*[425]	Prion	1	FTD-MND	Yes
2016	Cramm *et al*[98]	Molecular Neurobiology	2	1. AD; lost to follow-up	No
				2. Encephalopathy with seizures	No
2017	Hayashi *et al*[392]	Prion	1	Steroid-responsive encephalopathy, seizure	Yes
2017	Foutz *et al*[423]	Annals of Neurology	1	DLB + trace PrP	Yes
2017	Lattanzio *et al*[356]	Acta Neuropathologica	2	1. RPD + paraneoplastic syndrome (metastatic breast cancer)	No
				2. FTD	No
2020	Rhoads *et al*[242]	Neurology	1	AD + vascular dementia	Yes

Abbreviations. Alzheimer's disease. DLB, dementia with Lewy bodies. FTD, frontotemporal dementia. MND, motor neuron disease. PrP, prion protein. RPD, rapidly-progressive dementia. SDH, subdural haematoma.

Two were identified by Japanese authors. The first, reported in 2016, had frontotemporal dementia (FTD) with motor neuron disease (MND) overlap syndrome, with absent PSWCs on EEG, absent CJD-related changes on MRI and normal 14-3-3 and t-tau in CSF[425]. RT-QuIC was positive, with slow amplification. Total disease duration was one year. Autopsy demonstrated TDP43 protein and was negative for PrP^{Sc}. The second, reported in 2017, presented with acute-onset encephalopathy and seizures; after initial apparent improvement with corticosteroids this individual's condition deteriorated[392]. Post-ictal changes were noted on DWI and FLAIR; 14-3-3 and t-tau were elevated, and RT-QuIC amplified slowly. This individual died 3 months post-onset, with autopsy excluding PrP^{Sc} and revealing seizure-related changes.

Another individual was reported in a 2017 study which included 81 autopsied non-cases[423]. CSF RT-QuIC repeatedly tested positive. Autopsy demonstrated dementia with Lewy bodies (DLB), but further analysis using western blot demonstrated low levels of abnormal PrP^{Sc}. The authors noted that this individual may therefore have had dual pathology with subclinical CJD.

The most recently-identified individual was published in a 2020 study which included 70 individuals with neuropathological exclusion of prion disease who had undergone RT-QuIC testing; this was the single member of this group with a false positive assay[242]. Autopsy demonstrated comorbid Alzheimer's disease (AD) and vascular dementia.

Other reported RT-QuIC positive non-case individuals did not undergo autopsy and thus cannot be definitively stated to have had exclusion of prion disease. One was reported in a 2012 study in which this individual was part of an n=52 non-case cohort; this individual had progressive dementia with total disease duration of between 10-17 months[360]. 14-3-3 was positive, while EEG changes were non-specific and MRI was notable for small vessel disease and bilateral subdural haematomas. The final diagnosis was vascular dementia. Two individuals were reported in a 2016 study[98]. One had a clinical diagnosis of AD, negative 14-3-3 and t-tau, and was lost to follow-up. The other's diagnosis was outstanding at the time of reporting and to my knowledge this has not been subsequently reported definitively; notably this individual had a positive MRI and elevated tau and 14-3-3. The remaining two individuals were reported in a 2017 study[356]. One had rapidly-progressive dementia in the context of metastatic breast cancer, thought to be paraneoplastic: this individual had positive 14-3-3 and t-tau in the CSF and died 4 months after onset. The other had a clinical profile compatible with FTD, supported by nuclear imaging, with negative 14-3-3, raised CST tau, and did not undergo DWI-MRI. Total duration was 18 months.

Thus, from a large denominator of published RT-QuIC assays among non-cases, only four have been definitively reported to be false-positives with neuropathological exclusion of CJD, and one of these actually had trace amounts of PrP^{Sc} (possibly indicating dual pathology). This suggests that the assay is indeed highly specific, but ongoing longitudinal studies featuring neuropathologically-confirmed non-cases are needed to definitively quantify the specificity.

It is worth considering how the above individuals might have been classified during life according to diagnostic criteria[307]. Strictly, individuals with a progressive neurological disorder and a positive RT-QuIC assay qualify as *probable* sCJD. Such syndromes might include dementia, with clinical and neuropsychological phenotypes overlapping with AD, DLB and FTD. The individual with FTD-MND had upper motor neuron features (i.e. pyramidal signs) which would also indicate potential CJD, and lacked lower motor neuron features such as atrophy and fasciculations which would point away from CJD[425]. Assessing clinicians would also consider supportive evidence for the diagnosis, including rapid disease progression and positive biomarkers such as 14-3-3, present in a number of these individuals[98,356,392], (one of whom also had positive MRI[98]) which would also strengthen the diagnostic certainty of CJD during life. In my study, all of these individuals would have been classified during life as *probable* sCJD, though I would only have included the four individuals with neuropathological examination performed. As above, the diagnostic criteria do not stipulate lack of evidence of an alternative diagnosis, and in many of these individuals such evidence may have been present.

In summary, this thesis study supports the many published studies demonstrating specificity 100% for RT-QuIC, but isolated false positive results exist in the literature. A 2021 meta-analysis[358] of 12

studies reported an aggregate specificity of 100% for RT-QuIC, but excluded the above case reports[392,425] as well as the studies by Cramm *et al*[98] and McGuire *et al*[360]. This meta-analysis quoted a false positive rate of 0 of 703 non-case CSF samples undergoing RT-QuIC in the study by Lattanzio *et al*[356], but this was not the same as the result in the study (2 of 348, i.e. specificity 99.4%). The denominator quoted was that for 14-3-3 and t-tau. In this meta-analysis all 12 studies included were reported to have shown 100% specificity for RT-QuIC. Thus, further longitudinal work with neuropathologically-confirmed non-cases as a control group is necessary to evaluate the specificity of RT-QuIC, with studies assessed in aggregate as part of robust meta-analyses.

One advantage of the RT-QuIC assay is its apparent robust performance despite CSF storage conditions including multiple freeze-thaw cycles and different temperature conditions[98]. This is advantageous as assays may require prolonged transportation to surveillance sites, including for international research studies[98] and in future situations when established systems might provide testing services to other nations[1]. In some situations, CSF may have been stored in open bench or refrigerator conditions prior to CJD being raised as a diagnostic consideration, and RT-QuIC can still be performed in such situations until up to 8 days[97].

However, two factors should be considered in relation to the usage of CSF analysis for CJD diagnosis. Firstly, there may be challenges in relation to performing lumbar puncture in patients with advanced neurological conditions, including behavioural agitation, requirements for sedation, and in some cases, end-of-life status precluding invasive testing on ethical and compassionate grounds. Secondly, important CSF constituent factors can impede assay performance. In some situations, CSF samples may be contaminated with red blood cells following traumatic lumbar puncture. This can inhibit the assay and poses a risk of false-negative outcomes[98]. It is therefore advisable for clinicians performing CSF sampling to save the last-collected specimen bottle for RT-QuIC analysis, as this is the least likely to be heavily contaminated with blood. However, despite this approach, samples may be excessively contaminated, and unable to be tested. Repeat sampling may be necessary, if this is an option. In addition, CSF samples with high protein concentrations or elevated white blood cell numbers may pose challenges to RT-QuIC and be declared untestable. However, it is worth noting that these features are important basic investigation clues to a non-prion diagnosis such as autoimmune, infectious or neoplastic/paraneoplastic disease[349,384,426-428]. In the experience of the UK NCJDRSU, CJD is very rarely associated with elevated protein or white cell counts, and workup is best redirected to alternative causes.

14-3-3

As an established biomarker in all four participating nations, 14-3-3 was the second-most frequently performed investigation in both cases (n=453) and the most frequent in non-cases (n=123). 14-3-3

displayed moderately high sensitivity (72.0%) and modest specificity (45.5%; false-positives were seen in the majority of non-cases). The sensitivity was lower than in most of the original studies of this assay, typically ≥82%[95,96,319,325,332,336]. This was the case in all four nations, with marked variations seen between nations (see **Chapter 8**). The reasons for this are not certain.

One possibility is a selection bias favouring individuals with negative 14-3-3 to proceed to neuropathological examination, whereas those with a positive assay might have been felt to satisfy diagnostic criteria in life. Factors influencing this might have included slower progression. However, the survival duration among cases in this series is typical or even slightly shorter[6] than usual for sCJD (median 118 days in the study) which suggests slow progression was not a factor. The distribution of sCJD subtypes, explored in **Chapter 5**, does not suggest an atypical sample comprising an excess of atypical subtypes for which 14-3-3 is less sensitive (although it should be noted that the initial studies yielding the above figure of >82% predominantly comprised MM1/MV1 sCJD cases, perhaps influencing apparent sensitivity). Lastly, in contrast to the strict definition of a positive 14-3-3 assay used for this study (**Chapter 3)**, it is possible that some previous studies permitted classification of weakly positive or equivocal outcomes as positive, yielding higher sensitivity in aggregate by including these assays.

The overall specificity of 14-3-3 (45.5%) is low compared to established studies[95,286,328,332]. 14-3-3 is an intracellular protein with diagnostic utility as a non-specific marker of rapid neuronal injury[334]. Non-prion causes of raised 14-3-3 include cerebrovascular disease, neurodegenerative diseases, encephalitis and metabolic or hypoxic brain insults [68,326,332,429] . In the study cohort, non-cases associated with a positive 14-3-3 had disease aetiologies including Alzheimer's disease, anoxic brain injuries, encephalitis, seizures and cerebrovascular disease. Median survival duration in non-cases was 85 days, and rapid neuronal injury will likely have been a feature in many; 95.2% (n=139) were autopsy-confirmed non-cases, hence experienced rapidly-lethal diseases. The non-case cohort may therefore have been biased in contrast to the real-world experience of surveillance, in which many individuals referred for evaluation do not experience rapidly-lethal diseases, and some may improve with treatment for reversible aetiologies such as seizures, autoimmune encephalitis and deranged metabolic states[288,384]. Hence the low specificity may have reflected selection bias. Furthermore, it may have been that individuals with positive 14-3-3 and atypical phenotypes proceeded to autopsy given features pointing away from the diagnosis of CJD during life, in order to definitively exclude or confirm prion disease. An additional contributing factor included the different methods used between nations, explored in **Chapters 3 & 8**.

The factors influencing 14-3-3 positivity in cases, including c129 genotypes and disease duration, are explored in **Chapters 5 & 6**.

EEG

EEG was the second-most frequently performed investigation in cases (n=448, second to MRI at n=455) and non-cases (n=118, second to 14-3-3 at n=123). EEG carries the advantage of being non-invasive. However, while the specificity was high (88.1%), the sensitivity of EEG in the series was poor (46.2%). These results resemble those of numerous other studies[92,100,304,321,349]. This vastly limits its utility for surveillance as a minority of cases will display the characteristic abnormalities, further compounded by subtype-specific variations in sensitivity and those seen between different age and disease duration groups [6,63,92,304,308,430] (see **Chapters 5 & 6**). Additional limitations include its limited sensitivity across earlier disease stages: PWSCs do not emerge until advanced disease status[313].

Furthermore, as with MRI, CJD-related changes on EEG tend to be focal in earlier stages of disease[302]. This can cause the potential for misdiagnosis, often as focal non-convulsive status epilepticus[324], leading to aggressive medical management with anticonvulsants and sometimes intubation and ventilation in intensive care units[324]. This is also true of the generalised PSWCs seen in advanced sCJD which can be mistaken for seizures, often accompanied by diffuse and frequent myoclonus. In my experience this is a challenging situation, leading to difficult discussions around withdrawal of advanced care measures when sCJD is diagnosed.

An additional limitation regarding EEG for sCJD diagnosis is the requirement for dedicated neurophysiology services to perform the test in a timely manner. This is not always the case, for example in hospitals outside of tertiary centres, where EEG may not be available. Some centres may require patients to be transferred for EEG testing, which may not be appropriate for agitated patients, those receiving mechanical ventilation, or those at end-of-life stages.

Very few cases in the series (n=2; 0.4% of cohort) were classified as *probable* sCJD solely on the basis of a positive EEG. The investigation may still have utility in some circumstances, for example when MRI and LP are not tolerated by patients due to agitation. However, the requirement to tolerate EEG leads attached to the scalp is also likely to apply in such individuals. Only 1 case diagnosed as *probable* sCJD had EEG performed without any other diagnostic tests. The other case also underwent a negative 14-3-3 test and did not undergo RT-QuIC testing. Notably however, performance of EEG with other investigations as part of a partial workup can boost sensitivity (**table 4.10**).

These features, along with subtype-dependence and other characteristics influencing EEG outcomes in sCJD, limit its utility for surveillance. However, it is worth stating that the confirmation of PSWCs is not the only purpose of EEG in this setting: the potential to demonstrate abnormalities associated with alternative, potentially-treatable diagnoses such as seizure activity in NCSE[431] or extreme delta brush pattern in anti-NMDA receptor encephalitis[432] is a useful justification to pursue EEG in individuals given the broad differential diagnosis for CJD-like presentations[287]. I do not recommend abandoning EEG in the diagnostic workup for sCJD, but suggest its usage should be directed towards

refuting alternative aetiologies as a ‘rule-out’ (e.g. disproving subclinical seizures, noting the caveat regarding overlap with sCJD-related abnormalities as above) rather than as a ‘rule-in’ for sCJD, for which it is of limited value.

Conclusions

This study demonstrated high sensitivity (92.2%) and specificity (80.8%) of the revised diagnostic criteria, increasing to a maximal sensitivity (97.8%) when all investigations are performed, though with a reduction in specificity (67.3%). The overall sensitivity significantly increased compared to prior criteria, with no loss of specificity, indicating that the inclusion of RT-QuIC and cortical ribboning on MRI have enhanced capacity to detect sCJD without leading to increases in false-positive diagnoses. Major gains were seen in cases being reclassified from *unclear* to *probable* sCJD owing to the RT-QuIC assay. Findings have provided valuable clinico-pathological correlation of non-cases with abnormalities on diagnostic investigations.

Chapters 5-7 explore important subgroups such as different *PRNP* c129 genotypes, cases stratified by age and disease duration, and cases with specific investigation outcomes.

Chapter 5: Impact of *PRNP* codon 129 genotype and prion protein glycotype

This **Chapter** expands on the overall analysis of the international cohort, exploring important biological factors known to influence clinical phenotypic heterogeneity, namely codon 129 (c129) genotype in the prion protein gene (*PRNP*) and prion protein (PrP) glycotypes, with combinations of these yielding subtypes as defined by the Parchi classification[6]. These factors have a profound influence on all aspects of sCJD, including clinical manifestations, disease duration, investigation outcomes and neuropathological features, and are explored in the introductory **Chapters** of this thesis[6,63,92,180,181,304,305,308,433]. Thus, it was important to perform a sub-analysis of this diagnostic criteria validation study grouping individuals according to these factors.

Aims: to explore the performance of the diagnostic criteria and investigations in sCJD cases grouped according to subtypes defined by c129 genotype and PrP glycotype.

Hypotheses: variations in all assessed features would be seen between sCJD case groups, including overall diagnostic criteria sensitivity.

Methods: the international sCJD case cohort was grouped in two stages: first according to c129 genotype, secondly by combinations of c129 genotype and PrP glycotype (Parchi subtypes). The analysis performed in each stage was similar to that in the overall case cohort. Demographic features including survival duration were compared between groups. Clinical features were quantified. Cases' in-life statuses were classified by prior and revised criteria, using both the 'all' and 'any' investigations performed methods as in **Chapter 4**, allowing quantification of the change in sensitivity for *probable* classification in each group. Investigation sensitivity was quantified, including the different MRI profiles between groups.

Results:

These are first presented for cases grouped by *PRNP* c129 genotype, then for cases grouped by Parchi subtypes.

PRNP Codon 129 genotype

Demographics

Cases were grouped according to *PRNP* c129 genotype where possible (301, 60.0%) (**table 5.1**). Homozygosity for methionine (MM) was the most frequently encountered genotype with 196 (65.1%) cases, followed by heterozygosity for methionine and valine (MV) (n=57, 18.9%) and homozygosity for valine (VV) (n=48, 16.0%). Sex ratios were broadly equal in MM (male n=99, 50.5%), MV (male

n=25, 43.9%) and VV (male n=24, 50.0%) groups (P = 0.34). Mean age of onset did not significantly differ between groups (MM, 68.1 years (SD 10.3); MV, 67.3 years (SD 9.4) ; VV, 68.4 years (SD 8.4) ; P=0.67). Median disease duration differed significantly between groups (**P<0.001)**, with post-hoc analysis demonstrating significantly shorter duration in MM individuals compared to MV (112 vs 376 days, **P=0.014**) and MM compared to VV (112 vs 170 days**, P<0.001**) (**figure 5.1**). The difference between MV and VV cases (170 vs 376 days) was not statistically significant (P=0.154).

Table 5.1. Demographics of *PRNP* c129 polymorphism subgroups

	MM (n=196)	MV (n=57)	VV (n=48)	*P*
Sex, n (%)				
Male	99 (50.5)	25 (43.9)	24 (50.0)	0.34
Female	97 (49.5)	32 (56.1)	24 (50.0)	
Mean age, years (SD)	68.1 (10.3)	67.3 (9.4)	68.4 (8.4)	0.67
Median duration, days (IQR)	112 (74-212)	376 (141-527)	170 (130-213)	**0.001†**

P value from X^2 test for sex, ANOVA for age and Kruskall-Wallis test for duration.

†Post-hoc analysis with Dunn's test using Bonferroni correction factors demonstrated significant differences between MM and MV (P=0.1) and MM and VV (P<0.001). The difference between VV and MV groups was not significant (P=0.15).

Abbreviations. C129, codon 129. IQR, inter-quartile range. *PRNP*, prion protein gene. SD, standard deviation.

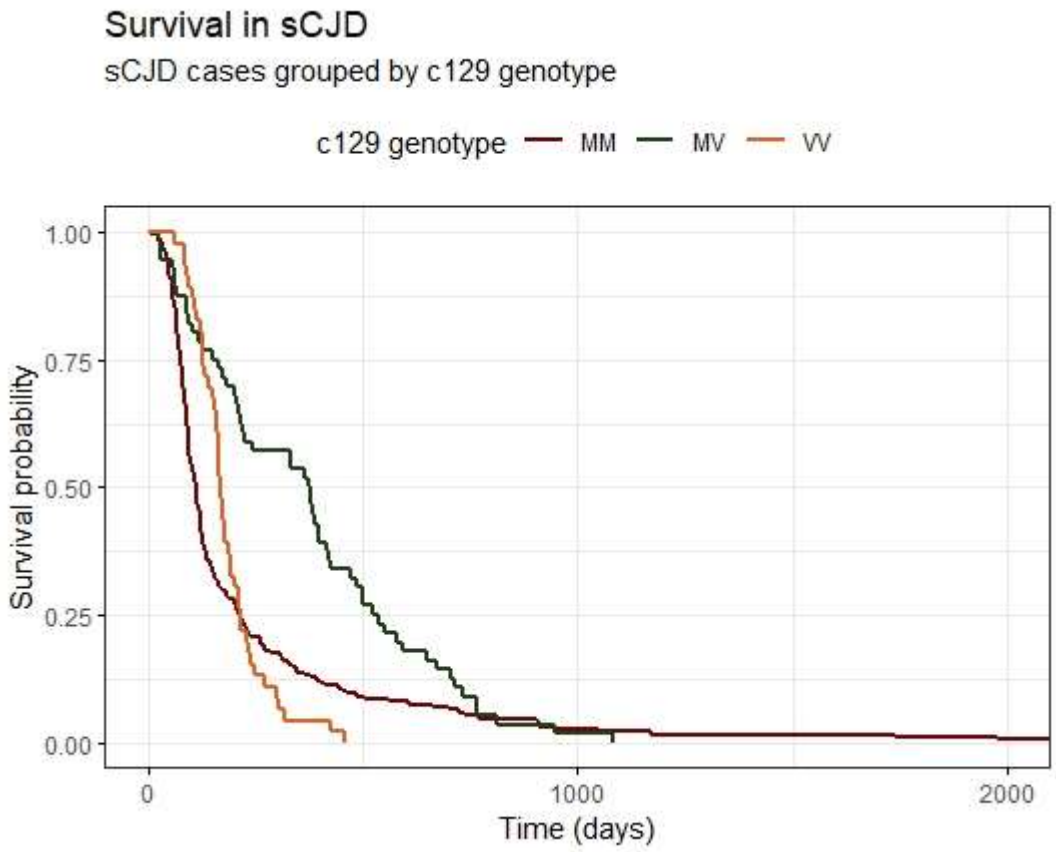


Figure 5.1. Survival in sCJD cases grouped by *PRNP* c129 polymorphism genotype.

Survival is shown in days from symptom onset. The longest median survival duration was seen in MV cases.

Abbreviations. c129, codon 129. MV, methionine-valine homozygous. *PRNP,* prion protein gene.

Clinical features

The frequency of cardinal clinical features was calculated for individual *PRNP* c129 genotype groups where available (**table 5.2, figure 5.2**). Rapidly-progressive cognitive decline was the most frequent feature, seen in 189 (99.5%) MM cases, 57 (100.0%) MV cases and 47 (100.0%) VV cases (P=1.0). Cerebellar features were most common in VV cases, seen in 87.2% in contrast to 80.7% of MV and 63.7% of MM cases (**P=0.001**). No other statistically-significant differences were observed between genotype groups, although there was a trend towards a higher frequency of akinetic mutism among MM cases (P=0.08).

Table 5.2. Clinical features, cases grouped by *PRNP* c129 genotype

	MM		MV		VV		
	n	%	n	%	n	%	*P*
Available (%)	190	96.9	57	100	47	97.9	
Feature, n (%)							
RPCD	189	99.5	57	100	47	100	1.0
Myoclonus	135	71.1	36	63.2	35	74.5	0.4
Visual	98	51.6	26	45.6	23	48.9	0.83
Cerebellar	121	63.7	46	80.7	41	87.2	**0.001**
Pyramidal	96	50.5	25	43.9	23	48.9	0.67
Extrapyramidal	109	57.4	28	49.1	27	57.4	0.53
Akinetic Mutism	100	52.6	21	36.8	20	42.6	0.08

Figures calculated using individuals for whom clinical data were available.
Fisher's exact test used for RPCD

Abbreviations. RPCD, rapidly progressive cognitive decline. MM, methionine homozygous. MV, methionine-valine heterozygous. VV, valine homozygous.

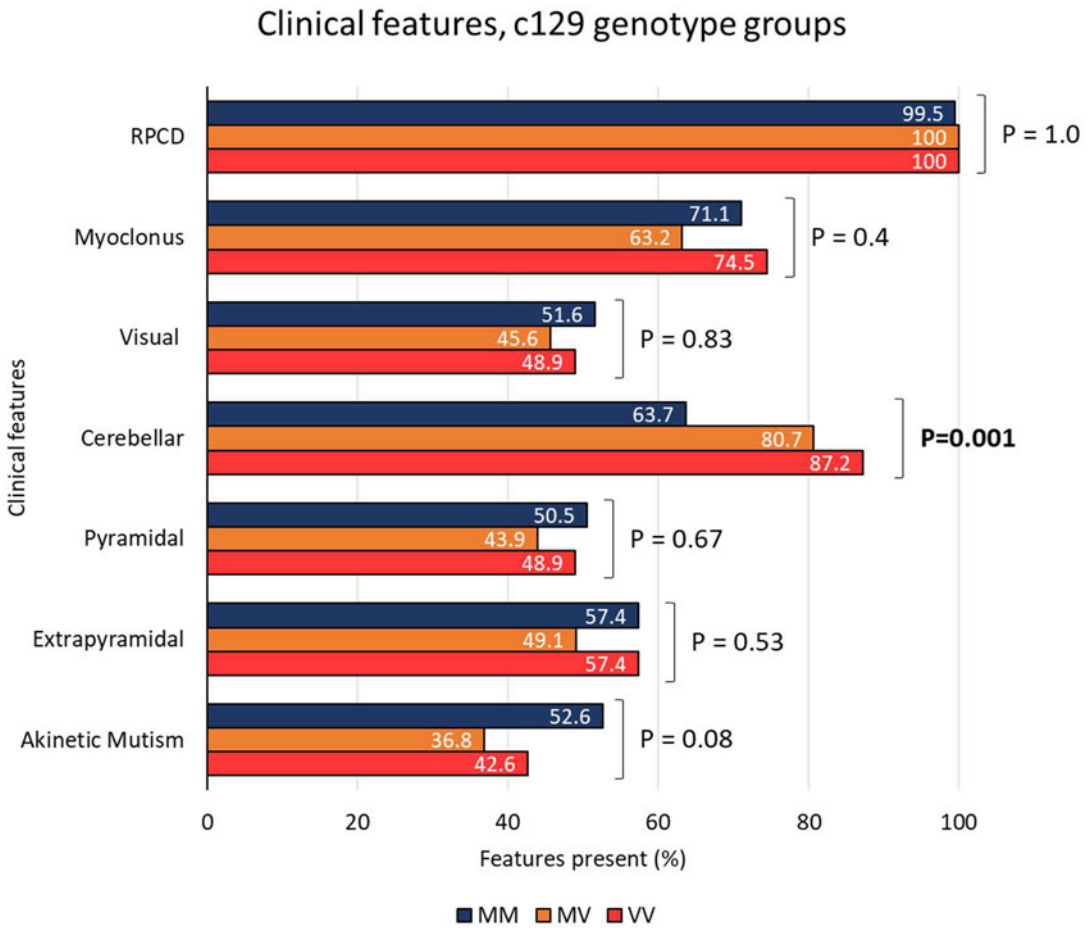


Figure 5.2. Clinical features in sCJD cases grouped by *PRNP* c129 polymorphism genotype.

The frequency of sCJD cases with clinical features is shown between polymorphism groups. The only statistically-significant difference was seen for cerebellar features, commonest in VV homozygous cases, followed by MV heterozygotes, and least common in MM homozygotes.

Abbreviations. MM, methionine homozygous. MV, methionine-valine heterozygous. RPCD, rapidly progressive cognitive decline. VV, valine homozygous.

Case classification

Cases grouped by *PRNP* c129 genotype were classified according to prior and revised diagnostic criteria (**tables 5.3, 5.4 & 5.5**). I assessed the proportion of cases classified as *probable* sCJD in each genotype group (i.e. the sensitivity). I first analysed all cases able to be classified regardless of the number of investigations performed ('any' analysis), then analysed all cases with the full panel of investigations performed ('all' analysis).

Table 5.3. Classification by diagnostic criteria, any investigation

Classification	Prior		Revised		Change (%)
	n	% [95% CI]	n	% [95% CI]	
MM					
Probable	145/190	76.3 [69.6-82.2]	179/190	94.2 [89.9-97.1]	17.9
Possible	20/190	10.5 [6.6-15.8]	3/190	1.6 [0.3-4.6]	-8.9
Unclear	25/190	13.2 [8.7-18.8]	8/190	4.2 [1.8-8.1]	-8.9
MV					
Probable	43/57	75.4 [62.2-85.9]	54/57	94.7 [85.4-98.9]	19.3
Possible	7/57	12.3 [5.1-23.7]	1/57	1.8 [0-9.4]	-10.5
Unclear	7/57	12.3 [5.1-23.7]	2/57	3.5 [0.4-12.1]	-8.8
VV					
Probable	43/47	91.5 [79.6-97.6]	46/47	97.9 [88.7-100]	6.4
Possible	1/47	2.1 [0-11.3]	1/47	2.1 [0-11.3]	0
Unclear	3/47	6.4 [1.3-17.5]	0/47	0 [0-7.6]	-6.4

Abbreviations. MM, methionine homozygous. MV, methionine-valine heterozygous. VV, valine homozygous.

Table 5.4. Classification by diagnostic criteria, all investigations

	Prior		Revised		
Classification	**n**	**% [95% CI]**	**n**	**% [95% CI]**	**Change (%)**
MM					
Probable	72/96	75.0 [65.1-83.3]	95/96	99.0 [94.3-100]	24
Possible	7/96	7.3 [3.0-14.5]	0/96	0 [0-3.8]	-7.3
Unclear	17/96	17.7 [10.7-26.8]	1/96	1 [0-5.7]	-16.7
MV					
Probable	20/27	74.1 [53.7-88.9]	27/27	100 [87.2-100]	25.9
Possible	3/27	11.1 [2.4-29.2]	0/27	0 [0-12.8]	-11.1
Unclear	4/27	14.8 [4.2-33.7]	0/27	0 [0-12.8]	-14.8
VV					
Probable	22/25	88.0 [68.8-97.5]	25/25	100 [86.3-100]	12
Possible	0/25	0 [0-13.7]	0/25	0 [0-13.7]	0
Unclear	3/25	12 [2.6-31.2]	0/25	0 [0-13.7]	-12

Abbreviations. MM, methionine homozygous. MV, methionine-valine heterozygous. VV, valine homozygous

Table 5.5. Diagnostic criteria sensitivity among *PRNP* c129 polymorphism subtypes

	MM (n=196)			MV (n=57)			VV (n=48)			*P*
	N	%	95% CI	n	%	95% CI	n	%	95% CI	
Clinical features (*possible* sCJD)	165/190	86.8	81.2-91.3	50/57	87.7	76.3-94.9	44/47	93.6	82.5-98.7	0.44
Diagnostic criteria (*probable* sCJD)										
Any										
Revised	179/190	94.2	89.9-97.1	54/57	94.7	85.4-98.9	46/47	97.9	88.7-100	0.68
Prior	145/190	76.3	69.6-82.2	43/57	75.4	62.2-85.9	43/47	91.5	79.6-97.6	0.06
All										
Revised	95/96	99	94.3-100	27/27	100	87.2-100	25/25	100	86.3-100	0.76
Prior	72/96	75	65.1-83.3	20/27	74.1	53.7-88.9	22/25	88	68.8-97.5	0.36

Abbreviations. c129, codon 129. CI, confidence interval. MM, methionine homozygous. MV, methionine-valine heterozygous. *PRNP,* prion protein gene. sCJD, sporadic Creutzfeldt-Jakob disease. VV, valine homozygous

Table 5.6. Investigation sensitivity among *PRNP* c129 polymorphism subtypes

	MM (n=196)			MV (n=57)			VV (n=48)			*P*
	N	%	95% CI	n	%	95% CI	n	%	95% CI	
Investigations										
EEG	93/175	53.1	45.5-60.7	20/50	40	26.4-54.8	6/36	16.7	6.4-32.8	**<0.001**
MRI (all)	154/177	87	81.1-91.6	49/54	90.7	79.7-96.9	45/46	97.8	88.5-99.9	0.085
CR & BG	72/177	40.7	33.4-48.3	26/54	48.1	34.3-62.2	20/46	43.5	28.9-58.9	0.62
CR alone	66/177	37.3	30.2-44.9	9/54	16.7	7.9-29.3	1/46	2.2	0.1-11.5	**<0.001**
BG alone	16/177	9	5.3-14.3	14/54	25.9	15.0-39.7	24/46	52.2	37.0-67.1	**<0.001**
CR (any)	138/177	78	71.1-83.3	35/54	64.8	50.6-77.3	21/46	45.7	30.9-61.0	**<0.001**
BG (any)	88/177	49.7	42.1-77.9	40/54	74.1	60.3-85.0	44/46	95.7	85.2-99.5	**<0.001**
14-3-3	127/178	71.4	64.1-77.9	23/50	46	31.8-60.7	38/43	88.4	74.9-96.1	**<0.001**
RT-QuIC	110/117	94	88.0-97.6	31/34	91.2	76.3-98.1	27/29	93.1	77.2-99.2	0.08

Abbreviations. BG, basal ganglia hyperintensity. c129, codon 129. CI, confidence interval. CR, cortical ribboning. EEG, electroencephalography. MM, methionine homozygous. MV, methionine-valine heterozygous. *PRNP*, prion protein gene. RT-QuIC, real-time quaking-induced conversion. sCJD, sporadic Creutzfeldt-Jakob disease. VV, valine homozygous

In the ‘any’ analysis, the sensitivity of prior criteria was highest in VV cases (43 of 47, 91.5%) and lowest in MV (43 of 57, 75.4%); this difference did not reach statistical significance (P=0.06). No differences were observed between groups with revised criteria (P=0.68).

In the ‘all’ analysis, no differences were observed in the classification rates of prior (P=0.36) and revised (P=0.76) criteria for a *probable* sCJD diagnosis between c129 groups.

Classification as *probable* sCJD (i.e. the sensitivity of criteria) increased in all groups with revised criteria in both ‘any’ and ‘all’ analyses (**tables 5.3 and 5.4**). The largest rises were observed in the ‘all’ analysis, and the biggest increase was observed in MV cases (20 of 27 [74.1%] to 27 of 27 [100%]; 25.9% increase), followed by MM (72 of 96 [75.0%] to 95 of 96 [99.0%]; 24.0% increase) and VV (22 of 25 [88.0%] to 25 of 25 [100.0%]; 12.0% increase). Similar but less pronounced increases were seen in the ‘any’ analysis, with the largest rises in MV cases (19.3%) and the smallest in VV (6.4%).

Clinical features

The frequency of cases with adequate clinical features to fulfil a diagnosis of *possible* sCJD by criteria is shown in **table 5.5**. The sensitivity did not significantly differ between groups and was highest in VV (44 of 47 [93.6%; 95% CI, 82.5%-98.7%]), followed by MV (50 of 57 [87.7%; 95% CI, 76.3%-94.9%]) and MM (165 of 190 [86.8%; 95% CI, 81.2%-91.3%]; P=0.44).

Diagnostic investigations

Diagnostic investigation results are shown for the three *PRNP* c129 genotype groups in **table 5.6**.

The sensitivity of MRI did not significantly differ between genotypes (P=0.085), although there was a trend towards highest sensitivity in VV (45 of 46 [97.8%; 95% CI, 88.5%-99.5%]) followed by MV (49 of 54 [90.7%; 95% CI, 79.7%-96.9%]) and MM (154 of 177 [87.0%; 95% CI, 81.1%-91.6%]) genotypes. Sensitivity of cortical ribboning was highest in MM (138 of 177 [78.0%, 95% CI, 71.1%-83.8%]) followed by MV (35 of 54 [64.8%, 95% CI, 50.6%-77.3%]) and VV (21 of 46 [45.7%, 95% CI, 30.9%-61.0%]; **P<0.001**) genotypes, while sensitivity of basal ganglia hyperintensity was highest in VV (44 of 46 [95.7%, 95% CI, 85.2%-99.5%]) followed by MV (40 of 54 [74.1%, 95% CI, 60.3%-85.0%]) and MM (88 of 147 [49.7%, 95% CI, 42.1%-57.3%]; **P<0.001**) genotypes. The distribution of MRI abnormalities is shown in **figure 5.3**.

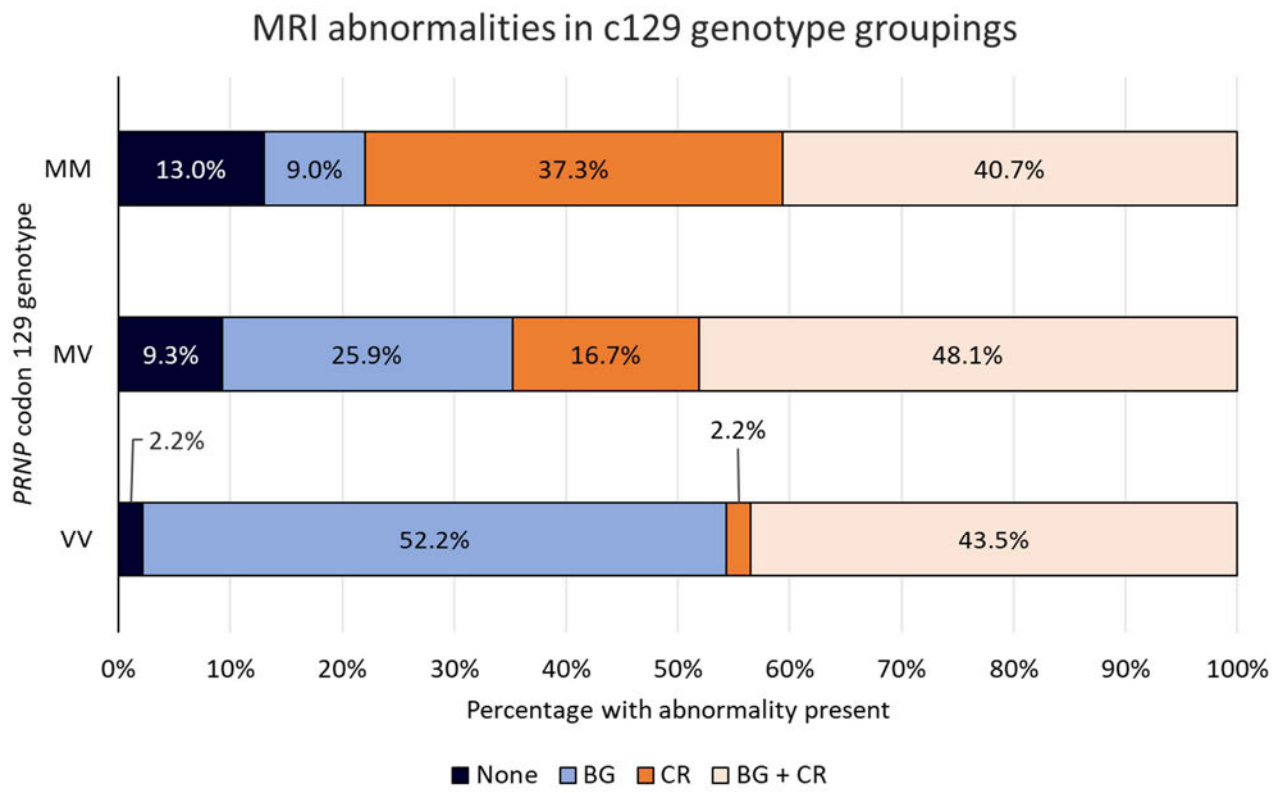


Figure 5.3. MRI abnormalities in sCJD cases grouped by *PRNP* c129 polymorphism genotype.

Isolated basal ganglia hyperintensities were uncommon in MM cases whereas they were the most frequent finding in VV cases. In contrast, isolated cortical ribboning was frequent in MM and rare in VV cases.

Abbreviations. BG, basal ganglia hyperintensity. c129, codon 129. CR, cortical ribboning. MM, methionine homozygous. MV, methionine-valine heterozygous. *PRNP,* prion protein gene. VV, valine homozygous.

Sensitivity of RT-QuIC did not vary between genotypes (P=0.08). The sensitivity of 14-3-3 was highest in VV (38 of 43 [93.1%; 95% CI, 77.2%-99.2%]) followed by MM (127 of 178 [71.4%; 95% CI, 64.1%-77.9%]) and least sensitive in MV (23 of 50 [46.0%; 95% CI, 31.8%-60.7%]; **P<0.001**).

Sensitivity of EEG was highest in MM (93 of 175 [53.1%; 95% CI, 45.5%-60.7%]) followed by MV (20 of 50 [40.0%; 95% CI, 26.4%-54.8%]) and lastly VV (6 of 36 [16.7%; 95% CI, 6.4%-32.8%]; **P<0.001**).

sCJD genotype-glycotype combinations (subtypes)

Data on PrP glycotype were available in 346 cases (69.1%). Type 1 PrP^{Sc} was seen in 208 (60.1%) and was the most frequent glycotype. 97 (28.0%) of cases had type 2A PrP^{Sc}, and the remaining 48 (13.9%) had co-occurrence of type 1 and 2A PrP^{Sc}.

Cases were further grouped by *PRNP* c129 genotype and PrP^{Sc} type[6] where possible (258, 51.5% of cohort) (**table 5.7, figure 5.4**). 9 combinations were observed, of which the most frequent was MM1 (n=130, 50.4%) and the least frequent was VV1 (n=1, 0.4%). I did not classify cases with co-occuring PrP glycotypes according to dominant glycotypes present (for example, MM2+1 if PrP^{Sc} type 2A was predominant) as this data was not available in the study.

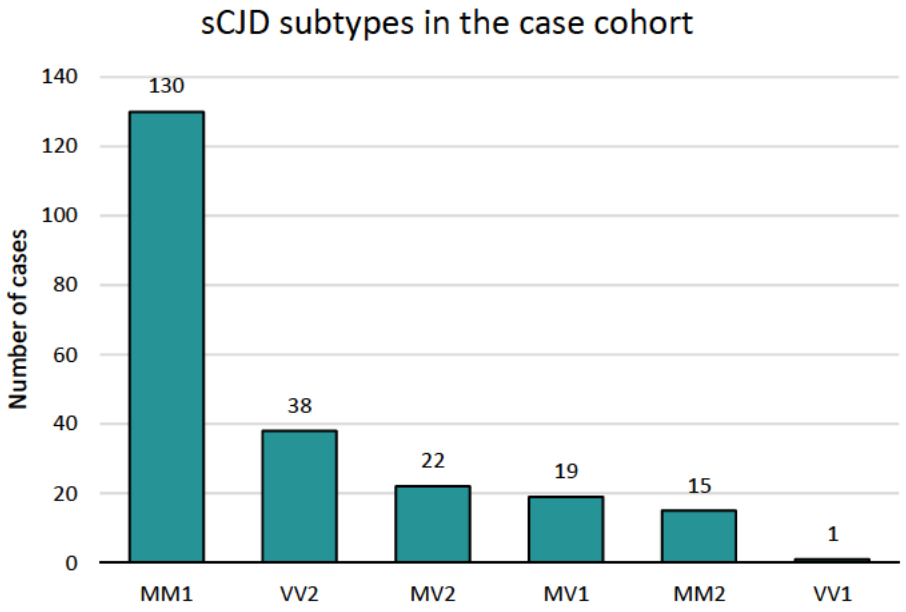


Figure 5.4. sCJD subtypes in the case cohort

sCJD cases grouped by subtype, i.e. combinations of *PRNP* c129 genotype and prion protein glycotype. Only 'pure' subtypes are shown, i.e. those without mixed glycotypes.

Abbreviations. c129, codon 129. *PRNP,* prion protein codon 129.

Table 5.7. Demographics of sCJD cases grouped by subtype

	MM1	MM2	MM1+2	MV1	MV2	MV1+2	VV1	VV2	VV1+2
Total (%)	130 (50.4)	15 (5.8)	24 (9.3)	19 (7.4)	22 (8.5)	7 (2.7)	1 (0.4)	38 (14.7)	2 (0.8)
Male	70 (53.8)	5 (33.3)	8 (33.3)	9 (47.4)	8 (36.4)	2 (28.6)	1 (100.0)	18 (47.4)	1 (50.0)
Female	60 (46.2)	10 (66.7)	16 (66.7)	10 (52.6)	14 (63.6)	5 (71.4)	-	20 (52.6)	1 (50.0)
Age	68.4	64.0	69.6	73.3	68.0	59.2	60.0	68.7	70.5
SD	10.3	12.2	9.4	5.5	9	14.1	-	9	5
Duration (median)	92.5	451	120	173	411.5	539	320	170	230, 232
IQR	67.8-150	307-661	88-223	65-374	341.5-668.25	309.5-649.5	-	128-203	-

Percentages in first row represent percentage of overall cohort with subtyping data available. Other percentages represent percentage within individual group.
Age shown as mean measured in years. Duration shown as median measured in days. For VV1+2, there were only two individuals, so durations are shown.
Subtypes defined by codon 129 genotype and prion protein glycotype 1, 2 or both.

Abbreviations. CI, confidence interval. IQR, interquartile range. MM, methionine homozygous. MRI, magnetic resonance imaging. MV, methionine-valine heterozygous. RT-QuIC, real-time quaking-induced conversion. sCJD, sporadic Creutzfeldt-Jakob disease. VV, valine homozygous.

Demographics

Demographic data for cases grouped by subtype are shown in **table 5.7**. Sex ratios were approximately equal in MM1, MV1 and VV2 groups, while there was a minor preponderance of females in MM2 (n=10, 66.7%) and MV2 (n=14, 63.6%) groups as well as in MM1+2 (n=16, 66.7%) and MV1+2 (n=5, 71.4%), groups with dual PrP glycotypes present. Mean age measured in years was youngest in MV1+2 (59.2 [SD 14.1]) and VV1 cases (60.0 [SD not calculated as this was the only VV1 case]), and eldest in MV1 (73.3 [SD 5.5]) followed by VV1+2 (70.5 [SD 5] cases. Median disease duration measured in days was shortest in MM1 (92.5 [IQR 67.8-150]) cases and longest in MV1+2 (539 [309.5-649.5]) and MV2 (411.5 [341.5-668.25]) cases (**figure 5.5)**.

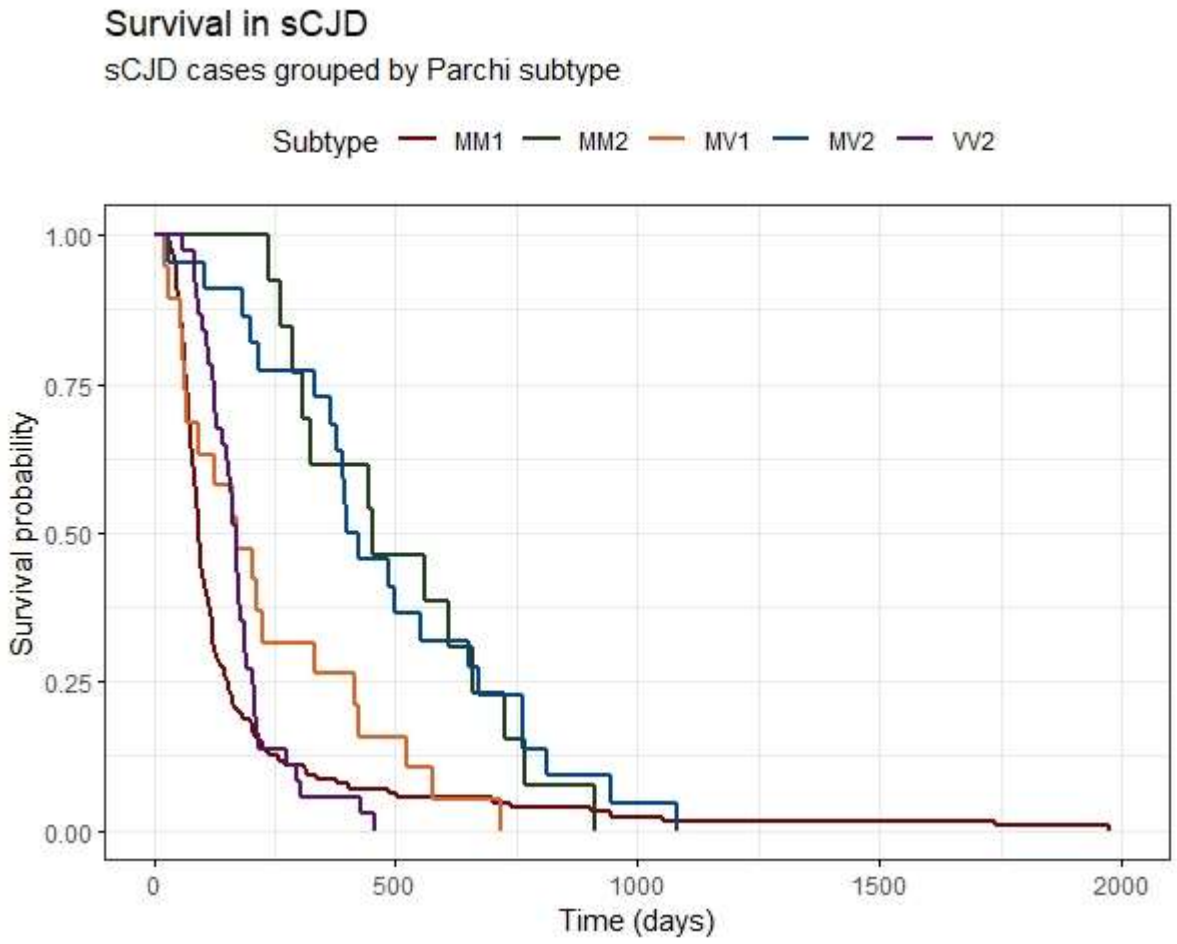


Figure 5.5. Survival in sCJD cases grouped by subtype.

Survival in sCJD shown by cases grouped according to subtype, i.e. combinations of *PRNP* c129 genotype and prion protein glycotype. The longest survival was seen in MV2 and MM2 cases. Only 'pure' subtypes are shown, i.e. those without mixed glycotypes. Only one VV1 case was present in the cohort, hence a curve is not depicted.

Clinical features

Clinical features are displayed in **table 5.8** for sCJD cases categorized by subtype as above, where this data was available. Rapidly-progressive cognitive decline was present in all individuals within each group. All other features were present at varied frequencies between groups. With the exceptions of VV1 (n=1) and VV1+2 (n=2) groups for which the small sample size limited the validity of descriptive analysis, myoclonus was most frequently reported in MM1 individuals (n=92, 72.4%). For subtypes not featuring co-occurrence of dual PrP glycotypes, cerebellar features were most frequently reported in VV2 (n=33, 89.2%) and MV2 (n=18, 81.8%) cases, and were comparatively rarer among MM1 (n=81, 63.8%) cases.

Table 5.8. Clinical features of cases grouped by subtype

	RPCD	Myoclonus	Visual	Cerebellar	Pyramidal	Extrapyramidal	Akinetic mutism
Subtype							
MM1	127 (100.0)	92 (72.4)	72 (56.7)	81 (63.8)	71 (55.9)	77 (60.6)	73 (57.5)
MM2	15 (100.0)	7 (46.7)	4 (26.7)	9 (60.0)	5 (33.3)	6 (40.0)	4 (26.7)
MM1+2	24 (100.0)	17 (70.8)	10 (41.7)	16 (66.7)	15 (62.5)	15 (62.5)	12 (50.0)
MV1	19 (100.0)	13 (68.4)	12 (63.2)	15 (78.9)	10 (52.6)	9 (47.4)	8 (42.1)
MV2	22 (100.0)	15 (68.2)	8 (36.4)	18 (81.8)	9 (40.9)	12 (54.5)	7 (31.8)
MV1+2	7 (100.0)	3 (42.9)	4 (57.1)	6 (85.7)	3 (42.9)	4 (57.1)	4 (57.1)
VV1	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)
VV2	37 (100.0)	26 (70.3)	17 (45.9)	33 (89.2)	20 (54.1)	23 (62.2)	16 (43.2)
VV1+2	2 (100.0)	2 (100.0)	1 (50.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)

Figures calculated using individuals for whom clinical data were available.
Subtypes defined by codon 129 genotype and prion protein glycotype 1, 2 or both.

Abbreviations. MM, methionine homozygous.MV, methionine-valine heterozygous. RPCD, rapidly-progressive cognitive decline. VV, valine homozygous.

Investigations

Sensitivity data for various investigations is displayed in **table 5.9**. No statistical testing was performed owing to the high number of groups and the small numbers in some groups (for example VV1 with only one case). Sensitivity of basal ganglia hyperintensities was highest in VV2 (94.6%) cases, while cortical ribboning was most sensitive in MM2 (78.6%) and MM1 (76.7%) cases. RT-QuIC showed sensitivity 95-100% in all groups except MM2 (66.7%) and VV1 (negative in only case).

Table 5.9. Investigation sensitivity in sCJD cases grouped by subtype

	MM1		MM2		MM1+2		MV1		MV2		MV1+2		VV1		VV2		VV1+2	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
EEG	69/118	58.5	4/12	33.3	11/22	50	11/19	57.9	4/18	22.2	3/6	50	0/1	0	4/34	11.8	0/2	0
MRI (all)	102/116	87.9	12/14	85.7	20/24	83.3	14/17	82.4	21/22	95.5	7/7	100	1/1	100	36/37	97.3	2/2	100
CR & BG	53/116	45.7	3/14	21.4	8/24	33.3	10/17	58.8	7/22	31.8	6/7	85.7	1/1	100	15/37	40.5	1/2	50
CR alone	36/116	31	8/14	57.1	10/24	41.7	2/17	11.8	4/22	18.2	1/7	14.3	0/1	0	1/37	2.7	0/2	0
BG alone	13/116	11.2	1/14	7.1	2/24	8.3	2/17	11.8	10/22	45.5	0/7	0	0/1	0	20/37	54.1	1/2	50
CR (any)	89/116	76.7	11/14	78.6	18/24	75	12/17	70.6	11/22	50	7/7	100	1/1	100	16/37	43.2	1/2	50
BG (any)	66/116	56.9	4/14	28.6	10/24	41.7	12/17	70.6	17/22	77.3	7/7	85.7	1/1	100	35/37	94.6	2/2	100
14-3-3	94/117	80.3	3/13	23.1	12/23	52.2	11/16	68.8	6/19	31.6	2/6	33.3	1/1	100	30/34	88.2	2/2	100
RT-QuIC	79/83	95.2	4/6	66.7	16/17	94.1	14/14	100	14/14	100	2/2	100	0/1	0	22/23	95.7	2/2	100

Subtypes defined by combination of c129 genotype and prion protein glycotype.

Abbreviations. BG, basal ganglia. CR, cortical ribboning. EEG, electroencephalography. MM, methionine homozygous. MRI, magnetic resonance imaging. MV, methionine-valine heterozygous. RT-QuIC, real-time quaking-induced conversion. VV, valine homozygous.

Discussion

In this **Chapter** I explored the characteristics of sCJD cases grouped according to *PRNP* c129 genotype and subsequently by subtypes defined by the Parchi classification[6]. A number of important findings warrant discussion.

I identified an important impact of *PRNP* c129 genotypes on duration, being longest in MV cases. This has been previously reported in the literature[3,434]. The more in-depth assessment of cases grouped by subtypes indicated marked variations in duration, shortest in MM1 cases (median 92.5 days) and longest in MV1+2 cases (539); of 'pure' cases (i.e. without dual PrP glycotypes) the longest duration was in MM2 cases (451). These findings are in keeping with the established literature on the biological factors underlying disease duration. I interrogate the features of sCJD cases stratified by duration in **Chapter 6**.

The clinical features of cases did not differ by *PRNP* c129 genotype with the exception of cerebellar features, most frequent (87.2%) in VV cases. Given that 97.4% (38 of 39) of VV cases with PrP glycotype data available were VV2, this indicates a strong association of cerebellar disorders with this subtype, which is well-known in the literature[6,308] and was the case in the series (present in 89.2% of VV2 cases) (see **vignette 4.4**). Other variations were seen in the frequency of clinical features with specific subtypes, for example myoclonus in 72.4% of MM1 and 46.7% of MM2 cases, in line with established studies[6,430,433]. However, with limited data available for subtyping, I was unable to perform formal statistical analysis on clinical features between subtypes. The frequencies of specific subtypes in the series corresponded to established frequencies, for example with only one VV1 case present[6,181] (**vignette 5.1**).

When cases were grouped by *PRNP* c129 genotype, no differences in sensitivity of prior or revised diagnostic criteria were observed between groups, both for the 'any' investigation analysis and the 'all' analysis restricted to cases with the full investigation panel performed. However, in the 'any' analysis there was a trend towards lower sensitivity for MV cases (P=0.06). The sensitivity of revised criteria had risen for all groups, most markedly for MV cases (25.9% by 'all' analysis). Thus while the results do not demonstrate that the prior criteria were less sensitive for these cases, the relatively large increase in sensitivity with revised criteria may enhance case ascertainment for this genotype in the modern era of surveillance.

In contrast to the sensitivity of criteria in aggregate, marked variations were observed in investigation sensitivity with *PRNP* c129 genotypes. While MRI sensitivity overall (i.e. with any pattern of sCJD-related abnormality) did not vary between groups there was a trend towards highest sensitivity in VV cases (P=0.085). However, VV cases displayed the highest frequency of basal ganglia

hyperintensities, both overall (i.e. with or without associated cortical ribboning) at 95.7% and in isolation at 52.5%. Given that the prior criteria did not classify cortical ribboning as diagnostic of sCJD, it follows that the sensitivity of MRI by prior criteria was significantly more sensitive for VV cases and least sensitive in MM cases (49.7%; the frequency of such cases with basal ganglia involvement). In contrast, the revised criteria sensitivity for positive MRI does not significantly differ by genotypes. This may partly account for the relatively greater rise in overall criteria sensitivity in MV (25.9%) and MM (24%) cases compared to VV (12%). Similar was seen in a 2009 study by Zerr *et al* which proposed the inclusion of cortical ribboning in novel criteria[92].

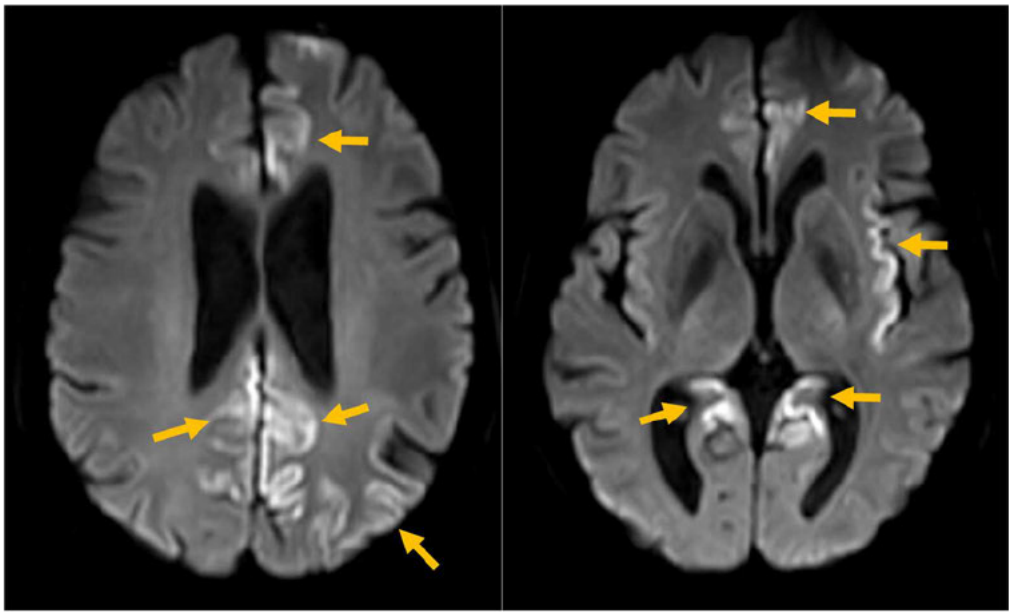
Vignette 5.1. VV1 sCJD

A 60 year-old man developed impaired short-term memory over 3 months. He subsequently developed expressive dysphasia and global cognitive decline. His gait became slow and shuffling. He developed hyperorality, eating paper and emollient creams.

9 months after onset he was admitted to hospital. At this stage he had myoclonus. When reviewed by the NCJDRSU clinician he was bedbound, encephalopathic, and displayed inappropriate laughter. He had generalised rigidity, upper limb myoclonus and brisk lower limb reflexes.

MRI showed bilateral cortical ribboning (b=1000 DWI shown). EEG had diffuse slowing with occasional triphasic complexes. Initial CSF testing was negative, but a repeat sample demonstrated positive 14-3-3 and negative RT-QuIC.

The patient died 320 days after onset. Post-mortem identified VV1 sCJD.



In contrast to basal ganglia hyperintensity, cortical ribboning was most frequent among MM cases (78.0%) and least frequent among VV (45.7%). Looking at specific subtypes the frequency was high among MM1 (76.7%) and MM2 (78.6%) cases. This association of MM cases with cortical pathology and MRI abnormalities is known[343,430,433,435]. The results add to the established literature concerning the biological basis for phenotypical variations on imaging in sCJD. The highest frequency of cases with isolated cortical ribboning was seen in MM sCJD (37.3%), indicating an association with this subtype. Characteristics of cases with isolated cortical ribboning are explored in **Chapter 7**.

Given the phenotypic variability arising between sCJD subtypes, a question arises over whether this can be used to predict genotypes during life. A 2020 study explored the potential of MRI for *in vivo* subtyping in sCJD[435]. The study used a machine learning approach following blinded lesion profiling of MRI data by an expert neuroradiologist; the accuracy of algorithms ranged from 82-89% accuracy. The authors proposed that this approach would have utility for prognostication and potential stratification of targeted therapies. This is an intriguing prospect, and certainly such antemortem non-invasive subtyping might have potential ramifications as the authors suggest, although there remains a need for any viable disease-modifying therapy for sCJD, and the existence of this would likely be a necessary initial condition prior to the development of subtype-targeted interventions.

Data from this thesis study support pre-existing studies that report differing MRI lesion patterns between sCJD subtypes[305], but this study was not designed to validate the use of MRI for *in vivo* subtyping. I quantified the presence or absence of cortical and/or basal ganglia abnormalities, whereas the study by Bizzi *et al* looked in depth at the distribution of changes, their relative involvement, and the involvement of additional regions such as the thalamus[435]. The authors proposed a diagnostic algorithm for subtyping via MRI. This is an attractive prospect, but clearly requires further work to allow robust in-life subtyping in this minimally-invasive manner.

The sensitivity of RT-QuIC did not differ between genotypes in this study. There was a trend towards highest frequency in MM (94%) but the overall sensitivity was very similar across groups. While I observed some variation between sCJD subtypes, only 258 individuals (51.5% of the cohort) had the necessary investigations performed, and my analysis was likely underpowered to formally analyse these, particularly in rare subtypes.

Only one confirmed VV1 individual was included in the study in whom RT-QuIC was negative. Studies assessing RT-QuIC sensitivity across different sCJD subtypes have been limited by small numbers of VV1 cases. Findings have varied between studies. In the 2020 study by Rhoads *et al*[242], sensitivity was 0% (positive in 0 of 3 cases). In contrast, the 2017 study by Foutz *et al* calculated sensitivity 75% (6 of 8), while two other studies published in the same year[356,363] each contained 1 individual with VV1 sCJD for whom the RT-QuIC was positive (i.e. sensitivity 100%). The results for the single VV1 case in this series add the established literature on the subject, but ultimately large,

multinational series will be necessary to definitively evaluate sensitivity of RT-QuIC in this rare subgroup.

There was a lower observed RT-QuIC sensitivity (4 of 6, 66.7%) in MM2 cases in this study (**vignette 5.2**). This pattern of lower sensitivity has been reported in the literature. In the 2017 study by Lattanzio *et al* the sensitivity was 44.4% in 9 individuals with MM2-C sCJD, and 33.3% in 3 individuals with MM2-T[356]. Two other studies were published in the same year. The first, by Foutz *et al*, calculated sensitivity 71.4% (5 of 7) in a retrospectively-analysed cohort, and 100% (2 of 2) in a prospective cohort. The other study, by Franceschini *et al*, calculated sensitivity 66.7% (6 of 9) for MM2-C and 75.0% (3 of 4) for MM2-T. The 2020 study by Rhoads *et al* featured a larger sample and calculated sensitivity 78.3% (18 of 23). Thus, with the exception of the two cases in the prospective cohort by Foutz *et al*, the sensitivity is typically estimated to be below 80%. The findings reflect the emerging pattern in the literature for MM2 sCJD, the second-rarest subtype[6], and provide a valuable contribution to this literature. As with VV1 however, there is a need for ongoing multinational prospective research into this observed pattern. An additional limitation of this approach was that I did not further sub-classify these individuals into MM2-C and MM2-T; this division may further influence sensitivity as with the above studies.

Vignette 5.2. MM2 sCJD

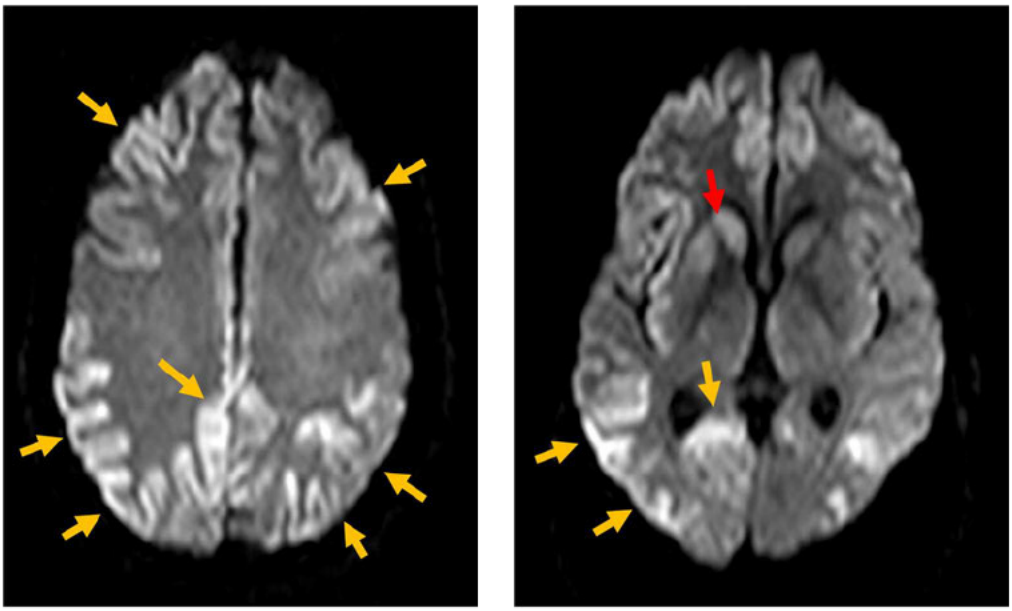
A 53-year old woman developed impaired ability to read a clock, accompanied by apathy, behavioural changes and a depressed affect. Over the following 6 months she developed delusions concerning her partner. She became paranoid and suspicious.

She was admitted to a psychiatric hospital 9 months into her illness. She developed visual hallucinations, a startle response and globally worsening cognition.

During the NCJDRSU assessment she was disorientated, had brisk reflexes and extensor plantars and no other focal signs.

b=1000 DWI showed bilateral cortical ribboning (yellow arrows) and right-sided basal ganglia hyperintensities (red arrowhead). EEG demonstrated generalized slowing. CSF 14-3-3, S100b and RT-QuIC were negative.

The patient died 911 days after onset. Post-mortem demonstrated MM2 sCJD.



The 14-3-3 assay displayed significant variations in sensitivity, being highest in VV genotype cases (88.4%) and lowest in MV (46%), in line with prior literature[304]. 14-3-3 sensitivity is known to vary with sCJD subtypes, although the extent varies in studies[96,179,336,430]. It is notable that sensitivity was higher in VV2 (88.2%) than MM1 (80.3%) and MV1 (69.9%), in contrast to some prior studies[96,336] but in keeping with a 2006 study by Collins *et al*, albeit with lower values observed in all three groups than in that study[304]. The sensitivity in MM2 cases was only 23.1% in this thesis study, and for MV2 cases it was 31.6%. While these subtypes have been associated with inferior sensitivity in studies[96,180,304,430], sensitivities in this series were particularly low. Reasons for this are uncertain. I cannot comment on investigation timing, and some cases may have undergone LP at a relatively early

stage given the long median survival in MM2 (451 days) and MV (411.5 days) cases; sensitivity of 14-3-3 is known to increase with disease progression[337]. Another possibility is that this series might have had a selection bias towards the minority of individuals undergoing autopsy; these individuals may have been more likely to have had negative investigations, making the in-life diagnosis uncertain. This conjecture is not supported by the higher sensitivities of other investigations such as MRI (85.7% in MM2, 95.5% in MV2) and RT-QuIC (100.0% in MV2, 66.7% in MM2), nor by the high in-life aggregate sensitivity for *probable* sCJD in this cohort (see **Chapter 4**).

EEG showed poor sensitivity in all genotypes and was least sensitive in VV cases (16.7%). Analysis in subtypes indicated poor sensitivity in VV2 (11.8%) and MV2 (22.2%) cases, which are less commonly associated with PSWCs on EEG[6,63,92,304,308,430]. The overall sensitivity of EEG was poor in this study (see **Chapter 4**), and subgroup analysis indicates marked variations between groups, further limiting its utility. One question for surveillance systems assessing cases of prion disease is the possibility of vCJD, and these results suggest that EEG would have poor ability to distinguish between sporadic and variant cases. The criteria for vCJD stipulate the absence of PSWCs[436], yet for many individuals with sCJD (including VV2, the second-commonest subtype) this would also be the case, as in this series. Furthermore, a number of atypical subtypes feature prolonged duration and psychiatric features, which can overlap with vCJD[6,430]. Distinction from vCJD would rely on other supportive features favouring either diagnosis, such as the pulvinar sign on MRI[136] or a positive RT-QuIC.

Summary

In this **Chapter** I have explored the demographic, clinical and investigation variations between sCJD cases grouped firstly by *PRNP* c129 genotype and subsequently by paired c129 and PrP^{Sc} glycotypes. Neither prior nor revised diagnostic criteria significantly differed in sensitivity for specific genotypes, but the relatively largest increases with revision were seen in MV and MM cases, which might partly have reflected the expanded criteria for positive MRI. Importantly, the RT-QuIC assay was robust across genotypes, in contrast to more established investigations for sCJD[304], and interestingly the results support the growing body of evidence that MM2 cases are less likely to display a positive RT-QuIC, the biological reasons for which are not known at the time of writing.

Chapter 6 explores the performance of the diagnostic criteria and investigations in sCJD cases stratified according to age and total disease duration.

Chapter 6. Disease duration and age

This **Chapter** presents the results of analyses of sCJD cases grouped according to two important factors, total disease duration (i.e. survival) and age at death. It has previously been demonstrated that these factors are associated with differing clinical features as well as investigation performances, which may impact on overall performance of the revised diagnostic criteria assessed in this thesis[3,304]. The previous single-centre validation study by Hermann *et al* did not explore these important factors[91].

Aims: to divide the sCJD case cohort by disease duration and subsequently by age, allowing assessment of key demographic features, clinical features, investigation performance and overall diagnostic criteria performance, as well as the impact of revision on in-life classification between groups.

Hypotheses: Individuals with atypical characteristics, i.e. atypically short or prolonged disease duration, or young or old age, would display atypical clinical features and lower sensitivity for investigations and diagnostic criteria. The revision of the criteria may have altered sensitivity to differing extents between groups.

Methods: I analysed the case cohort in two steps.

Firstly, grouping cases according to disease duration (measured in days from symptom onset to death) divided in quartiles, defining short survivors as those in the shortest duration quartile, long survivors in the longest quartile, and typical survivors as those in quartiles 2-3. This approach was taken to ensure robust sample sizes in each group. Restricting analysis to a more polarised subset (e.g. the shortest and longest deciles for survival) would have limited the sample size and hindered analysis. The use of quartiles also ensured statistically defined definitions of short and long, rather than arbitrarily chosen cutoffs (e.g. >300 days as 'long'). Median and quartiles were used as duration was positively skewed, with most cases deceased within in a short number of weeks.

Secondly, grouping cases by age at death (measured in years), with young cases defined as those more than one standard deviation below the mean, and older greater than one standard deviation above it. This approach was taken after age was found to be normally distributed within the case cohort, and the use of standard deviations was chosen to ensure i) a statistically sound sample in each group (rather than restricting to smaller outlier groups from a more polarised cutoff) and ii) statistically-defined cutoffs (rather than arbitrary definitions, e.g. 'young' as <50 years).

Analyses were performed in the same manner as in **Chapters 4 & 5**, assessing clinical features, investigation sensitivity and overall diagnostic criteria performance, both for individuals with all investigations performed and those with any combination of investigations.

Results: these are presented within this **Chapter**, first presented for disease duration, then age.

Part 1. Disease duration

1. **Demographics**
2. **Clinical features**
3. **Case classification**
4. **Investigations**
5. **Codon 129**
6. **sCJD subtype**

sCJD cases were grouped according to survival duration. Those with survival duration in the shortest quartile were categorized as 'short' survivors; those with duration in quartiles 2-3 were categorized as 'typical' survivors, and the remaining cases had survival duration in the longest quartile and were termed 'long' survivors.

1. Demographics

Data on duration were available in 484 (96.6%) cases (**table 6.1**). Short survival was calculated as disease duration less than 75 days, long survival greater than 222 days, and typical survival any duration between these outer quartiles. The short survival group consisted of 121 cases (25.0%, i.e. the shortest quartile), while the typical survival group featured 242 cases and the long survival group 121 cases. There was a male preponderance among short survivors (n=74, 61.2%) compared to typical (n=116, 47.9%) and long (n=54, 44.6%; **P= 0.02**) survivors among whom a slight excess of female cases was seen. Mean age was 70.4 years in short survivors compared to 69.1 and 66.6 in typical and long survivors respectively (**P=0.004**) (**table 6.1**). Post-hoc analysis indicated that the difference in means was statistically significant between short and long survivors (P=0.002) but not typical and long (P=0.1) or typical and short (P=0.15) survivors.

Table 6.1. sCJD cases stratified by duration

	Short	Typical	Long	*P*
Total	121	242	121	
Male	74 (61.2)	116 (47.9)	54 (44.6)	**0.02**
Female	47 (38.8)	126 (52.1)	67 (55.4)	
Age, years (SD)	70.4 (8.5)	69.1 (9.5)	66.6 (10.7)	**0.004**

Post-hoc: short vs long statistically significant (P=0.002) but not typical vs long (P=0.1) or typical vs short (P=0.15)
Abbreviations. SD, standard deviation

2. Clinical features

The frequency of cardinal clinical features in sCJD cases grouped by duration is shown in **table 6.2** and **figure 6.1**. Rapidly-progressive cognitive decline (RPCD) was the most frequent feature in all groups and the frequency did not vary between them (P=0.9). Pyramidal features and akinetic mutism were the least frequent features in the cohort. Myoclonus was most frequently seen in short survivors (n=87, 73.1%) followed by typical (n=170, 71.7%) and long survivors (n=73, 61.3%), but this difference did not reach statistical significance (P=0.08). In contrast, extrapyramidal features were most frequent in long survivors (n=50, 42.0%) followed by typical (n=122, 51.5%) and short survivors (n=51, 42.9%; **P=0.04**). Visual features were most frequent in typical survivors (**P=0.004**) as was akinetic mutism (**P=0.04**).

Table 6.2. Clinical features, cases grouped by duration

	Short (<75)	Typical (75-222)	Long (>222)	*P*
Available (%)	119 (98.4)	237 (97.9)	119 (98.4)	
RPCD	117 (98.3)	233 (98.3)	118 (99.2)	0.9
Myoclonus	87 (73.1)	170 (71.7)	73 (61.3)	0.08
Visual	56 (47.1)	131 (55.3)	44 (37.0)	**0.004**
Cerebellar	91 (76.5)	169 (71.3)	92 (77.3)	0.37
Pyramidal	52 (43.7)	111 (46.8)	50 (42.0)	0.66
Extrapyramidal	51 (42.9)	122 (51.5)	70 (58.8)	**0.04**
Akinetic Mutism	47 (39.5)	120 (50.6)	46 (38.7)	**0.04**

Figures calculated using individuals for whom clinical data were available. Duration measured in days.
Fisher's exact test used for RPCD

Abbreviations. RPCD, rapidly-progressive cognitive decline.

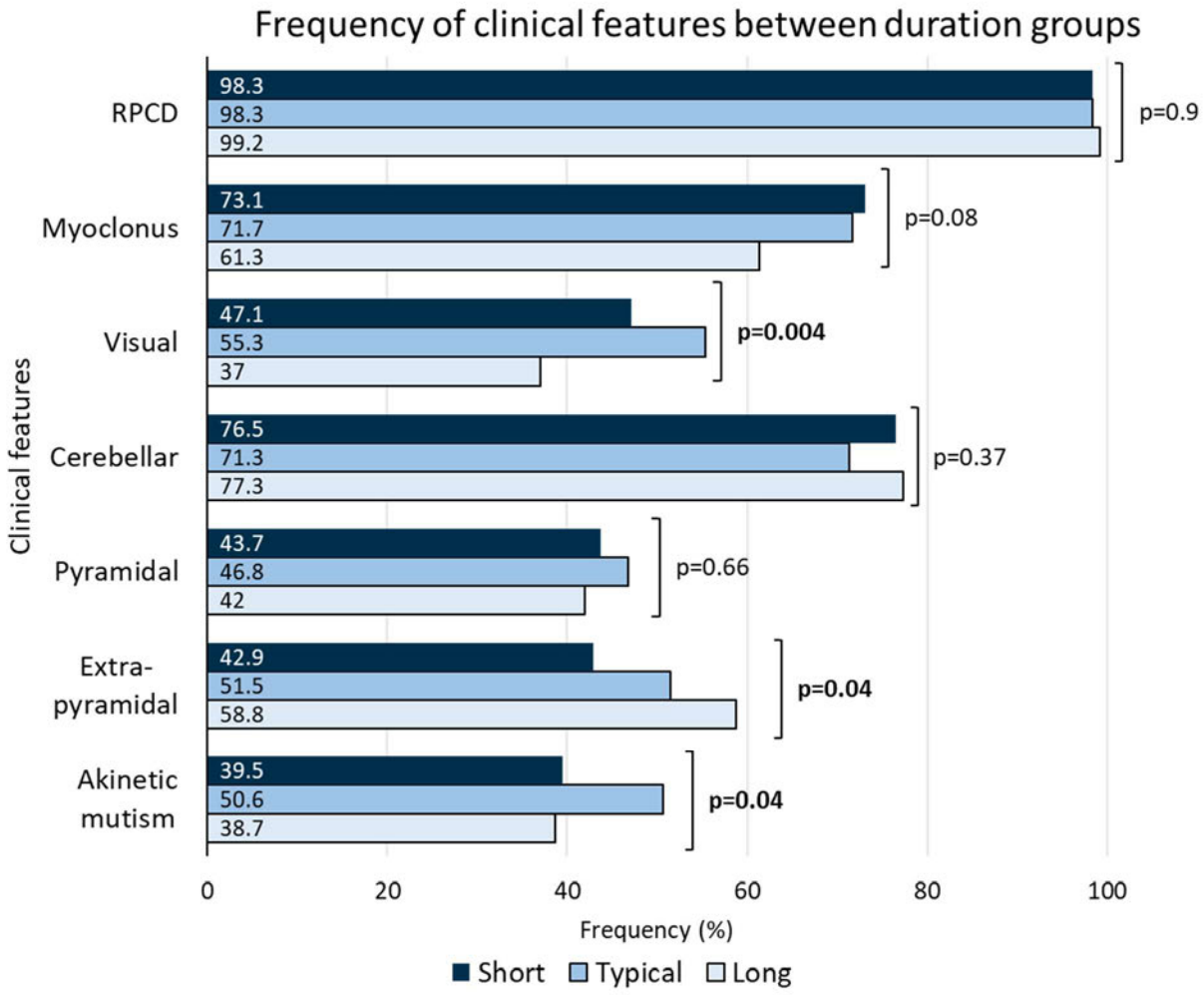


Figure 6.1. Frequency of cardinal clinical features among sCJD cases grouped by duration

Clinical features varied between cases grouped by disease duration. Visual features and akinetic mutism were most common among those with typical duration, while extrapyramidal features were commonest among those with prolonged duration.

Abbreviations. RPCD, rapidly-progressive cognitive decline.

Table 6.3. Diagnostic criteria sensitivity, cases grouped by duration

	Short (<75 days) (n=121)			Typical (75-222 days) (n=242)			Prolonged (>222 days) (n=121)			
	n	%	95% CI	n	%	95% CI	n	%	95% CI	*P*
Clinical features (*possible sCJD*)	102/119	85.7	77.3-90.7	213/239	89.1	84.5-92.8	98/119	82.4	74.3-88.7	0.19
Probable sCJD										
Any										
Revised	111/119	93.3	87.2-97.1	226/239	94.6	90.9-97.1	106/119	89.1	82.4-94.1	0.16
Prior	96/119	80.7	72.4-87.3	203/239	84.9	79.8-89.2	73/119	61.3	52.0-70.1	**<0.001**
All										
Revised	51/52	98.1	89.8-100	109/110	99.1	95.0-100	53/56	94.6	85.1-98.9	0.19
Prior	43/52	82.7	69.7-91.8	91/110	82.7	74.4-89.3	32/56	57.1	43.2-70.3	**0.001**

Sensitivity defined as positive outcome/total for cases. Duration measured in days.
First row relates to cases with sufficient clinical features to warrant classification as *possible* sCJD.
'Any' refers to cases with any combination of investigations performed. 'All' refers to those with the full panel performed.

Abbreviations. CI, confidence interval. sCJD, sporadic Creutzfeldt-Jakob disease.

3. Case classification

The diagnostic criteria were applied to cases when grouped by survival duration. In line with the method used for analysis of the overall cohort I first assessed case classification using cases with any investigation performed ('any'), then using cases with the full panel of tests ('all'). Results are illustrated in **table 6.3**.

In the 'any' analysis, the previous diagnostic criteria showed greater sensitivity for cases with typical (203 of 239, 84.9% [95% CI, 79.8%-89.2%] duration followed by short (96 of 119, 80.7% [72.4%-87.3%]) duration and was least sensitive among those with prolonged duration (73 of 119, 61.3% [52.0%-70.1%]; **P<0.001**). In contrast the revised criteria did not demonstrate any between-group differences in sensitivity (P=0.16).

In the 'all' analysis, the previous diagnostic criteria were most sensitive in cases with short (43 of 52, 82.7% [95% CI, 69.7%-91.8%]) and typical (91 of 110, 82.7% [95% CI, 74.4%-89.3%]) duration compared to those with prolonged duration (32 of 56, 57.1% [95% CI, 43.2%-70.3%]; **P=0.001**) (**table 6.3)** As with the 'any' analysis, no between-group differences were observed with revised criteria in sCJD cases with all investigations performed (P=0.19).

I quantified the magnitude and direction of change for the three classification categories (*probable, possible* and *unclear*) between duration groups for both the 'any' and 'all' analyses (**table 6.4, figure 6.2**). The frequency classified as *probable* sCJD with revised criteria increased in all duration groups. The most striking increase was seen in long survivors in the 'all' analysis (37.5%). Relatively smaller increases were seen in cases with typical and short survival (16.4% and 15.4% respectively). In all groups in the 'all' analysis, the largest change was seen among cases undergoing re-classification from *unclear* to *probable* sCJD.

Table 6.4. Case classification by diagnostic criteria, cases stratified by duration

	Short (<75 days)			Typical (75-222 days)			Prolonged (>222 days)		
Classification	**Prior**	**Revised**	**Change (%)**	**Prior**	**Revised**	**Change (%)**	**Prior**	**Revised**	**Change (%)**
Any test									
Probable	96 (80.7)	111 (93.3)	12.6	203 (84.9)	226 (94.6)	9.6	73 (61.3)	106 (89.1)	27.7
Possible	6 (5.0)	3 (2.5)	-2.5	10 (4.2)	2 (0.8)	-3.3	25 (21.0)	4 (3.4)	-17.6
Unclear	17 (14.3)	5 (4.2)	-10.1	26 (10.9)	11 (4.6)	-6.3	21 (17.6)	9 (7.6)	-10.1
All tests									
Probable	43 (82.7)	51 (98.1)	15.4	91 (82.7)	109 (99.1)	16.4	32 (57.1)	53 (94.6)	37.5
Possible	2 (3.8)	1 (1.9)	-1.9	3 (2.7)	-	-2.7	9 (16.1)	-	-16.1
Unclear	7 (13.5)	-	-13.5	16 (14.5)	1 (0.9)	-13.6	15 (26.8)	3 (5.4)	-21.4

Survival duration measured in days. Numbers shown denote cases within each duration group who underwent either any diagnostic test or the full battery of tests, according to their in-life classification by diagnostic criteria; percentages shown in brackets.

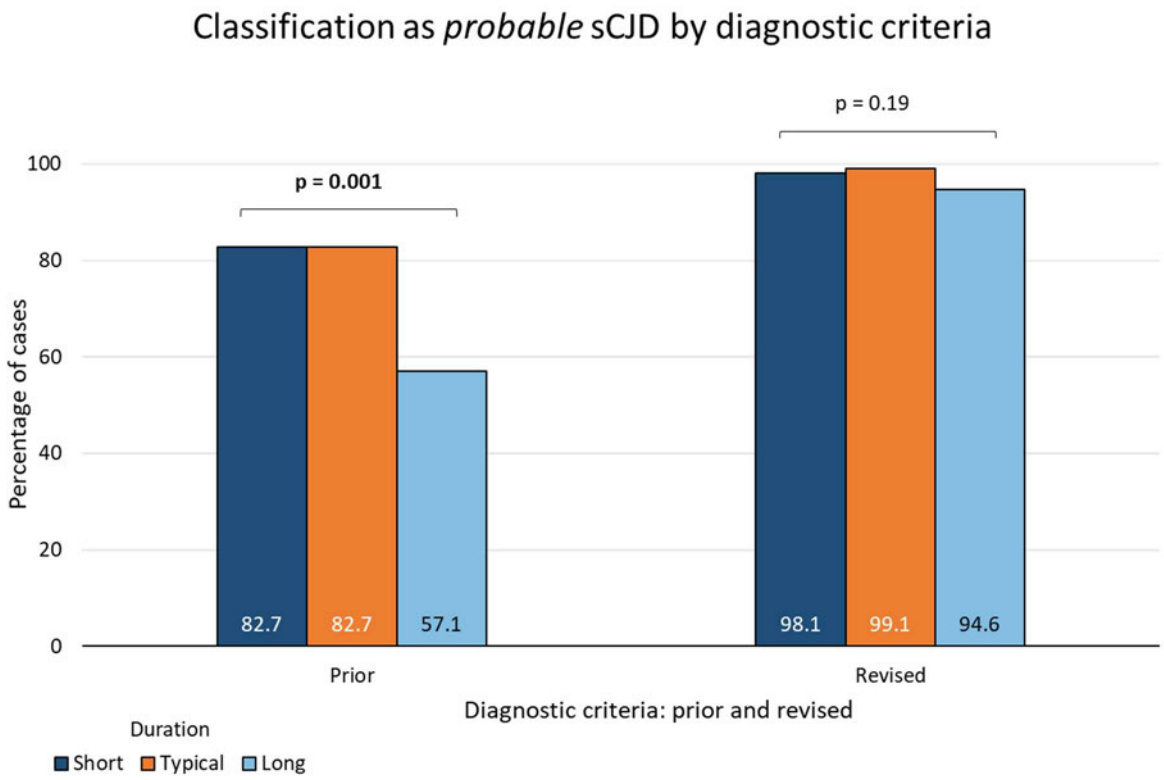


Figure 6.2. Classification of cases grouped by duration

Cases who underwent the full panel of investigations ('all' analysis) were grouped by disease duration from onset to death. Prior criteria were significantly less sensitive for cases with prolonged disease duration, while revised criteria showed no differences in sensitivity between duration groups.

In the 'any' analysis, the frequency of long survivors classified as *probable* sCJD underwent a 27.7% increase with 33 cases being re-classified. The majority (n=21) were previously classified as *possible* sCJD. Of these, 11 (52.4%) were reclassified due to cortical ribboning (2 had negative RT-QuIC; the remainder did not undergo RT-QuIC), 3 (14.3%) were due to RT-QuIC alone (2 had negative MRI and the other did not undergo MRI), and 7 (33.3%) were due to both.

4. Investigations

The sensitivity of diagnostic investigations was compared between sCJD cases grouped by survival duration (**table 6.5**).

There were no differences in sensitivity of MRI based on disease duration, including for MRI overall (i.e. with basal ganglia hyperintensity and/or cortical ribboning) and for individual outcomes (for example basal ganglia hyperintensity and cortical ribboning), with the exception of isolated basal

ganglia hyperintensity which was most frequent in cases with long duration (28 of 115, 24.4% [95% CI, 16.8%-33.2%] followed by typical (45 of 223, 20.2% [95% CI, 15.1%-26.1%] and short duration (11 of 106, 10.4% [95% CI, 5.3%-17.8%]; **P=0.02**).

RT-QuIC was most sensitive in cases with typical (126 of 131, 96.2% [95% CI, 91.3%-98.7%])) followed by prolonged (59 of 67, 88.1% [95% CI, 77.8%-94.7%]) and short (59 of 68, 86.8% [95% CI, 76.4%-93.8%]); **P=0.03**) duration. 14-3-3 and EEG were both most sensitive in short survival groups (**P<0.001** for both).

Table 6.5. Investigation sensitivity, cases stratified by duration

	Short (<75 days) (n=121)			Typical (75-222 days) (n=242)			Prolonged (>222 days) (n=121)			
	N	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	***P***
EEG	68/109	62.4	52.6-71.5	105/225	46.7	44.2-58.2	28/105	26.7	18.5-36.2	**<0.001**
MRI	92/106	86.8	78.8-92.6	190/223	85.3	79.9-89.6	104/115	90.4	83.5-95.1	0.41
CR & BG	48/106	45.3	35.6-55.3	86/223	38.6	32.1-45.3	43/115	37.4	28.6-46.9	0.42
CR alone	33/106	31.1	22.5-40.9	59/223	26.5	20.8-32.8	33/115	28.7	20.7-37.9	0.67
BG alone	11/106	10.4	5.3-17.8	45/223	20.2	15.1-26.1	28/115	24.4	16.8-33.2	**0.02**
CR (any)	81/106	76.4	67.2-84.1	145/223	65	58.4-71.3	76/115	66.1	56.7-74.7	0.94
BG (any)	59/106	55.7	45.7-65.3	131/223	58.7	52.0-65.3	71/115	61.7	52.2-70.7	0.66
14-3-3	90/107	84.1	75.8-90.5	175/226	77.4	71.4-82.7	53/108	49.1	39.3-58.9	**<0.001**
RT-QuIC	59/68	86.8	76.4-93.8	126/131	96.2	91.3-98.7	59/67	88.1	77.8-94.7	**0.03**

Sensitivity defined as positive outcome/total for cases. Duration measured in days.
'Any' in brackets refers to the frequency of cases displaying the MRI abnormality regardless of additional imaging features (for example, cortical ribboning with or without basal ganglia hyperintensities).

Abbreviations. BG, basal ganglia. CR, cortical ribboning. RT-QuIC, real-time quaking-induced conversion.

5. *PRNP* Codon 129 genotype

The frequencies of different *PRNP* c129 genotypes between survival groups are shown in **table 6.6** and **figure 6.3.** The distribution of genotypes varied significantly with duration (**P<0.001**). While MM was the commonest genotype and the most frequent in all three groups, the relative frequency of MM cases was highest among short survivors (48 of 56, 85.7%). In contrast, the highest relative frequency of MV cases within an individual group was seen in long survivors (34 of 88, 38.6%).

Table 6.6. Codon 129 and subtype information, sCJD cases stratified by duration

	Short (<75)	Typical (75-222)	Long (>222)	*P*
c129 genotype				
Available	56	147	88	
MM	48 (85.7)	97 (66.0)	44 (50.0)	**<0.001**
MV	7 (12.5)	15 (10.2)	34 (38.6)	
VV	1 (1.8)	35 (23.8)	10 (11.4)	
Subtype				
MM1	41 (77.4)	68 (55.3)	19 (25.0)	
MM2	-	-	13 (17.1)	
MM1+2	4 (7.5)	13 (10.6)	6 (7.9)	
MV1	6 (11.3)	6 (4.9)	7 (9.2)	
MV2	1 (1.9)	4 (3.3)	17 (22.4)	
MV1+2	-	1 (0.8)	6 (7.9)	
VV1	-	-	1 (1.3)	
VV2	1 (1.9)	31 (25.2)	5 (6.6)	
VV1+2	-	-	2 (2.6)	

Subtypes refer to cases grouped by c129 genotype and prion protein glycotype 1, 2 or both. P value shown is that for the comparison of genotype distribution between duration groups.
Duration measured in days.

Abbreviations. c129, codon 129. MM, methionine homozygous. MV, methionine-valine heterozygous. sCJD, sporadic Creutzfeldt-Jakob disease. VV, valine homozygous.

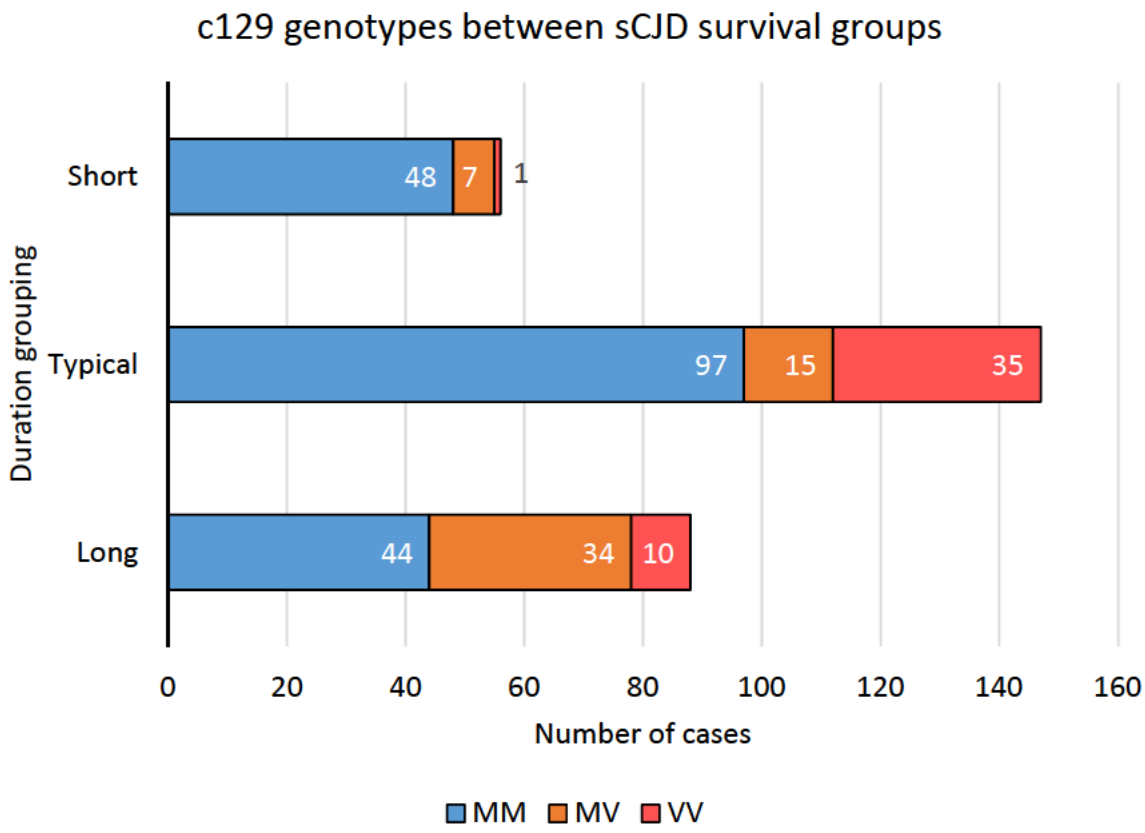


Figure 6.3. Frequency of individual c129 genotypes between duration groups

Among sCJD cases grouped by disease duration there were differences in observed rates of *PRNP* c129 genotypes. MV cases accounted for a disproportionate number of long survivors.

Duration measured in days; short <75, typical 75-222, long >222.

Abbreviations. c129, codon 129. MM, methionine homozygous. MV, methionine-valine heterozygous. sCJD, sporadic Creutzfeldt-Jakob disease. VV, valine homozygous.

6. sCJD subtypes defined by Parchi classification

The frequencies of sCJD subtypes defined by *PRNP* c129 genotype and PrP^{Sc} glycotype pairings (Parchi classification) between duration groups are shown in **table 6.6** and **figure 6.4**. The MM1 subtype was the commonest subtype in the overall cohort **(see Chapter 5 table 5.6**) and also in all duration groups, but the preponderance was most marked among short (n=41, 77.4%) followed by typical (n=68, 55.3%) and finally long survivors (n=19, 25.0%). The converse was seen in MV2 sCJD, most frequently seen in long (n=17, 22.4%), followed by typical (n=4, 3.3%) and short (n=1, 1.9%) survivors. VV2, the second-commonest subtype in the cohort, was associated with typical survival duration, accounting for 25.2% of cases in that group. MM2 and VV1 cases were only identified in the long survival group where they accounted for 13 (17.1%) and 1 (1.3%) of cases respectively.

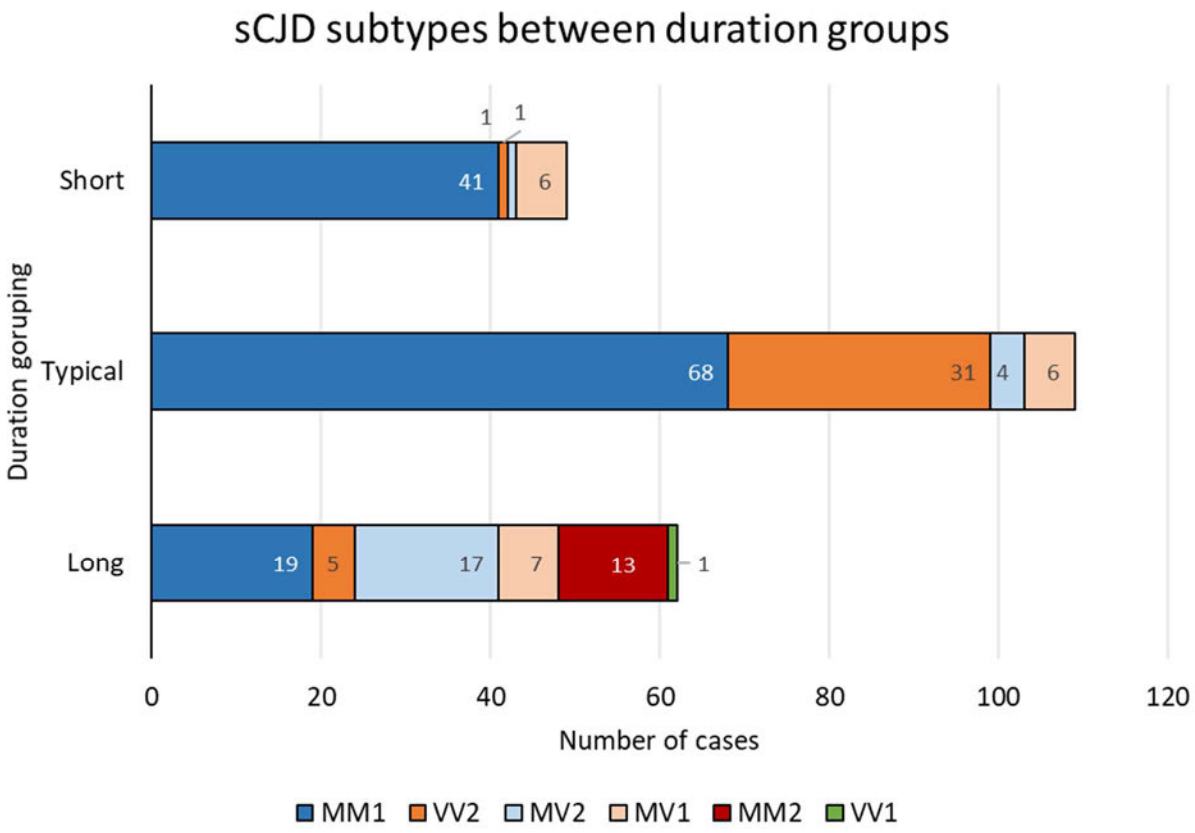


Figure 6.4. Frequency of individual sCJD subtypes between duration groups.

Among sCJD cases grouped by disease duration there were differences in observed rates subtypes defined by combinations of *PRNP* c129 genotypes and PrP^{Sc} glycotypes. MM2 and MV2 cases accounted for disproportionate numbers of long survivors.

Duration in days defined as short (<75), typical (75-222) and long (>222).

Abbreviations. c129, codon 129. MM, methionine homozygous. MV, methionine-valine heterozygous. *PRNP,* prion protein gene. VV, valine homozygous.

Part 2. Age

1. **Demographics**
2. **Clinical features**
3. **Case classification**
4. **Investigations**
5. ***PRNP* Codon 129 genotype**
6. **Investigation subtypes**

sCJD cases were stratified into groups according to age. Data on age were available in 497 individuals (99.2% of cohort). Young individuals were defined as those with age less than one SD from the mean (individuals <59 years old), while older individuals were those with age more than one SD from the mean (>78.6 years old); individuals with ages between these were defined as the standard age group.

1. Demographics

79 (15.9%) cases were in the young group, 333 (67.0%) in the standard group and 85 (17.1%) in the older group (**table 6.7**). 40 young cases were male (50.6%) compared to 169 (50.8%) and 42 (49.4%) in standard and older groups respectively, indicating no sex preponderance in any group (P=0.98). Median survival measured in days was shortest in the older group (101 [IQR 71.25-177]) followed by standard (115 [73.5-230]) and longest in the young group (161 [92.25-282.75]; **P=0.01**). Post-hoc analysis using Dunn's test with Bonferroni correction factors indicated significant differences in survival duration between young versus older cases (**P=0.009**), while the differences for young vs standard (P=0.06) and older vs standard (P=0.48) were not significant. Survival curves for the three groups of sCJD cases stratified by age are shown in **figure 6.5**.

Table 6.7. Cases stratified by age

	Young (<59.0)	Standard (59.0-78.6)	Older (>78.6)	*P*
Total	79	333	85	
Male	40 (50.6)	169 (50.8)	42 (49.4)	0.98
Female	39 (49.4)	164 (49.2)	43 (50.6)	
Duration (days)				
Median (IQR)	161 (92.25-282.75)	115 (73.5-230)	101 (71.25-177)	**0.01**

Age measured in years. For sex, brackets indicate percentages.

Post-hoc analysis using Dunn test demonstrated significant differences between older vs young cases (P=0.009). Differences were not significant for older vs standard (P=0.48) or standard vs young (P=0.06)

Abbreviations. IQR, inter-quartile range

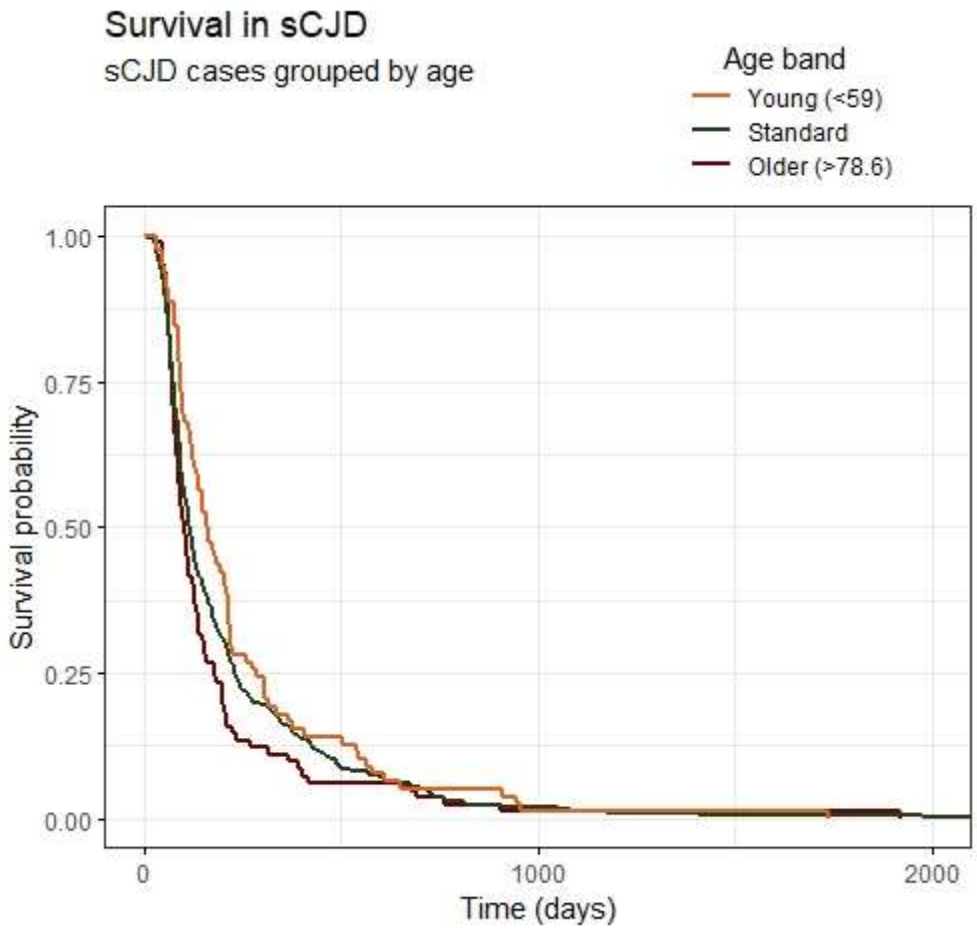


Figure 6.5. Survival in sCJD cases grouped by age

Survival curves for sCJD cases grouped according to age at death measured in years. The extended survival in young adults was statistically significant (P=0.01).

2. Clinical features:

The frequency of cardinal clinical features used in the diagnosis of sCJD for each age group is displayed in **table 6.8** and **figure 6.6**. The vast majority of cases in all groups had rapidly-progressive cognitive decline (RPCD) with no difference seen between groups (P=0.88). Cerebellar features were most frequent in young cases (66 of 79, 83.5%) followed by standard (237 of 322, 73.6%) and older cases (54 of 83, 65.1%; **P=0.03**). Visual features were most frequently seen in young cases (43 of 79, 54.4%) followed by standard (160 of 322, 49.7%) and older cases (31 of 83, 37.3%), but this difference did not reach statistical significance (P=0.06). No other features varied between groups to any degree approaching statistical significance.

Table 6.8. Clinical features, cases grouped by age

	Young (<59.0 years)	Standard (59.0-78.6 years)	Older (>78.6 years)	*P*
Available (%)	79 (100.0)	322 (96.7)	83 (97.7)	
Feature, n (%)				
RPCD	78 (98.7)	316 (98.1)	81 (97.6)	0.88
Myoclonus	54 (68.4)	222 (68.9)	56 (67.5)	0.97
Visual	43 (54.4)	160 (49.7)	31 (37.3)	0.06
Cerebellar	66 (83.5)	237 (73.6)	54 (65.1)	**0.03**
Pyramidal	29 (36.7)	146 (45.3)	40 (48.2)	0.29
Extrapyramidal	38 (48.1)	168 (52.2)	40 (48.2)	0.71
Akinetic Mutism	39 (49.4)	137 (42.5)	41 (49.4)	0.36

Figures calculated using individuals for whom clinical data were available, total number shown in the first row (with percentages referring to total number of cases in each age category).
Age measured in years.
Fisher's exact test used for RPCD

Abbreviations. RPCD, rapidly-progressive cognitive decline.

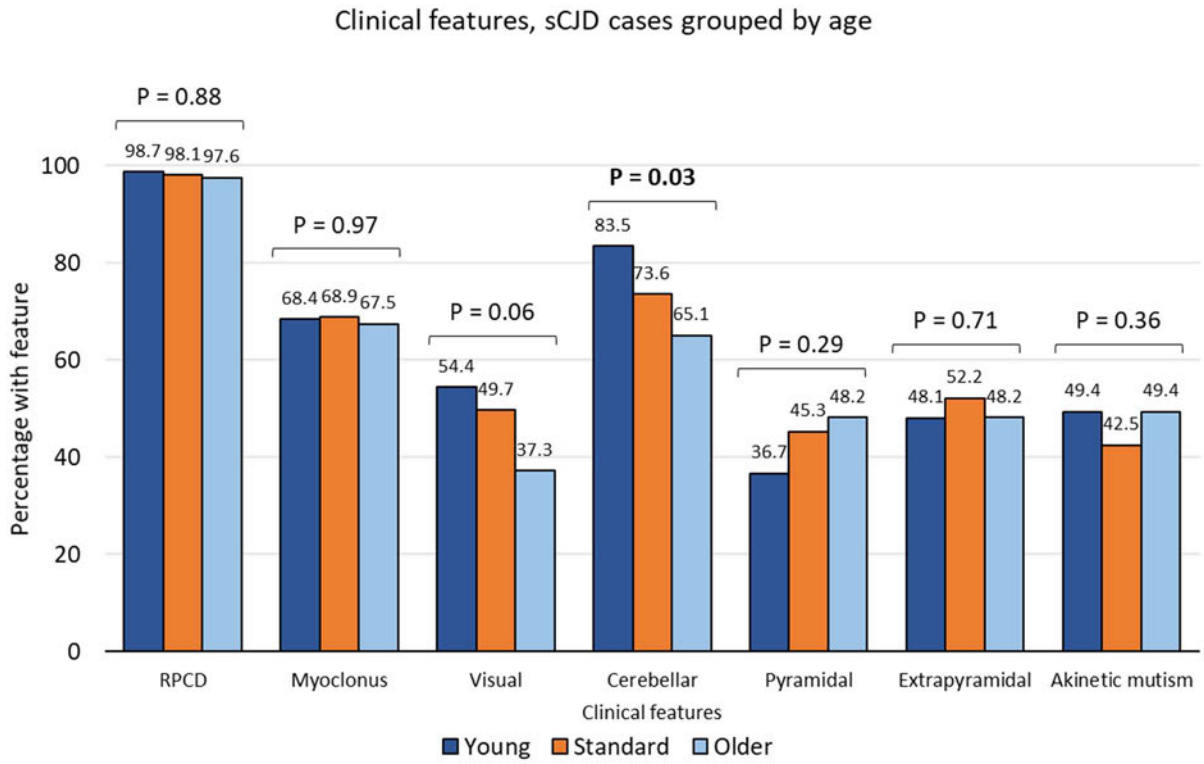


Figure 6.6. Clinical features, sCJD cases grouped by age

Clinical features shown in sCJD cases grouped by age (years) into young (<59.9), standard (59.0-78.6) and older (>78.6) groups. There was a significant association between younger age and cerebellar features (**P=0.03**). A similar trend towards an association with younger age and visual features was present but not statistically significant (P=0.06).

Abbreviations: RPCD, rapidly-progressive cognitive decline.

3. Case classification:

Cases were classified according to the diagnostic criteria after grouping by age (**table 6.9**). No statistically-significant differences were seen in the frequency of cases fulfilling criteria for *possible* sCJD diagnosis, i.e. those with sufficient clinical features (P=0.81). Continuing the approach described above and **Chapters 4 & 5**, I first performed an 'any' analysis. No differences were seen in the sensitivity of prior (P=0.15) or revised (P=0.73) criteria definitions of *probable* sCJD.

Table 6.9. Diagnostic criteria sensitivity, cases grouped by age

	Young (<59 years) (n=79)			Standard (59-78.6 years) (n=333)			Older (>78.6 years) (n=85)			
	n	%	95% CI	N	%	95% CI	n	%	95% CI	*P*
Clinical features (*possible* sCJD)	70/79	88.6	79.5-94.7	278/323	86.1	81.8-89.7	70/82	85.4	75.8-92.2	0.81
Probable sCJD										
Any										
Revised	73/79	92.4	84.2-97.2	300/323	92.9	89.5-95.4	74/82	90.2	81.7-95.7	0.73
Prior	68/79	86.1	76.5-92.8	246/323	76.2	71.1-80.7	62/82	75.6	70.9-88.0	0.15
All										
Revised	36/38	94.7	82.3-99.4	144/145	99.3	96.2-100.0	36/38	94.7	82.3-99.4	**0.049**
Prior	32/38	84.2	68.8-94.0	110/145	75.9	68.1-82.6	27/38	71.1	54.1-84.6	0.38

Sensitivity defined as positive outcome/total for cases.
'Any' refers to cases with any combination of investigations, 'all' those with the full panel performed.
Fisher's exact test used for 'all' analysis of revised criteria classification

Abbreviations. CI, confidence interval. sCJD, sporadic Creutzfeldt-Jakob disease.

In the 'all' analysis, a statistically significant difference in sensitivity for *probable* classification was seen between groups, being highest in the standard age group (144 of 145 [99.3%; 95% CI, 96.2%-100.0%]) and equal in both young (36 of 38 [94.7%; 95% CI, 82.3%-99.4%]) and older groups (36 of 38 [94.7%; 95% CI, 82.3%-99.4%]; **P=0.049**). However, no significant difference in sensitivity was observed with prior criteria (P=0.38).

In both the 'any' and 'all' analyses the frequency of cases classified as *probable* sCJD increased in all age groups (**table 6.10**). The largest gain was seen for older cases in the 'all' analysis with a 23.7% increase, due to both an 18.% decline in *unclear* cases and 5.3% decline in *possible* cases. In both analyses, the least substantial increases were observed in young cases (6.3% in the 'any' analysis and

10.5% in the ‘all’ analysis), indicating that the revised criteria have had the least impact on the re-classification of younger sCJD cases.

Table 6.10. Case classification by diagnostic criteria, cases stratified by age

	Young (<59.0 years)			Standard (59.0-78.6 years)			Older (>78.6 years)		
Classification	**Prior**	**Revised**	**Change (%)**	**Prior**	**Revised**	**Change (%)**	**Prior**	**Revised**	**Change (%)**
Any test									
Probable	68 (86.1)	73 (92.4)	6.3	246 (76.2)	300 (92.9)	16.7	62 (75.6)	74 (90.2)	14.6
Possible	2 (2.5)	0 (0)	-2.5	32 (9.9)	6 (1.9)	-8	8 (9.8)	4 (4.9)	-4.9
Unclear	9 (11.4)	6 (7.6)	-3.8	45 (13.9)	17 (5.3)	-8.7	12 (14.6)	4 (4.9)	-9.8
All tests									
Probable	32 (84.2)	36 (94.7)	10.5	110 (75.9)	144 (99.3)	23.4	27 (71.1)	36 (94.7)	23.7
Possible	1 (2.6)	0 (0)	-2.6	10 (6.9)	0 (0)	-6.9	3 (7.9)	1 (2.6)	-5.3
Unclear	5 (13.2)	2 (5.3)	-7.9	25 (17.2)	1 (0.7)	-16.6	8 (21.1)	1 (2.6)	-18.4

Age measured in years. Brackets show percentages.

4. Diagnostic investigations

The sensitivity of diagnostic investigations was calculated for the three age groups (**table 6.11**). RT-QuIC was highly sensitive in young (40 of 45 [88.9%; 95% CI, 80.0%-96.3%]), standard (168 of 181 [92.8%; 95% CI, 88.0%-96.1%]) and older (41 of 45 [91.1%; 95% CI, 78.8%-97.5%]) cases, with no statistically significant difference between groups (P=0.57).

There were statistically significant differences in the sensitivity of MRI, in terms of overall sensitivity as well as the sensitivity of individual diagnostic abnormalities (**figure 6.7)**. There was an age-dependent decline in sensitivity of MRI, highest in young cases (74 of 78 [94.9%; 95% CI, 87.4%-98.6%]) followed by standard (258 of 296 [87.2%; 95% CI, 82.8%-90.8%]) and older cases (60 of 77 [77.9%; 95% CI, 67.0%-86.6%]; **P=0.007**). Basal ganglia hyperintensities were most frequently reported in young cases (64 of 78 [82.1%; 95% CI, 73.2%-90.8%]) followed by standard (166 of 296 [56.1%; 95% CI, 50.2%-61.8%]) and older cases (35 of 77 [45.5%; 95% CI, 34.1%-57.2%]; **P<0.001**). Cortical ribboning was most frequent among cases with standard age (211 of 296 [71.3%; 95% CI, 65.8%-76.4%]) followed by young (53 of 78 [67.9%; 95% CI, 56.4%-78.1%]) and older cases (43 of 77 [55.8%; 95% CI, 44.1%-67.2%]; **P=0.04**). Isolated basal ganglia hyperintensities were most frequent in young cases (64 of 78 (26.9%; 95% CI, 17.5%-38.2%]) followed by older (17 of 77 [22.1%; 95% CI, 13.4%-33.0%]) and standard cases (47 of 296 [15.9%; 95% CI, 11.9%-20.6%]; **P=0.03**).

While differences were observed between the sensitivities of EEG and 14-3-3 between age groups, these were not statistically significant (P=0.15 for EEG and P=0.21 for 14-3-3) (**table 6.11**).

Table 6.11. Investigation sensitivity, cases grouped by age

	Young (<59 years) (n=79)			Standard (59-78.6 years) (n=333)			Older (>78.6 years) (n=85)			
	n	%	95% CI	n	%	95% CI	n	%	95% CI	*P*
EEG	26/72	36.1	25.1-48.3	142/298	47.7	41.9-53.5	38/75	50.7	38.9-62.4	0.15
MRI	74/78	94.9	87.4-98.6	258/296	87.2	82.8-90.8	60/77	77.9	67.0-86.6	**0.007**
CR & BG	43/78	55.1	43.4-66.4	119/296	40.2	34.6-46.0	18/77	23.4	14.5-34.4	**<0.001**
CR alone	10/78	12.8	6.3-22.3	92/296	31.1	25.9-36.7	25/77	32.5	22.2-44.1	**0.004**
BG alone	21/78	26.9	17.5-38.2	47/296	15.9	11.9-20.6	17/77	22.1	13.4-33.0	**0.03**
CR (any)	53/78	67.9	56.4-78.1	211/296	71.3	65.8-76.4	43/77	55.8	44.1-67.2	**0.04**
BG (any)	64/78	82.1	73.2-90.8	166/296	56.1	50.2-61.8	35/77	45.5	34.1-57.2	**<0.001**
14-3-3	56/77	72.7	61.4-82.3	210/301	69.8	64.2-74.9	57/71	80.3	69.1-88.8	0.21
RT-QuIC	40/45	88.9	80.0-96.3	168/181	92.8	88.0-96.1	41/45	91.1	78.8-97.5	0.57

Sensitivity defined as positive outcome/total for cases.
Fisher's exact test used for RT-QuIC.

Abbreviations. BG, basal ganglia hyperintensity. CI, confidence interval. CR, cortical ribboning. EEG, electroencephalography. MRI, magnetic resonance imaging. RT-QuIC, real-time quaking-induced conversion.

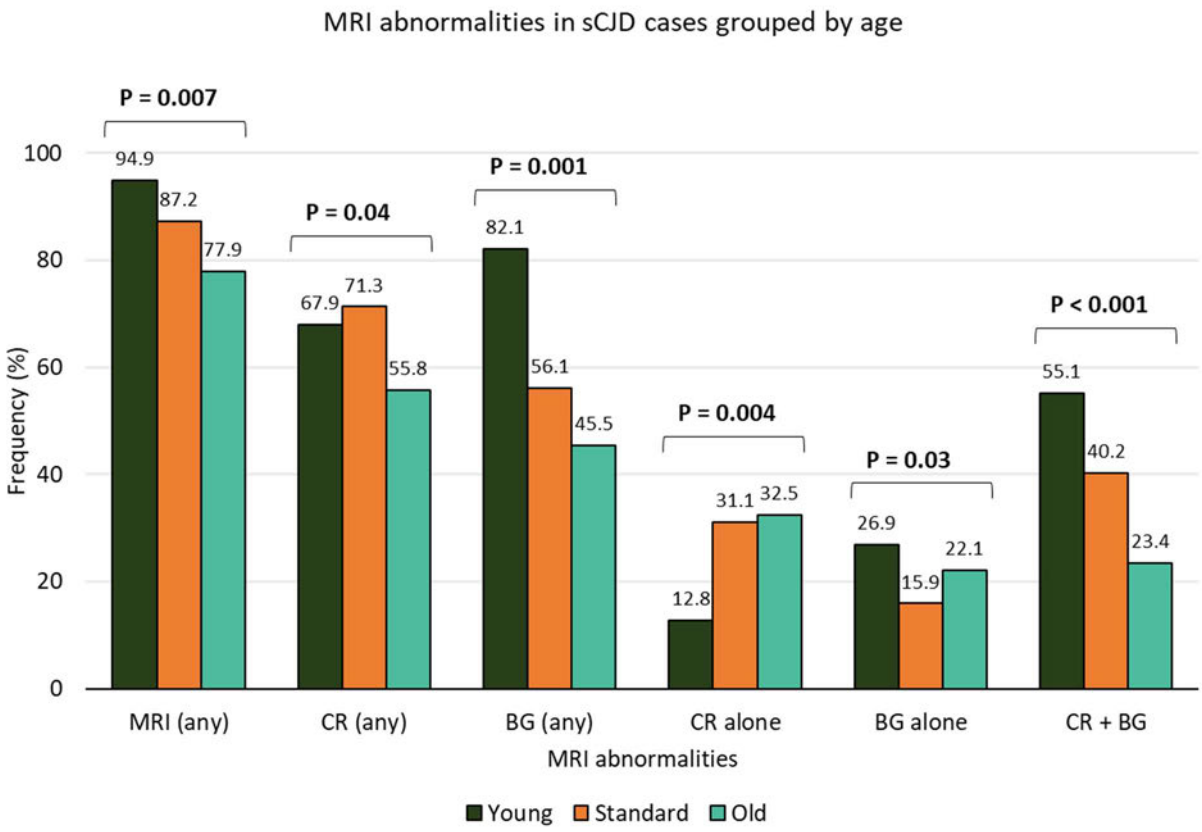


Figure 6.7. MRI abnormalities in sCJD cases grouped by age

MRI abnormalities significantly varied with age for any individual abnormality or combination of abnormalities. Basal ganglia hyperintensities were generally associated with younger age. Isolated cortical ribboning was most frequently encountered in older cases.

Abbreviations. BG, basal ganglia hyperintensity. CR, cortical ribboning. MRI, magnetic resonance imaging. sCJD, sporadic Creutzfeldt-Jakob disease.

5. *PRNP* Codon 129 genotype

The frequencies of different *PRNP* c129 genotypes are shown in **table 6.12**. Data were available in 55 (69.6%) young, 200 (60.0%) standard and 45 (52.9%) older cases respectively. In all groups, c129 genotype MM was the most frequent, while the least frequent genotype varied between groups. In young cases MV was least frequent genotype (n=6, 10.9%) while VV was the least frequent genotype in standard (n=30, 15.0%) and older groups (n=5, 11.1%). However, the frequencies of difference c129 genotypes did not vary between groups to a statistically significant degree (P=0.18).

6. Subtypes defined by Parchi classification

sCJD subtypes defined by c129 genotype in combination with PrP glycotype are shown in **table 6.12**. In all groups the most frequent subtype was MM1. The rarest subtype in the cohort was VV1 with only 1 individual identified; this individual was in the standard age group. No MM2 cases were identified in the older cases group. Young cases had the highest frequency of VV2 subtypes (n=12, 25.5%), while older cases had the highest frequency of MV2 subtypes (n=5, 12.5%).

Table 6.12. Codon 129 and subtype information, cases stratified by age

	Young (<59.0)	Standard (59.0-78.6)	Older (>78.6)	*P*
***PRNP* c129 genotype**				
Available	55 (69.6)	200 (60.0)	45 (52.9)	
MM	36 (65.5)	127 (63.5)	33 (73.3)	0.18
MV	6 (10.9)	43 (21.5)	7 (15.6)	
VV	13 (23.6)	30 (15.0)	5 (11.1)	
Subtype				
MM1	23 (48.9)	84 (49.1)	23 (57.5)	
MM2	6 (12.8)	9 (5.3)	-	
MM1+2	2 (4.3)	17 (9.9)	5 (12.5)	
MV1	1 (2.1)	16 (9.4)	2 (5.0)	
MV2	1 (2.1)	16 (9.4)	5 (12.5)	
MV1+2	2 (4.3)	5 (2.9)	-	
VV1	-	1 (0.6)	-	
VV2	12 (25.5)	21 (12.3)	5 (12.5)	
VV1+2	-	2 (1.2)	-	

Age measured in years. Brackets list percentages.
MM, MV and VV refer to *PRNP* c129 genotypes. Subtypes are defined by these in combination with prion protein glycotype 1, 2 or both.

Abbreviations. c129, codon 129. MM, methionine homozygous. MV, methionine-valine heterozygous. *PRNP*, prion protein gene. sCJD, sporadic Creutzfeldt-Jakob disease. VV, valine homozygous.

Discussion

In this **Chapter** I stratified sCJD cases according to two important characteristics, age and disease duration, and interrogated the performance of the diagnostic criteria in these groups as well as the associated demographic, clinical and biological features associated with these. These were not able to be studied by the single-centre validation study by Hermann *et al*[91], and represent a novel aspect of my approach.

Rising sensitivity among long survivors

There was a marked improvement in sensitivity for *probable* sCJD classification among long survivors, representing a 37.5% increase when all investigations were performed (**tables 6.3 & 6.4, figure 6.2**). The previous criteria were significantly less sensitive in this group (57.1%) compared to both short and typical survivors (82.7% for each, **P=0.001** for difference), yet this variation was not observed with revised criteria. A similar but less dramatic rise of 27.7% was observed in cases who underwent at least one investigation, indicating that the effect did not require the full workup to take place. The majority of cases in the cohort (276 of 501 [55.1%]) underwent ≤3 investigations (**table 4.9** & **figure 4.5**), i.e. incomplete workup, indicating that this is the 'real-world' norm for most cases, and that the gain in sensitivity should apply to long survivors evaluated by surveillance centres even with varying methods and usage of individual investigations.

This marked rise in accurate in-life classification of sCJD cases with prolonged survival has major advantages for surveillance; many of these cases would have defied in-life classification by prior criteria, potentially escaping diagnosis had they not proceeded to neuropathological examination, which is only performed in a diminishing minority of cases in modern surveillance[1,42]. It is conceivable that such individuals with slower progression might be at risk of misdiagnosis as alternative neurodegenerative conditions such as Alzheimer's disease[81] (**vignette 6.1**). Provisional work by the NCJDRSU led by Dr John Centola has looked at cases only identified at post-mortem, and has indicated both longer survival and frequent in-life misdiagnosis as an alternative neurodegenerative aetiology (see **Chapter 9**).

In the 'any' analysis the largest change (17.6%) was seen in long survivors reclassified from *possible* to *probable* sCJD (n=21). This transition implies adequate clinical features and the presence of at least one of positive RT-QuIC and/or cortical ribboning, with negative results for 14-3-3 and/or EEG and the absence of basal ganglia hyperintensities on MRI (if these were performed), hence prior classification as *possible* sCJD. MRI with cortical ribboning was the largest contributor to this transition, both on its own (n=11) and accompanied by positive RT-QuIC (n=7).

This information is useful to understand the impact of the novel criteria. 14-3-3 and EEG display higher sensitivity in short survivors[304] and this was the case in this study (14-3-3 84.1%; EEG 62.4%). This was not the case for MRI outcomes including basal ganglia hyperintensities; no significant differences were seen between groups for the frequency of this abnormality, with the exception of isolated basal ganglia hyperintensities (i.e. not accompanied by cortical hyperintensities) which were more frequent among long-survivors, likely reflecting prevalent underlying sCJD subtypes such as MV2[180]. RT-QuIC was found to significantly vary in sensitivity between groups, being maximal in the typical survival group (96.2%) and least sensitive in short survivors (86.8%). The clinical relevance of this discrepancy is uncertain; the overall values were high in all three duration groups, and the lowest, 86.8%, is higher than almost all other investigations in the study for any duration group (bar MRI in long survivors at 90.4%); hence, this statistically-significant finding may have limited 'real-world' significance, but it is perhaps noteworthy that non-typical duration cases may have a higher probability of a negative RT-QuIC result.

The influence of disease duration on sensitivity of RT-QuIC is not yet fully known. A 2012 study found no relationship between duration or timing of sampling with RT-QuIC sensitivity[360], while a 2015 study found that shorter duration was associated with higher seeding activity[361]. More recently a 2020 study found longer survival and earlier sampling were associated with lower RT-QuIC sensitivity [242]. Thus the overall pattern is unclear and further longitudinal studies are necessary to establish if there is a relationship between duration and RT-QuIC sensitivity, ideally analysing such variations in a multivariable fashion to control for potentially-confounding variables such as *PRNP* c129 genotype and PrP^{Sc} glycotype combinations (as discussed below).

Vignette 6.1. Prolonged disease duration

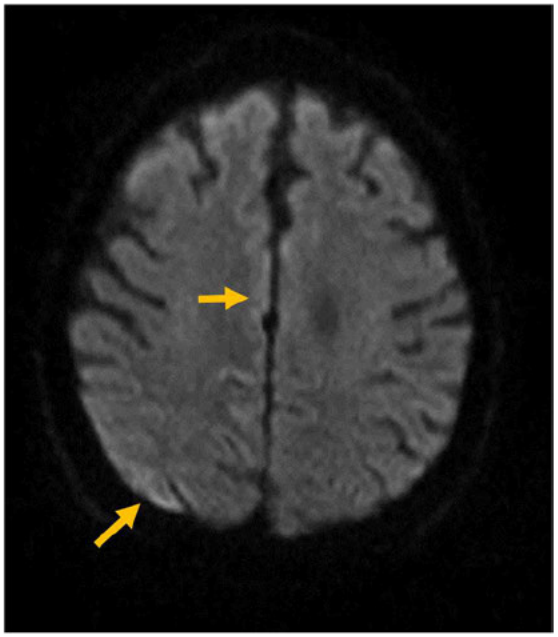
A 68-year-old man developed concentration difficulties and limb paraesthesias. In subsequent weeks he became paranoid and developed delusions about his partner, and displayed disinhibited sexual behaviour. Over the following months his recall and orientation deteriorated. He displayed poor judgement while driving, misjudging distances and driving too fast. His gait became unsteady and he began having falls. He became less interactive and emotionally blunted. He required prompting for basic daily tasks and developed urinary incontinence.

He was hospitalised 12 months post-onset due to cognitive decline and poor mobility. .

During the NCJDRSU assessment he was disorientated. He scored 75/100 on an Addenbrooke's cognitive examination, losing points for attention, memory and fluency. He had a frontal affect, displaying concrete and rigid thought patterns. He displayed saccadic intrusions, a positive jaw jerk, grasp and palmomental reflexes, left arm myoclonus, and bilateral postural and action tremors in the arms. He had bilateral lower limb rigidity and hyperreflexia with upgoing plantars. An exaggerated startle reflex was present.

b=1000 DWI demonstrated subtle cortical ribboning without basal ganglia hyperintensities. CSF 14-3-3 and S100b were negative; RT-QuIC was positive. Genetic testing demonstrated *PRNP* c129 genotype MV and excluded pathogenic mutations.

The patient died 764 days post-onset. Post-mortem demonstrated MV2 sCJD.



If it were to emerge that longer duration was associated with lower RT-QuIC sensitivity, it would be important to use multivariable analyses to explore if this was independent of the influence of

subtypes, particularly those associated with both longer survival and lower RT-QuIC sensitivity (such as MM2)[356,363,437]. In addition it would be valuable to explore the influence of timing of sampling; in patients with rapid progression, CSF sampling might be more likely to occur at a relatively later stage of the overall disease trajectory, which might partly influence the apparent influence of duration on sensitivity. It would be particularly valuable to interrogate any individuals in whom RT-QuIC converted from negative to positive with disease progression.

Evidently the addition of cortical ribboning, the sensitivity of which does not vary with duration, and RT-QuIC, which does but not to a degree likely to have major clinical impact, have enhanced the ability to appropriately classify long survivors with clinically-compatible feature as *probable* sCJD. Of note, 4 of the individuals re-classified by cortical ribboning had MM2 sCJD, a subtype associated with cortical ribboning and long-survival[179,430], and a group likely to be better classified by the revised criteria. RT-QuIC was less sensitive for this subtype (66.7%), as discussed in **Chapter 5**.

RT-QuIC enabled classification of 10.1% (n=12) of long-survivors lacking adequate clinical features (i.e. *unclear* classification) as *probable* sCJD (**table 6.4**). Interestingly, all of these cases had at least 1 other investigation (MRI in 11, 14-3-3 in 8, EEG in 3) supporting the diagnosis, but did not satisfy clinical criteria. I have discussed the challenges surrounding such clinically-limited cases in **Chapter 4,** and the major value of RT-QuIC in resolving such dilemmas, which clearly extends to long-survivors, a group which may be more likely to lack adequate clinical features for much of their disease trajectory, defying classification in the absence of RT-QuIC.

Biological basis for survival variations: *PRNP* c129 genotype and PrP^{Sc} Parchi subtype

The three duration groups featured different proportions of both *PRNP* c129 genotypes and individual Parchi subtypes. As explored in **Chapter 5**, MV cases had the longest survival durations among genotypes, and consequently were over-represented among long survivors (34 of 88 [38.6%]); the majority of MV cases were long survivors (34 of 56 [60.7%]. This association of MV genotype with increased survival is important from a prognostic perspective and has been reported in the literature[3,11,434]. MM cases made up the majority of short survivors (48 of 56 [85.7%]), although most MM cases within the cohort were in the typical survival group (97 of 189 MM cases [51.3%]).

The MM1 subtype accounted for 77.4% of cases with short survival and only 25.0% of long surviving cases. In contrast, the MM2 subtype was only identified in the long survival cohort, accounting for 17.1% of these cases. As in **Chapter 5**, the median survival among MM2 cases was 451 days in stark contrast to MM1 cases at 92.5 days. This contrast has been reported in the literature since the landmark clinicopathological studies by Parchi *et al* in the 1990s[6,179,433]. The VV2 subtype, the second commonest in my study cohort (n=38 [14.7%] and in the literature[6], was most prevalent among the

typical survival duration group, accounting for 25.2% of cases, while the VV1 subtype, the most rare[181], had only one individual with prolonged survival (320 days). The contrasts between MM1 and MM2 cases, likewise VV1 and VV2, in disease duration illustrate the profound influence of the biochemical properties of PrP^{Sc} on many aspects of the disease. The duration of overall survival is a critically important variable for affected individuals and their relatives in addition to medical service providers, and understanding the biological factors underpinning prognosis is vital; this study contributes to this important branch of the prion disease literature.

Clinical features

I compared the frequency of cardinal clinical features between disease duration groups. Myoclonus was most frequent among short survivors (73.1%) and least in long survivors (61.3%), although the trend did not achieve significance (P=0.08). The apparent predominance of myoclonus among short survivors may reflect two factors. Firstly, this feature was very common in MM1 cases in my series, reported in 72.4% of cases; MM1 was the subtype with the highest proportion of cases displaying myoclonus (see **Chapter 5**). This is similar to the reported literature: myoclonus was seen in 97% of MM1 cases in the original series by Parchi *et al*[6]. MM1 cases accounted for most short survivors, while myoclonus is less common among subtypes MV2[180] and VV2[308] which are associated with longer survival[6]. Hence the underlying disease subtypes comprising survivor groups may partly account for clinical feature prevalence. Secondly, my analysis did not report on the timing of emergence of clinical features; it is conceivable that individuals with shorter survival would have undergone assessment at a relatively later, more advanced stage in their illness, often at a stage when emergency hospitalisation is necessary (in comparison to long survivors who, in my experience, frequently underwent assessment in outpatient settings), and myoclonus is known to emerge with more advanced disease states[6,180,181,308,433,438-441].

Extrapyramidal features were most frequent in long survivors (58.8%); again, this may reflect sCJD subtypes associated both with longer survival and the presence of features such as Parkinsonism, present in 33% of MM2-C, 17% of MM2-T, 22% of MV2 and 33% of VV1 cases, yet only 7% of MM1 cases[6]. Case series have indicated high prevalence of extrapyramidal features in MV2[180], VV1[181] and MM2[433] cases. This is useful information; an ongoing NCJDRSU study assessing cases evading diagnosis during life has indicated higher proportions of cases with extrapyramidal features (see **Chapter 9**). Enhanced recognition of, and diagnostic capacity for, sCJD cases with longer survival and atypical phenotypes will likely have an impact on ongoing ascertainment of such cases.

My approach was defined using quartiles; the long survival group included individuals with duration >222 days (i.e. 7.4 months). The study was underpowered to assess the performance of diagnostic criteria in cases with very long survival, for example >3 years (n=6, 1.2% of cohort). It would be

interesting to explore the characteristics of such rare, outlying individuals, but this would require a long study period enrolling cases from many nations to recruit adequate numbers. Given the rarity of such cases, insights gained might be of limited real-world value in contrast to my approach, which included the 25% of cases with atypically long survival, who comprise a large proportion of real-world sCJD cases.

Age analysis

Stratifying cases into young, standard and older age groups demonstrated a significant association of younger age with longer survival, as has been reported previously[3,382]. Underlying factors may include the preponderance of atypical subtypes associated with longer duration in younger cases such as MM2 (12.8%) as well as potentially greater resilience amid degenerative neurological disease in younger adults (**vignette 6.2**). Another important confounding factor may have been a higher proportion of younger adults receiving life-prolonging therapies such as artificial feeding[18] or intensive care support[442]; I did not assess for these factors in the study and did not have the necessary data for this analysis.

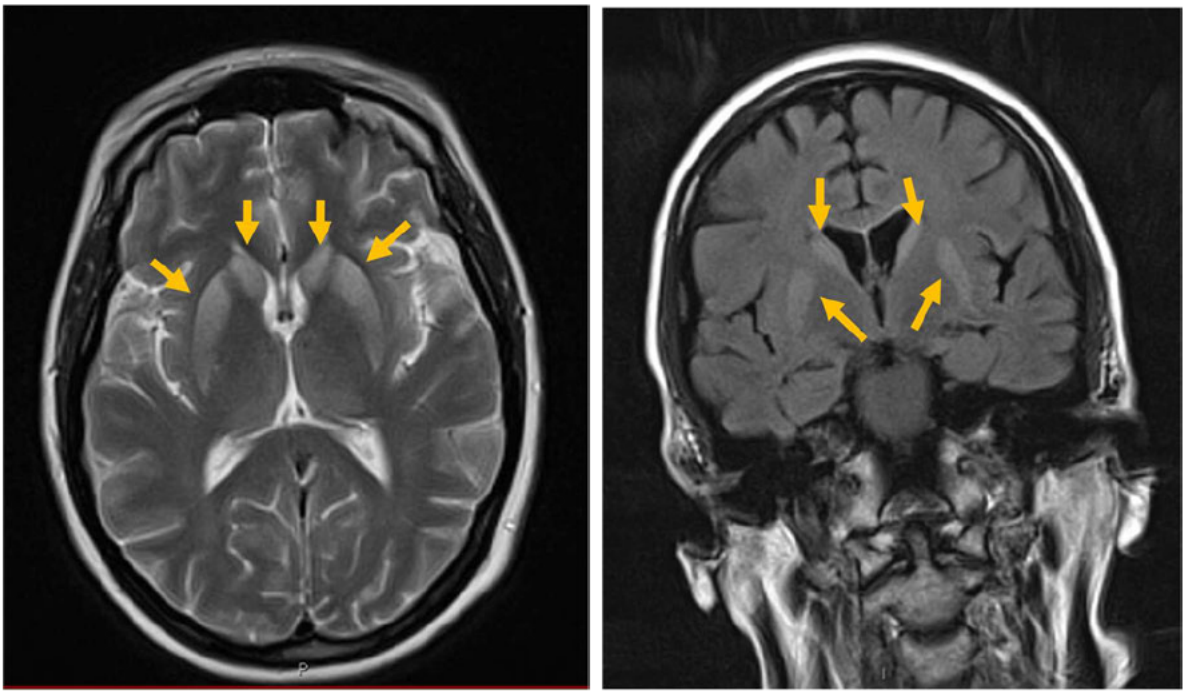
Vignette 6.2. Young-onset

A 47-year old woman developed gait ataxia over several months. Her cognition deteriorated with poor self-care. She became irritable and anxious, and developed a sweet tooth. She subsequently developed dysphasia, a unilateral arm tremor, and continued to deteriorate, eventually moving in with her parents.

She was eventually hospitalised. During the NCJDRSU assessment she was disorientated, scoring 16/30 on MMSE with deficits in orientation, attention and recall. She had dysarthric speech, saccadic intrusion, pout and grasp reflexes, myoclonus and upper limb ataxia.

MRI brain demonstrated bilateral basal ganglia hyperintensities on T2 (left) and FLAIR (right). EEG demonstrated diffuse slowing. CSF 14-3-3 and S100b were negative; RT-QuIC was positive. Genetic testing revealed c129 MV genotype and no pathogenic mutations.

She died 334 days from onset. Post-mortem demonstrated MV2 sCJD.



When individuals were stratified by age, increases were seen in all groups in the proportion classified as *probable* by revised criteria compared to prior criteria. The largest rise was seen in older cases at 23.7%, predominantly due to *unclear* cases being reclassified due to RT-QuIC. The least marked rises were in young cases. The prior criteria were not significantly different in sensitivity between age groups in the 'any' or 'all' analyses, while in the 'all' analysis the revised criteria were significantly most sensitive for standard-aged cases (99.3%) and were equivalent (94.7%) for young and older cases.

The large rise among older cases indicates a potentially enhanced capacity to classify this age group appropriately in-life. This poses interesting questions concerning its impact on modern sCJD

surveillance, as older adults represent a less commonly encountered subgroup. The observed lower incidence of sCJD in older adults is not fully understood; while there may be biological reasons for this, it may in part reflect limited ascertainment[42]. Plausible hypotheses for this might include older individuals not undergoing testing, sCJD not being considered as a diagnostic possibility (perhaps overshadowed by commoner aetiologies, or contributions from comorbidities), limited neurologist input in such older adults, and lower sensitivity of sCJD-related investigations in the elderly. Little has been published regarding the influence of older age on sCJD[168,443,444]. Of all cases of sCJD diagnosed from 2019 to 2021 in the UK, 189 of 388 (48.7%) of cases were aged ≥70 at death, and 12.1% ≥80 (Jan MacKenzie, personal communication) (**vignette 6.3**). A study evaluating the diagnosis of sCJD in older adults in the modern era of surveillance would be highly informative (see Further Work section in **Chapter 9**).

Some published studies have explored the features of younger adults with sCJD[382,445]. A 2005 study conducted by the German surveillance unit identified longer survival and prominent early psychiatric features in addition to overrepresentation of atypical subtypes including VV1[382]. As is explored in **Chapter 9** there is a need for a large and up-to-date study exploring in detail the features of sCJD cases with young-onset, including the performance of investigations including RT-QuIC. Indeed the NCJDRSU is currently undertaking such a study. However, it seems conceivable that the lower case rates in the young reflect underlying biological factors; the aetiology of sCJD is not known[1], but there may be a greater tendency with progressive ageing towards stochastic misfolding of PrP^C or somatic mutations in *PRNP* (leading hypotheses regarding sCJD aetiology).

Concerning investigation sensitivity, I did not identify statistically significant variations with age for RT-QuIC, 14-3-3 or EEG, whereas MRI sensitivity differed for overall and individual abnormality sensitivity (**table 6.11, figure 6.7**). Overall sensitivity declined with age as did that for basal ganglia hyperintensity and co-occurring basal ganglia hyperintensity and cortical ribboning. This trend is likely to reflect the preponderance of the VV2 subtype (94.6% of whom displayed this basal ganglia hyperintensity; see **Chapter 5**) in younger cases, 25.5% of whom were VV2 subtype. A less marked variation with age is seen for cortical ribboning overall, and older adults displayed the highest frequency of cases with isolated cortical ribboning (32.5%). This may partly underlie the relatively large increase in sensitivity for *probable* sCJD diagnosis with revised criteria in older adults.

Vignette 6.3. Elderly-onset

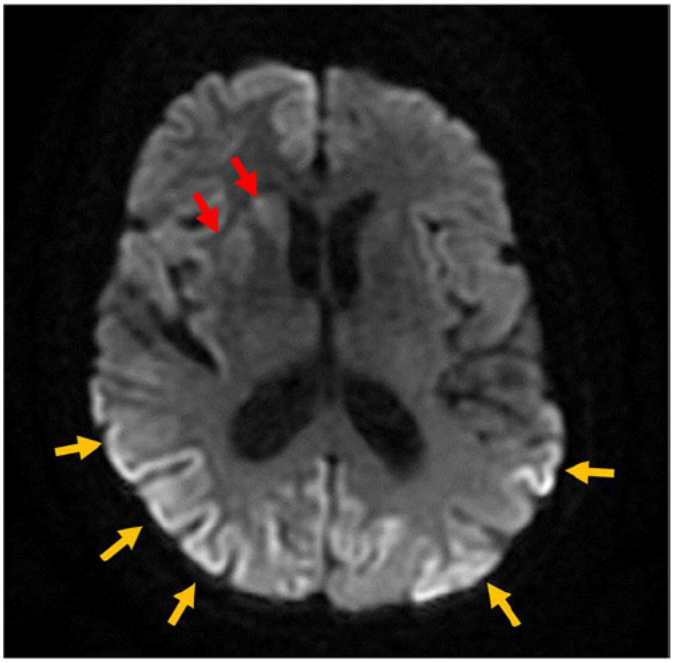
An 87 year-old woman developed anxiety and panic attacks. In subsequent she began getting lost on familiar routes. She misplaced items at home and struggled to navigate her house. She moved into supported accommodation. She was seen in a memory clinic and scored 86/100 on an Addenbrooke's test, losing points on visuospatial function.

She became increasingly withdrawn and less interactive. She developed clumsiness, her gait became slow and she had several falls. She developed daytime somnolence, declining self-care and was moved to a nursing home.

Her condition deteriorated rapidly and she was admitted to hospital. She developed marked rigidity, dysphagia and became mute and bedbound. During the NCJDRSU assessment she was akinetic and mute, displayed bilateral rigidity with brisk reflexes and upgoing plantars.

b=1000 DWI sequences on MRI brain demonstrated posterior-predominant cortical ribboning (yellow arrows) and hyperintensity of right caudate and putamen (red). The decision was made to forgo CSF sampling and EEG.

The patient died after 270 days in total. Post-mortem demonstrated PrP^{Sc} type 1 with co-occurrence of type 2A. Genetic testing revealed c129 status MM and no pathogenic mutations in *PRNP*.



The lack of age-dependence on RT-QuIC sensitivity has been reported in a 2012 study[360]. This is a major advantage of RT-QuIC over conventional biomarkers such as 14-3-3, S100b and NSE, which appear to be less sensitive in younger adults[96]. There are a number of benefits to this. Firstly, for surveillance programmes assessing younger individuals, an important consideration is vCJD, a disease for which 14-3-3 is less sensitive[330,446], and which does not appear to be associated with positive RT-QuIC (see earlier **Chapters**). Demonstration of a positive RT-QuIC assay in such individuals would

provide reliable ante-mortem evidence against vCJD. Secondly the recognition of 'chameleons': younger onset of sCJD may be associated with atypical features such as prolonged duration or prominent cerebellar or neuropsychiatric features at onset[382]. These features might suggest alternative aetiologies including non-prion diseases (e.g. spinocerebellar ataxia or frontotemporal dementia) and indeed vCJD. A positive RT-QuIC assay would resolve such diagnostic dilemmas.

The frequency of individual clinical features did not differ between sCJD cases grouped by age with the exception of cerebellar features, most frequent among younger adults. As with basal ganglia abnormalities on MRI this likely reflected a preponderance of the VV2 subtype[308]. However, the highest frequency of cases classified as *unclear* (i.e. with insufficient clinical features for *possible* or greater classification) was observed in older adults using prior criteria, indicating larger numbers of older adults with clinically-limited disease. This is a major advantage of the RT-QuIC assay as seen by the large proportions being reclassified to *probable* sCJD.

Summary

This analysis has demonstrated major increases in the ability of revised diagnostic criteria to appropriately classify sCJD cases with longer survival, driven by both MRI and RT-QuIC. The revised criteria were equivalent between age groups bar in the subset with full investigation panels performed for which the sensitivity was highest in the standard age group and was equivalent for young and older cases. The largest rise in sensitivity was seen among older cases.

The epidemiological consequences of these changes remain to be seen but it is likely that the revised criteria will improve ascertainment in these cases with atypical demographics. This is of major value for accurate disease monitoring as well as for enabling appropriate actions following a diagnosis, such as public health actions. I have demonstrated the biological factors associated with atypical age and duration, as well as the performance of individual investigations in these groups.

Chapter 7: Characteristics of cases with specific investigation outcomes

The RT-QuIC assay and the presence of cortical ribboning on MRI brain were the novel additions to the 2017 diagnostic criteria. In this **Chapter** I explore the characteristics of sCJD cases with specific outcomes: firstly, RT-QuIC positive and negative cases, and secondly cases with isolated cortical ribboning. These cases are of relevance to the criteria revision: those with positive RT-QuIC include cases which are able to be diagnosed with clinically-limited disease states, while RT-QuIC negative cases may represent specific subgroups for which this highly-specific assay lacks sensitivity. Cases with isolated cortical ribboning would not previously have been classified as having positive MRI sequences, and can now be appropriately identified through surveillance.

RT-QuIC

274 cases underwent CSF RT-QuIC testing (**table 7.1**). 251 (91.6%) tested positive and the remainder negative. Summary demographics of both groups are shown in **table 7.1**. While the majority (14 of 23, 60.9%) of RT-QuIC negative cases were male in contrast to a more even balance in RT-QuIC positive cases (male 131 of 251, 52.4%), this difference was not statistically significant (p=0.58). No differences were observed in mean age at death (p=0.5) or median duration (p=0.74) between cases grouped by RT-QuIC outcome.

Table 7.1. Demographics, cases grouped by RT-QuIC outcome

	RT-QuIC		
Feature	**Positive**	**Negative**	***P***
Cohort size	251	23	
Male (%)	131 (52.4)	14 (60.9)	0.58
Female (%)	119 (47.6)	9 (39.1)	
Mean age, years (SD)	68.8 (9.3)	66.3 (11.8)	0.50
Median duration, days (IQR)	120 (75.75-216.75)	88 (65.75-389.75)	0.74

Abbreviations. RT-QuIC, real-time quaking-induced conversion.

No differences were observed in the frequencies of individual *PRNP* c129 genotypes between groups (p=0.83), nor in the frequencies of PrP^{Sc} glycotypes (P=0.9) (**table 7.2**). When sCJD cases were grouped by the Parchi classification scheme, individual subtypes were seen to occur at differing frequencies; however, the sample size was too small to allow formal statistical testing. The MM2 group accounted for 22.2% (2 of 9) of RT-QuIC negative cases and 2.6% (4 of 153) of RT-QuIC positive cases. No MV1 or MV2 cases were identified in the RT-QuIC negative group.

Table 7.2. Subtype information, cases grouped by RT-QuIC outcome

	RT-QuIC positive		RT-QuIC negative		
	n	%	n	%	*P*
c129 genotype					
Available	168		12		
MM	110	65.5	7	58.3	0.83
MV	31	18.5	3	25.0	
VV	27	16.1	2	16.7	
PrP					
Available	175		12		
1	110	62.9	7	58.3	0.90
2A	47	26.9	4	33.3	
Dual	18	10.3	1	8.3	
sCJD subtype					
Available	153		9		
MM1	79	51.6	4	44.4	
MM2	4	2.6	2	22.2	
MM1+2	16	10.5	1	11.1	
MV1	14	9.2	0	0	
MV2	14	9.2	0	0	
MV1+2	2	1.3	0	0	
VV1	0	0	1	11.1	
VV2	22	14.4	1	11.1	
VV1+2	2	1.3	0	0	

Abbreviations. c129, prion protein gene codon 129. MM, methionine homozygous. MV, methionine-valine heterozygous. VV, valine homozygous. RT-QuIC, real-time quaking-induced conversion

Isolated cortical ribboning

Of 455 cases undergoing MRI, 128 (28.1%) displayed isolated cortical ribboning (**table 7.3**). 67 (52.3%) were male. Mean age was 71.2 years (SD 8.2), and median duration was 71 days (IQR 66.2-77). There was a strong predominance of the MM genotype (66 of 76, 86.8%; **table 7.4**). Assessing subtypes, MM1 cases accounted for 58.1% (36 of 62) of the group (**figure 7.1**). MM2 cases, which comprised 5.8% of the overall cohort, accounted for 12.9% (8 of 62) of cases with isolated cortical ribboning; this was the second-commonest 'pure' subtype (i.e. without mixed PrP^{Sc} glyotypes). Despite accounting for 14.7% of the overall sCJD cohort, VV2 cases only accounted for 1.6% (1 of 62) of cases with isolated cortical ribboning.

Table 7.3. Demographics, cases with isolated cortical ribboning

Feature	
Cohort size	128
Male (%)	67 (52.3)
Female (%)	61 (47.7)
Mean age, years (SD)	71.2 (8.2)
Median duration, days (IQR)	71 (66.2-77)

Abbreviations. IQR, interquartile range. sCJD, sporadic Creutzfeldt-Jakob disease. SD, standard deviation.

Table 7.4. Subtype information, cases with isolated cortical ribboning

	n	%
c129 genotype		
Available	76	
MM	66	86.8
MV	9	11.8
VV	1	1.3
PrP		
Available	85	
1	56	65.9
2A	13	15.3
Dual	16	18.8
sCJD subtype		
Available	62	
MM1	36	58.1
MM2	8	12.9
MM1+2	10	16.1
MV1	2	3.2
MV2	4	6.5
MV1+2	1	1.6
VV1	0	0
VV2	1	1.6
VV1+2	0	0

Abbreviations. c129, prion protein gene codon 129. MM, methionine homozygous. MV, methionine-valine heterozygous. VV, valine homozygous. RT-QuIC, real-time quaking-induced conversion

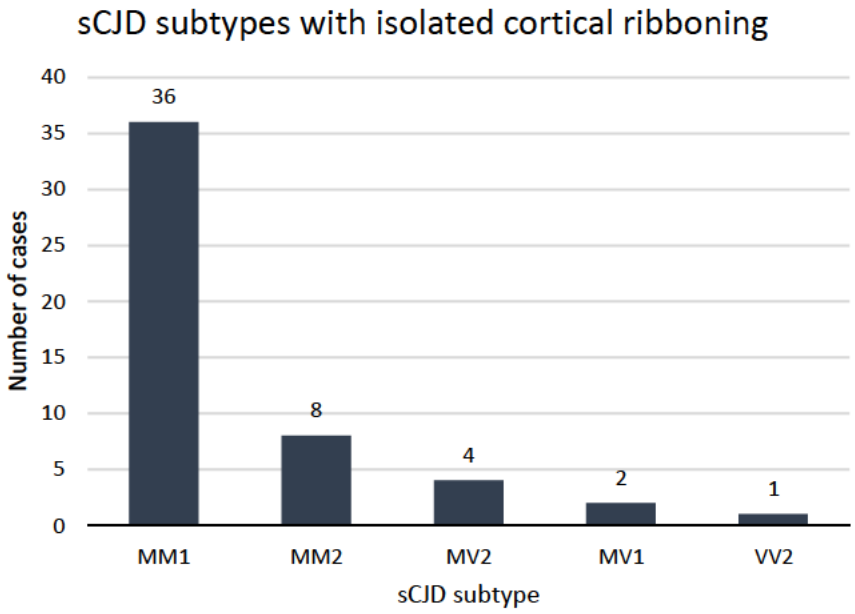


Figure 7.1. sCJD subtypes with isolated cortical ribboning

sCJD subtypes defined by combinations of *PRNP* c129 genotype and PrP^{Sc} glycotype are shown by frequency among cases with isolated cortical ribboning.

Discussion

In this analysis I explored characteristics of two important groups of relevance to the revised diagnostic criteria. Firstly, sCJD cases defined by RT-QuIC outcome, and secondly, cases with isolated cortical ribboning.

Cases with negative RT-QuIC are a minority in surveillance given its excellent sensitivity. In this series these represented 8.4% of cases. Thus, if performed, RT-QuIC is highly likely to enable a positive diagnosis in cases of sCJD and as discussed in **Chapter 4**, the assay has outstanding specificity, with almost no false-positive results reported in the literature[97]; hence a positive result can be relied on to secure the diagnosis. However, a number of challenges emerge when the assay returns negative, putting the diagnosis into question. In order to better understand the factors associated with a negative outcome (and not simply attribute it to chance), it was valuable to explore this subgroup of cases.

Biological sex had no influence on RT-QuIC outcomes, in contrast to a recent study by Rhoads *et al* in which indicated a higher odds ratio of positive RT-QuIC in female cases[242]. The same study indicated associations of negative RT-QuIC with younger age and longer disease duration, in contrast to my findings with neither age nor duration significantly associated with a particular outcome; this

was also the case in an earlier study[360]. In **Chapter 6** I found a significant association of positive RT-QuIC with typical disease duration, but the real-world significance of this was uncertain given the high sensitivity in all groups and similar sensitivity in short and long survivors.

As was the case in prior studies[242,360,361], *PRNP* c129 genotypes did not differ between RT-QuIC positive and negative cases. The distribution of sCJD subtypes did differ however, as evidenced by the preponderance of MM2 cases (22.2%) in the RT-QuIC negative group. I have discussed the association of MM2 cases with lower RT-QuIC sensitivity in **Chapter 5**. The only VV1 case was in the RT-QuIC negative group. Thus, the results indicate that RT-QuIC negative cases may reflect a subgroup with atypical subtypes. Examples are shown in **Chapter 5, vignettes 3 & 4.**

The question for surveillance systems and for clinicians assessing cases of potential sCJD is therefore whether a negative RT-QuIC outcome represents i) a 'false' negative (e.g. owing to an atypical subtype), ii) a case of non-prion disease, or iii) an alternative form of prion disease such as variant or genetic CJD.

RT-QuIC does not yield positive assay results in vCJD[97] – due to the inability of CSF from affected individuals to seed the traditional assay, which uses full-length hamster recombinant PrP as a substrate (Alison Green, personal communication). In addition, in one published study, RT-QuIC using second-generation truncated hamster recombinant PrP yielded a positive outcome in only 1 of 4 samples taken from individuals with vCJD[363]. This fact is valuable in the modern scenario of CJD surveillance, where future potential vCJD cases may have clinical and investigative features overlapping with sCJD, as was reported in the last primary reported case[55]**.** In this individual, RT-QuIC was negative. A negative RT-QuIC may therefore indicate a potential vCJD case, along with other features such as younger age or longer duration. The NCJDRSU approach has been to encourage consideration of vCJD in RT-QuIC negative cases, and in most of these cases to advocate for neuropathological examination to fully evaluate this consideration. The importance of this cannot be understated given the major public health implications of an ongoing vCJD epidemic as well as the additional transmissibility concerns in vCJD relating to peripheral PrP^{Sc} deposition[186]. If vCJD is a possibility the RT-QuIC test should be performed, and a negative outcome should be considered supportive evidence favouring the diagnosis. The PMCA assay may also be considered to support the diagnosis[214].

In addition to vCJD, gCJD is an important differential for sCJD and can present in a similar fashion[61]. Some commoner mutations are associated with positive RT-QuIC (sensitivity 100% in E200K and 95.2% in V210I[356]) whereas others such as D178N appear to be associated with negative RT-QuIC[356]. Features indicative of potential gCJD such as family history, prolonged duration and younger onset[61], are not 100% reliable. Sequencing of *PRNP* is encouraged by the NCJDRSU in all CJD cases, but I

would additionally advise consideration of gCJD in individuals with negative RT-QuIC (**see Vignette 7.1)**.

Besides these elements, the diagnosis of sCJD in an RT-QuIC negative case can be supported by other diagnostic features, such as a typical MRI.

Cases with isolated cortical ribboning (**Vignette 6**) represented 28.1% of those with MRI performed. There was an approximately equal sex ratio in this group, and these cases had a typical age at death. Disease duration was short: median survival was 71 days, and the interquartile range was narrow (66.2-77 days). The vast majority were MM cases, which is in keeping with the results reported in **Chapter 5**. These comprised MM1, MM2 and MM1+2 cases, all of which accounted for disproportionate numbers of cases with isolated cortical ribboning, while only 1 VV2 case was seen.

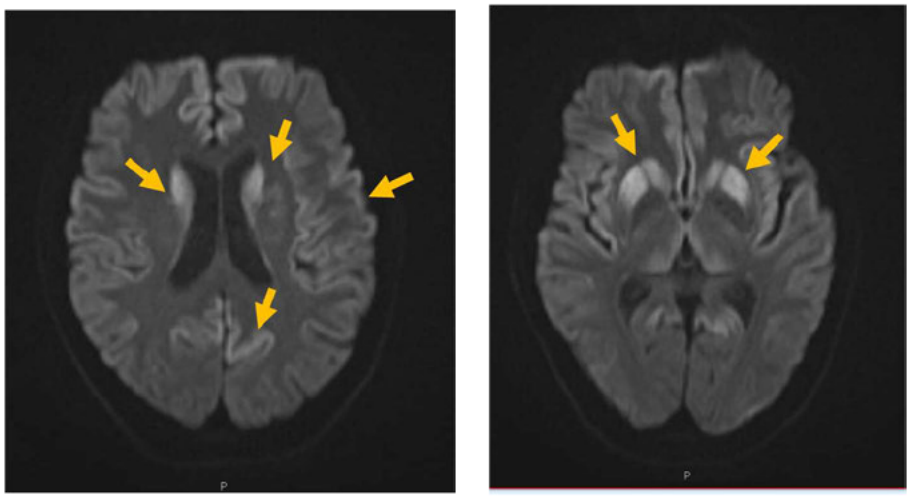
Vignette 7.1. D178N-129V genetic CJD

A 41-year-old woman presented with acute behavioural disturbance, confusion,. dysarthric speech and ataxia. Investigations demonstrated bilateral basal ganglia hyperintensities;CSF biomarkers including RT-QuIC were negative. She improved partially with intravenous vitamins and was discharged with a diagnosis of probable Wernicke's encephalopathy.

In subsequent weeks she had ongoing dysarthric speech and displayed unusual behaviours and impaired short-term episodic memory. Her condition deteriorated 3 months after her initial presentation and she was re-admitted. On assessment she had ataxia, disinhibition, hyperorality and visual hallucinations.

Repeat MRI showed bilateral basal ganglia hyperintensities with cortical ribboning. CSF biomarkers remained negative. *PRNP* sequencing demonstrated the D178N mutation; c129 status on the *cis*-allele was valine. No family history was reported; both parents remained alive. The patient died after a total of 347 days from onset.

This illustrates the importance of considering gCJD in patients with potential CJD in whom imaging may support the diagnosis, biomarkers may be negative, and a suggestive family history may be lacking.



This information is useful given that these cases would not previously have been considered to have had a positive MRI. MM1 cases are the most common subtype[6] and the sensitivity of investigations for these cases is high[304] (**table 5.8**), and thus such MM1 cases with isolated cortical changes would likely have been able to be diagnosed by other investigation. In contrast, the uncommon MM2 group is associated with lower sensitivity of other investigations including RT-QuIC, and few MM2 cases display basal ganglia hyperintensities; this group is likely to be better-classified using newer criteria incorporating cortical ribboning.

The imperfect specificity of cortical ribboning has been discussed in **Chapter 4**. Clinicians identifying individuals with this should be mindful of potential alternative diagnoses which can 'mimic' sCJD, as has been discussed. Important considerations include seizures, inflammation and infarction. Judicious clinical assessment is crucial, potentially supported by serial imaging in unclear circumstances.

Summary:

These results enhance the known literature on the characteristics of specific subgroups defined by outcomes on the newest investigations incorporated in the diagnostic criteria. These findings are of value to ongoing surveillance efforts, particularly when evaluating cases of suspected sCJD with isolated cortical ribboning and/or a negative RT-QuIC assay.

Chapter 8: Overview of data from individual nations

This study was performed using data from the national surveillance units of the UK, France, Germany and Italy. The combined approach ensured maximal statistical power. While the methods employed by each system were broadly similar (see **Chapter 3**), there were some important differences which led to variations in outcomes between nations which are worth exploring as a subanalysis.

The study results from individual nations are reported in this **Chapter**. Each national cohort was subjected to the same analysis as the overall cohort, interrogating demographics, non-case diagnoses, diagnostic criteria sensitivity and specificity and that of the individual investigations.

1. UK cohort

CJD surveillance in the UK is conducted by the National CJD Research & Surveillance Unit (NCJDRSU), based in Edinburgh. Referrals come from clinicians around the UK and are triaged by the NCJDRSU neurologists. Individuals with high suspicion of CJD receive dedicated clinical and epidemiological assessment visits. The NCJDRSU provides biomarker analysis for the entire UK, and in addition all MRI brain studies are reviewed blindly by an expert neuroradiologist. The unit also provides dedicated neuropathology services for the UK and *PRNP* genotyping for Scottish individuals.

1.1. Demographics

103 individuals were recruited from the NCJDRSU database. 84 (81.6%) were sCJD cases and the remaining 19 (18.4%) were non-cases with alternative diagnoses (**table 8.1)** There was near-equivalence of male and female cases (n=43, 51.2% and n=41, 48.8% respectively), while more non-cases were male (n=13, 68.4%) than female (n=6, 31.6%). The mean age of cases was 69.9 years (SD

8.1), slightly younger than non-cases (72.8, SD 11.2). Median duration was longer in cases at 138 days (IQR 84.25-276.75) compared to non-cases (60 days, IQR 34.5-169.5).

Table 8.1. National cohorts, demographic features

	France		Germany		Italy		UK	
	Cases	**Non-cases**	**Cases**	**Non-cases**	**Cases**	**Non-cases**	**Cases**	**Non-cases**
Cohort size	110	25	91	55	216	47	84	19
Male (%)	43 (39.1)	14 (56.0)	55 (60.4)	27 (49.1)	112 (51.9)	20 (42.6)	43 (51.2)	13 (68.4)
Female (%)	67 (60.9)	11 (44.0)	36 (39.6)	28 (50.9)	103 (47.7)	27 (57.4)	41 (48.8)	6 (31.6)
Mean age, years (SD)	69 (9.8)	71.1 (11.6)	67.6 (9.7)	72.6 (10.4)	69.5 (10.6)	73.7 (8.9)	69.9 (8.1)	72.8 (11.2)
Median duration, days (IQR)	113 (82-205)	95 (47-269.25)	88 (64-153)	74 (50.5 - 168.5)	130 (78.25-241.25)	101 (65-209.5)	138 (84.25-276.75)	60 (34.5 - 169.5)
Neuropathology, n (%)								
Biopsy	-	-	-	3 (5.5%)	-	-	-	4 (21.1)
Autopsy	110 (100.0)	25 (100.0)	91 (100.0)	52 (94.5)	216 (100.0)	47 (100.0)	84 (100.0)	15 (78.9)

Abbreviations. IQR, interquartile range. SD, standard deviation. UK, United Kingdom.

1.2. Diagnoses in non-cases

Among 19 non-cases, 18 (94.7%) had neuropathological diagnostic information available (**table 8.2, figure 8.1**). The commonest aetiology was neurodegenerative disorders (n=7, 38.9%). Of these, 3 (16.7%) non-cases had AD, 1 with co-existing cerebral amyloid angiopathy (CAA); 2 (11.1%) had DLB, and 2 (11.1%) had co-occurring AD and DLB. The second commonest aetiology was vascular disease (n=5, 27.8%); 4 (22.2%) had cerebrovascular disease (including stroke and diffuse small vessel disease), and 1 (5.6%) had cerebral vasculitis. Rare aetiologies included metastatic renal cell carcinoma (n=1, 5.6%).

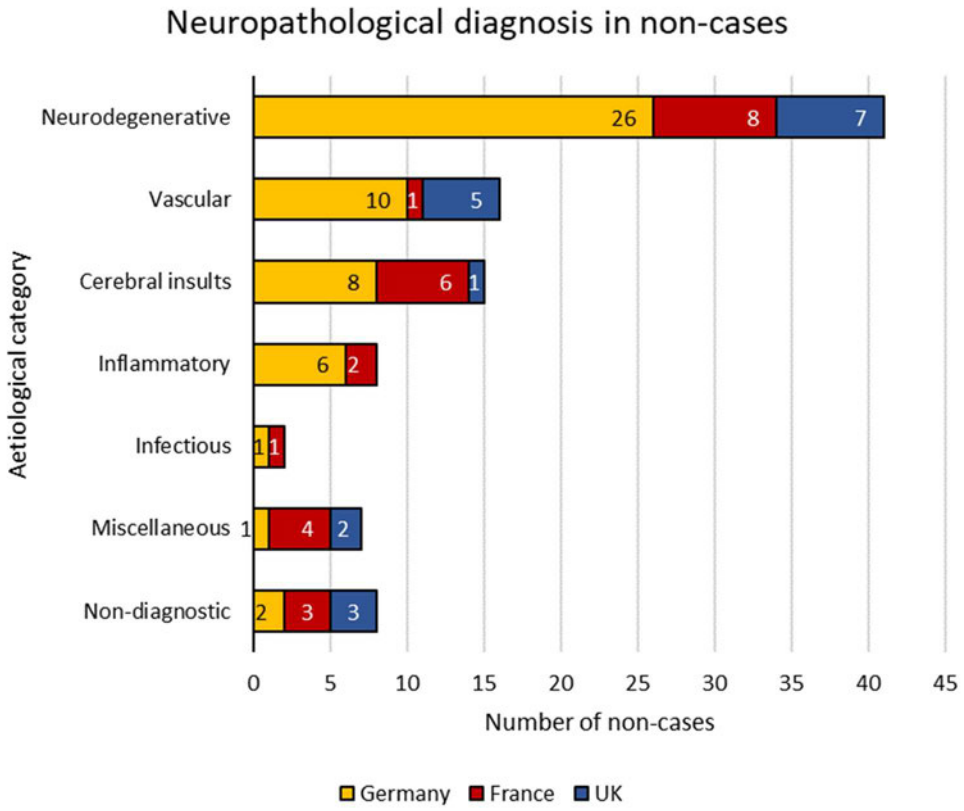


Figure 8.1. Neuropathological diagnoses in non-cases

Neurodegenerative disorders were the commonest non-case diagnoses, A small number of non-cases had non-diagnostic autopsy or biopsy examinations. The Italian centre was unable to contribute data on tissue diagnoses.

Table 8.2. Neuropathological examination results among non-cases (where reports available)

	Neurodegenerative			Vascular			Cerebral insult			Inflammation		
	Dx	n	%	Dx	n	%	Dx	n	%	Dx	n	%
France												
	Total	8	32.0	Total	1	4.0	Total	6	24.0	Total	2	8.0
	AD	5	20.0	APS	1	4.0	Anoxia	2	8.0	AIE	1	4.0
	Dual	2	8.0				Anoxia + seizure	1	4.0	Behcet's	1	4.0
	AD + DLB	1	4.0				HE + seizure	1	4.0			
	AD + MSA	1	4.0				Hypoglycaemia	1	4.0			
	CBD	1	4.0				Metabolic, NOS	1	4.0			
Germany												
	Total	26	48.1	Total	10	18.5	Total	8	14.8	Total	6	11.1
	AD	13	24.1	Cerebro-vascular	8	14.8	Anoxia	5	9.3	CD8+ encephalitis	3	5.6
	Dual	8	14.8	CAA	1	1.9	Seizure	1	1.9	AIE	1	1.9
	AD + DLB	7	13.0	Vasculitis	1	1.9	Metabolic, NOS	1	1.9	Influenza-associated ANE	1	1.9
	AD + Tau	1	1.9				Subcortical necrosis	1	1.9	Inflammation, NOS	1	1.9
	Tauopathy	2	3.7									
	TDP43	2	3.7									
	DLB	1	1.9									
UK												
	Total	7	38.9	Total	5	27.8	Total	1	5.6	Total	0	0.0
	AD	3	16.7	Cerebro-vascular	4	22.2	Anoxia	1	5.6			
	DLB	2	11.1	Vasculitis	1	5.6						
	Dual	2	11.1									
	AD+DLB	2	11.1									

Abbreviations. AD, Alzheimer's disease. AIE, autoimmune encephalitis. ANE, acute necrotizing encephalitis. APS, antiphospholipid syndrome. CBD, corticobasal degeneration. Dx, diagnosis. HE, hepatic encephalopathy. NOS, not otherwise specified. RCC, renal cell carcinoma.

Table 8.2 (continued). Neuropathological examination results among non-cases (where reports available)

	Infection			Miscellaneous			Non-diagnostic	
	Dx	n	%	Dx	n	%	n	%
France								
	Total	1	4.0	Total	4	16.0	3	12.0
	Toxoplasmosis	1	4.0	Other, NOS	2	8.0		
				Encephalopathy, NOS	1	4.0		
				Fahr disease	1	4.0		
Germany								
	Total	1	1.9	Total	1	1.9	2	3.7
	Abscess	1	1.9	Leuko-encephalopathy	1	1.9		
UK								
	Total	0	0.0	Total	2	11.1	3	16.7
				Gliosis	1	5.6		
				Metastatic RCC	1	5.6		

Abbreviations. AD, Alzheimer's disease. AIE, autoimmune encephalitis. ANE, acute necrotizing encephalitis. APS, antiphospholipid syndrome. CBD, corticobasal degeneration. Dx, diagnosis. HE, hepatic encephalopathy. NOS, not otherwise specified. RCC, renal cell carcinoma.

1.3. Clinical features

Data on clinical features were available in 79 (94.1%) cases and 19 (100.0%) non-cases (**table 8.3**). All features were more frequently encountered in cases than non-cases. The most common feature was rapidly progressive cognitive decline, present in 79 (100.0%) cases and 18 (94.7%) non-cases. The least commonly-encountered feature was akinetic mutism, reported in 30 (38.0%) cases and 5 (26.3%) non-cases. Compared to non-cases, cases showed a preponderance of myoclonus (84.8% vs 36.8%), visual (57.0% vs 0%), cerebellar (69.6% vs 31.6%), pyramidal (65.8% vs 31.6%) and extrapyramidal (70.9% vs 42.1%) features.

1.4. Case classification

The 79 (94.1%) cases with available clinical information were classified as per diagnostic criteria (**tables 8.4 & 8.5**). The number classified as *probable* sCJD was 61 (77.2%) on prior criteria, rising 13.9% to 72 (91.1%) with revised criteria. The number classified *possible* sCJD declined from 14 (17.7%) with prior criteria to 4 (5.1%, a 12.7% decrease) via revised criteria, while the number classified as *unclear* decreased from 4 (5.1%) to 3 (3.8%, 1.3% decrease).

38 (45.2%) cases had undergone the full panel of investigations and were used to assess the performance of the diagnostic criteria in aggregate via the 'all' analysis. By prior criteria, 33 (86.8%) were classified as *probable*, rising to 37 (97.4%, 10.5% increase) via revised criteria. Comparing prior and revised criteria, the number of cases classified as *possible* sCJD declined from 3 (7.9%) to 0 (0.0%, 7.9% decrease) respectively, while the number classified as *unclear* declined from 2 (5.3%) to 1 (2.6%, a 2.6% decrease).

19 (100%) of non-cases were able to be classified by criteria. No changes were observed in the frequencies classified as *probable* (10.5%), *possible* (42.1%) or *unclear* (47.4%) when comparing the prior and revised criteria, yielding specificity 89.5% (**table 8.4 & 8.5)**. In the 'all' analysis, only 6 non-cases had adequate clinical data and underwent all investigations, and the classifications were unchanged with both criteria: 1 (16.7%) was classified *probable*, 4 (66.7%) *possible* and 1 (16.7%) *unclear*, and thus the specificity with both criteria was 83.3%.

As demonstrated in the previous section, 75 (89.3%) cases had adequate clinical features to be classified as *probable* or *possible* by prior criteria (which do not include RT-QuIC); this figure represents the sensitivity of clinical features for at least a *possible* sCJD diagnosis. Among non-cases, 10 (52.6%) had sufficient clinical features for *possible* or *probable* sCJD diagnosis, while the remaining 9 (47.4%) did not, and thus the specificity of clinical features was 47.4% in the UK cohort.

Table 8.3. National cohorts, clinical features

	France		Germany		Italy		UK	
Feature	**Cases**	**Non-cases**	**Cases**	**Non-cases**	**Cases**	**Non-cases**	**Cases**	**Non-cases**
RPCD	107 (99.1)	4 (44.4)	80 (94.1)	51 (94.4)	213 (99.1)	39 (86.7)	79 (100)	18 (94.7)
Myoclonus	82 (75.9)	5 (55.6)	44 (51.8)	23 (42.6)	141 (65.6)	17 (37.8)	67 (84.8)	7 (36.8)
Visual	59 (54.6)	1 (11.1)	31 (36.5)	5 (9.3)	100 (46.5)	5 (11.1)	45 (57.0)	0 (0)
Cerebellar	82 (75.9)	1 (11.1)	70 (82.4)	13 (24.1)	153 (71.2)	14 (31.1)	55 (69.6)	6 (31.6)
Pyramidal	55 (50.9)	2 (22.2)	18 (21.2)	6 (11.1)	90 (41.9)	19 (42.2)	52 (65.8)	6 (31.6)
Extrapyramidal	61 (56.5)	3 (33.3)	22 (25.9)	13 (24.1)	108 (50.2)	19 (42.2)	56 (70.9)	8 (42.1)
Akinetic Mutism	78 (72.2)	1 (11.1)	12 (14.1)	8 (14.8)	97 (45.1)	16 (35.6)	30 (38.0)	5 (26.3)

Brackets show percentages of individuals displaying each clinical feature per nation.

Abbreviations. RPCD, rapidly-progressive cognitive decline. UK, United Kingdom.

Table 8.4. National cohorts, case classification ('any' analysis)

	France			Germany			Italy			UK		
Classification	**Prior**	**Revised**	**Change (%)**	**Prior**	**Revised**	**Change (%)**	**Prior**	**Revised**	**Change (%)**	**Prior**	**Revised**	**Change (%)**
Cases												
Probable	89 (82.4)	97 (89.8)	7.4	56 (65.9)	75 (88.2)	22.4	172 (80.0)	206 (95.8)	15.8	61 (77.2)	72 (91.1)	13.9
Possible	13 (12.0)	5 (4.6)	-7.4	3 (3.5)	0 (0)	-3.5	12 (5.6)	1 (0.5)	-5.1	14 (17.7)	4 (5.1)	-12.7
Unclear	6 (5.6)	6 (5.6)	0	26 (30.6)	10 (11.8)	-18.8	31 (14.4)	8 (3.7)	-10.7	4 (5.1)	3 (3.8)	-1.3
Non-cases												
Probable	1 (11.1)	1 (11.1)	0	13 (24.1)	13 (24.1)	0	7 (16.3)	8 (18.6)	2.3	2 (10.5)	2 (10.5)	0
Possible	0	0	0	5 (9.3)	5 (9.3)	0	13 (30.2)	12 (27.9)	-2.3	8 (42.1)	8 (42.1)	0
Unclear	8 (88.9)	8 (88.9)	0	36 (66.7)	36 (66.7)	0	23 (53.5)	23 (53.5)	0	9 (47.4)	9 (47.4)	0

Table 8.5. National cohorts, diagnostic criteria performance

	France		Germany		Italy		UK	
	Sensitivity	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
Criteria								
Clinical features (*possible* sCJD)	102/108 (94.4)	8/9 (88.9)	59/85 (69.4)	36/54 (66.7)	184/215 (85.6)	23/43 (53.5)	75/79 (94.9)	9/19 (47.4)
Probable sCJD								
Any investigations								
Revised	97/108 (89.8)	8/9 (88.9)	75/85 (88.2)	41/54 (75.9)	206/215 (95.8)	35/43 (81.4)	72/79 (91.1)	17/19 (89.5)
Prior	89/108 (82.4)	8/9 (88.9)	56/85 (65.9)	41/54 (75.9)	172/215 (80.0)	36/43 (83.7)	61/79 (77.2)	17 /19 (89.5)
All investigations								
Revised	-	-	51/53 (96.2)	16/28 (57.1)	130/132 (98.5)	14/18 (77.8)	37/38 (97.4)	5/6 (83.3)
Prior	-	-	35/53 (66.0)	16/28 (57.1)	102/132 (77.3)	15/18 (83.3)	33/38 (86.8)	5/6 (83.3)

Sensitivity defined as positive outcome/total for cases. Specificity defined as negative outcome/total for non-cases. Brackets show percentages

Abbreviations. BG, basal ganglia. CR, cortical ribboning. sCJD, sporadic Creutzfeldt-Jakob disease.

1.5. Diagnostic investigations

The performance of individual investigations is shown in **table 8.6**. The most sensitive investigation in the UK cohort was MRI (67 of 70, 95.7%), which was 93.8% (15 of 16) specific. The sensitivity and specificity values of separate MRI abnormalities arising alone or in combination is shown in **table 8.6**. The second most sensitive investigation was RT-QuIC (52 of 58, 89.7%) which was 100.0% specific (13 of 13). EEG was by far the least sensitive investigation (12 of 55, 21.8%), but had 100.0% (12 of 12) specificity in the UK cohort.

1.6. Genotype and neuropathology

PRNP c129 genotype was available for 76 (90.5%) cases (**table 8.7**). MM was the commonest genotype (n=47, 61.8%) followed by VV (n=17, 22.4%) and lastly MV (n=12, 15.8%). PrP glycotype information was available in 65 (77.4%) cases. Type 1 PrP was present in 39 (60.0%) cases. Type 2A PrP was present in 19 (29.2%), while co-occurrence of types was present in 7 (10.8%). The frequencies of individual sCJD subtypes defined by genotype-glycotype combinations are shown in **table 8.7.**

Table 8.6. National cohorts, investigation sensitivity and specificity

	France		Germany		Italy		UK	
Investigation	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**	**Sensitivity**	**Specificity**
EEG	55/103 (53.4)	14/20 (70.0)	37/77 (48.1)	41/43 (95.3)	102/213 (48.4)	37/43 (86.0)	12/55 (21.8)	12/12 (100)
MRI (all)	94/104 (90.4)	10/17 (58.8)	70/81 (86.4)	39/44 (88.6)	164/201 (81.6)	27/34 (79.4)	67/70 (95.7)	15/16 (93.8)
CR & BG	48/104 (46.2)	16/17 (94.1)	32/81 (39.5)	43/44 (97.7)	55/201 (27.4)	32/34 (94.1)	46/70 (65.7)	16/16 (100.0)
CR alone	28/104 (26.9)	11/17 (64.7)	29/81 (35.8)	42/44 (95.5)	56/201 (27.9)	31/34 (91.2)	15/70 (21.4)	16/16 (100.0)
BG alone	16/104 (15.4)	17/17 (100.0)	9/81 (11.1)	42/44 (95.5)	53/201 (26.4)	32/34 (94.1)	6/70 (8.6)	15/16 (93.8)
CR (any)	76/104 (73.1)	10/17 (58.8)	61/81 (75.3)	41/44 (93.2)	111/201 (55.2)	29/34 (85.3)	61/70 (87.1)	16/16 (100.0)
BG (any)	66/104 (63.5)	16/17 (94.1)	40/81 (49.4)	41/44 (93.2)	108/201 (53.7)	30/34 (88.2)	52/70 (74.3)	15/16 (93.8)
14-3-3	57/104 (54.8)	15/24 (62.5)	69/87 (79.3)	18/48 (37.5)	163/204 (79.7)	23/38 (60.5)	37/58 (63.8)	11/13 (84.6)
RT-QuIC	-	-	61/71 (85.9)	37/37 (100.0)	138/145 (95.2)	27/27 (100.0)	52/58 (89.7)	13/13 (100.0)

Sensitivity defined as positive outcome/total for cases. Specificity defined as negative outcome/total for non-cases. Brackets show percentages

Abbreviations. BG, basal ganglia. CR, cortical ribboning. EEG, electroencephalography. MRI, magnetic resonance imaging. RT-QuIC, real-time quaking-induced conversion. UK, United Kingdom.

Table 8.7. National cohorts, cases grouped by *PRNP* c129 genotype and PrP glycotype

	France		Germany		Italy		UK	
	n	%	n	%	n	%	n	%
***PRNP* c129 genotype**								
Available	63	57.3	12	13.1	150	69.4	76	90.5
MM	42	66.7	10	83.3	97	64.7	47	61.8
MV	13	20.6	2	16.7	30	20.0	17	22.4
VV	8	12.7	0	0.0	23	15.3	12	15.8
PrP								
Available	101	91.8	3	3.3	175	81.0	65	77.4
1	60	59.4	2	66.7	106	60.0	39	60.0
2A	26	25.7	1	33.3	50	28.6	19	29.2
Dual	15	14.9	0	0.0	19	10.9	7	10.8
sCJD subtype								
Available	58	52.7	2	2.2	137	63.4	65	77.4
MM1	28	48.3	1	50.0	66	48.2	35	53.8
MM2	4	12.1	1	50.0	7	5.1	3	4.6
MM1+2	7	5.2			18	13.1	3	4.6
MV1	3	5.2			12	8.8	4	6.2
MV2	4	6.9			13	9.5	5	7.7
MV1+2	4	6.9			2	1.5	1	1.5
VV1	0	0.0			0	0	1	1.5
VV2	8	13.8			18	13.1	12	18.5
VV1+2	0	0.0			1	0.7	1	1.5

Abbreviations. c129, prion protein codon 129. MM, methionine homozygous. MV, methionine-valine heterozygous. PrP, prion protein glycotype. VV, valine homozygous. UK, United Kingdom.

2. French Cohort

CJD surveillance in France is conducted by the Cellule Nationale de référence des maladies de Creutzfeldt-Jakob (CNRMCJ), centred in Paris. Cases of suspected CJD are referred to the unit. Dedicated clinical assessments are performed in specific circumstances, for example cases with suspicion of vCJD, while other cases are evaluated using medical records and reports from referring clinicians. Biomarker analysis is performed in several laboratories around the country, but predominantly in Paris, and during the study period the RT-QuIC assay was only being performed in Paris. Neuroimaging reporting is delivered by local radiologists although the CNRMCJ neurologist reviews a number of sequences. Genetic testing is performed in an affiliated laboratory, while neuropathology is performed by numerous centres in France with additional analyses performed in centres in Paris and Lyon.

2.1. Demographics

The French surveillance centre provided data on 135 individuals, of which 110 (81.5% were sCJD cases and the remaining 25 (18.5%) were non-cases with alternative neuropathological conditions. Demographic features are summarised in **table 8.1**. There was a preponderance of female (n=65, 60.9%) individuals among cases, in contrast to non-cases (n=11, 44.0%). The mean age of cases was 69 (SD 9.8) years, while in non-cases it was 71.1 (11.6) years. Disease duration was shorter in non-cases at 95 days (IQR 47-269.5) versus 113 days (82-205) in non-cases. In all individuals, neuropathological examination was performed following autopsy; no biopsies were performed in the French cohort.

2.2.Diagnoses in non-cases

In non-cases, neuropathological data were available in all 25 (100.0%) individuals (**table 8.2, figure 8.1**). The commonest aetiology was neurodegenerative disorder (ND) (n=8, 32.0%), of which 5 (20.0%) were AD, 2 were AD with co-occurrence of another ND (DLB in 1, 4.0%; MSA in 1, 4.0%).

Cerebral insults were diagnosed in 6 (24.0%) non-cases; diagnoses were anoxic brain injury (n=2, 8.0%), anoxia and seizures (n=1, 4.0%), hypoglycaemia (n=1, 4.0%), hepatic encephalopathy with seizures (n=1, 4.0%) and a metabolic disorder not otherwise specified (n=1, 4.0%). Rarer aetiologies identified in the French non-case cohort included Fahr disease (n=1, 4.0%), Behcet's disease (n=1, 4.0%) and toxoplasmosis (n=1, 4.0%).

2.3. Clinical features

The French centre provided information regarding the presence or absence of the cardinal clinical features for diagnosis of sCJD in 108 (98.2%) cases, but were only able to provide this for 9 (36.0%) non-cases. The frequency of individual features is outlined in **table 8.3**. While all features were more frequently noted among cases than non-cases, the high proportion of missing data (64.0%) among the latter group limited the validity of a comparative analysis.

2.4. Case classification

Case classification via diagnostic criteria was possible in 108 (98.2%) of cases in the French cohort (**table 8.4 & 8.5**). Comparing prior and revised criteria, 89 (82.4%) versus 97 (89.8%, 7.4% increase) fulfilled *probable* sCJD classification, while 13 (12.0%) versus 5 (4.6%, 7.4% decrease) fulfilled *possible* sCJD classification. There was no change in the number (6, 5.6%) meeting *unclear* classification owing to the absence of RT-QUIC assay performance in the French cohort. Thus, the 7.4% rise in *probable* classification was entirely due to the presence of cortical ribboning on MRI brain.

Case classification was only possible in 9 (36.0%) of non-cases owing to limited available clinical data as outlined above. In these 9 individuals, only 1 (11.1%) fulfilled *probable* sCJD diagnosis; the remaining 8 (88.9%) were classified *unclear*. Hence the specificity for probable diagnosis was 88.9%.

It was not possible to perform an enhanced analysis using cases and non-cases who had undergone the full panel of investigations due to the absence of RT-QuIC assays in this cohort.

By criteria definitions, all individuals meeting *probable* or *possible* diagnosis on prior criteria have sufficient clinical features present. Thus, as outlined above, the sensitivity of clinical features in the French cohort was 94.4% (102 of 108), while the specificity was 88.9% (8 of 9 appropriately not fulfilling *probable* or *possible* diagnosis).

2.5. Diagnostic investigations

The performance of individual diagnostic investigations is outlined in **table 8.6**. MRI (positive outcome defined as any possible combination of sCJD-related abnormalities) was the most sensitive investigation (94 of 104 cases, 90.4%), while its specificity was 58.8% (negative in 10 of 17 non-cases). The most specific investigation outcome was MRI revealing isolated basal ganglia hyperintensities (not seen in any of 17 non-cases, 100.0%). Only 1 MRI demonstrated basal ganglia hyperintensities which were co-present with cortical ribboning, hence the specificity of this combination was 94.1% (absent in 16 of 17). The least sensitive investigation was EEG (55 of 103, 53.4%); this investigation was 70.0% (14 of 20) specific.

2.6. Genotype and neuropathology

Data on *PNRP* c129 genotype were available in 63 (57.3%) cases. The commonest genotype was MM (n=42, 66.7%), followed by MV (n=13, 20.6%) and VV (n=8, 12.7%). PrP glycotype data were available in 101 (91.8%) of cases. Type 1 was most frequent (n=60, 59.4%) followed by type 2 (n=26, 25.7%) and lastly co-occurrence of both types (n=15, 14.9%). The relative frequencies of c129 and PrP combinations (sCJD) subtypes are shown in **table 8.7**.

3. German Cohort

German CJD surveillance is delivered by the Nationales Referenzzentrum fur TSE (NRZ-TSE), centred in Göttingen. Entry points for referral include direct enquiries from clinicians around the nation as well as a high number of referrals following regional 14-3-3 testing. Clinical visits are only performed in special circumstances (e.g. potential vCJD), while individuals are assessed using dedicated questionnaires and physician reports. RT-QuIC testing is underaken by the unit. MRI reporting is delivered by regional radiologists, but most images are reviewed by the NRZ-TSE neurologists (who have extensive experience; the NRZ-TSE has been world-leading in prion disease imaging research). Finally, neuropathology services are delivered by two separate sites allied to the NRZ-TSE, with most performed by one site which does not usually perform PrP glycotyping.

3.1. Demographics

3.2. Diagnoses in non-cases

3.3. Clinical features

3.4. Case classification

3.5. Diagnostic investigations

3.6. Genotype & neuropathology

3.1. Demographics

The German surveillance centre provided data on 146 individuals. 91 (62.3%) were cases and the remaining 55 (37.7%) non-cases with alternative diagnoses. Demographic features are shown in **table 8.1.** There was a preponderance of male cases (n=55, 60.4%) compared to non-cases (n=27, 49.1%). The mean age of cases (67.6 years, SD 9.7) was younger than non-cases (72.6, SD 10.4). Median disease duration was longer in non-cases at 74 days (IQR 50.5-168.5) versus 88 days (IQR 64-153) in cases. Biopsy was performed in 3 (5.5%) non-cases, with the remaining 52 (94.5%) undergoing autopsy. No cases underwent biopsy.

Neuropathological diagnostic information was available in 54 (98.2%) non-cases (**table 8.2, figure 8.1**). Neurodegenerative disorders (NDs) were the most frequently encountered aetiology (n=26, 48.1%), of which the commonest diagnosis was AD (n=13, 24.1%); 4 (7.4%) of these individuals had co-existing CAA. Co-existing NDs were identified in 8 (14.8%) of non-cases, including AD and DLB (n=7, 13.0%).

3.2. Clinical features

Information on clinical features was available in 85 (93.4%) cases and 54 (98.2%) non-cases (**table 8.3**). While all features were more frequently encountered in cases than non-cases, visual disturbance (n=31, 36.5% vs n=5, 9.3%) and cerebellar features (n=70, 82.4% vs n=13, 24.1%) were particularly over-represented among cases. Akinetic mutism, a phenomenon seen in advanced disease states in CJD and other neurological disorders, was similarly infrequent in cases (n=12, 14.1%) and non-cases (n=8, 14.8%).

3.3. Case classification

85 (93.4%) of cases were able to be classified by diagnostic criteria. 56 (65.9%) met criteria for *probable* sCJD diagnosis using prior criteria, rising by 22.4% to 75 (88.2%) on revised criteria (**table 8.4 & 8.5**). 3 (3.5%) cases were initially classified as *possible* sCJD, decreasing to 0 with the revised criteria. Finally, 26 (30.6%) were classified as unclear, declining to 10 (11.8%, 18.8% decrease) with revision.

53 (58.2%) cases had available clinical information and underwent the full panel of investigations. In these cases, 35 (66.0%) met *probable* sCJD diagnosis on prior criteria, rising to 51 (96.2%) on revised criteria, a 30.2% increase. 2 (3.8%) were classified *possible* sCJD on prior criteria; both of these cases

were re-classified as *probable* sCJD by revised criteria. Finally, 16 (30.2%) cases were classified as *unclear,* declining 26.4% to 2 (3.8%) with the criteria revision.

54 (98.2%) non-cases were able to be classified by criteria. The revised diagnostic criteria did not alter the classification of cases as *probable* (n=13, 24.1%), *possible* (n=5, 9.3%) and *unclear* (n=36, 66.7%) sCJD. The reason for this was that no positive RT-QuICs were seen in this group, and all non-cases with cortical ribboning on MRI already fulfilled *probable* classification on prior criteria due to other investigations. The specificity of prior and revised criteria was therefore 75.9%.

28 non-cases had adequate clinical data and underwent the full complement of investigations; classification by both criteria was identical, with 12 (42.9%) fulfilling *probable* classification, 2 (7.1%) *possible* and 14 (50.0%) *unclear,* and hence the specificity for *probable* classification was 57.1%.

Following the information above, the sensitivity of clinical features was 69.4% (59 of 85), as demonstrated by all individuals meeting *possible* or *probable* diagnosis by prior criteria. The specificity of clinical features was 66.7% (36 of 54), i.e. the proportion of non-cases classified as *unclear*.

3.5. Diagnostic investigations

The most sensitive investigation in the German cohort was MRI (86.4%, 70 of 81), with specificity 88.6% (negative in 39 of 44 non-cases) (**table 8.6**). RT-QuIC was 85.9% sensitive (61 of 71) and had the highest specificity among investigations, 100.0% (negative in 37 of 37 non-cases). The least sensitive individual investigation was EEG (48.1%, 37 of 77), although EEG had high specificity at 95.3% (negative in 41 of 43). 14-3-3 had sensitivity 79.3% (69 of 87), and was the least specific investigation (37.5%, 18 of 48), reflecting its widespread usage in Germany and frequent role as a basis for referral to the national surveillance centre following positive results.

3.6. Genotype & neuropathology

Owing to preferences among German surveillance neuropathologists, *PRNP* c129 genotyping and PrP glycotyping were only infrequently performed in the German cohort (**table 8.7)**. c129 genotypes were available in 12 (13.1%), of which 10 (83.3%) were MM and 2 (16.7%) were MV. PrP glycotype data were available in 3 (3.3%) cases; 2 (66.7%) were type 1 and the remaining 1 (33.3%) type 2A. sCJD strain subtypes were only able to be defined in 2 (2.2%) cases; 1 (50.0%) was MM1 and the other (50.0%) MM2.

4. Italian Cohort

CJD surveillance in Italy is coordinated by the Registry of Creuztfeldt-Jakob disease, based in the Istituto Superiore di Sanita (ISS) in Rome. Cases of suspected CJD are referred by clinicians across Italy and are assessed remotely using a questionnaire and submitted physician reports, although a small number of cases undergo direct visits. Biomarkers are processed in numerous sites around Italy, including RT-QuIC. Imaging reports are largely generated by local radiologists; only a minority of sequences are reviewed by the unit team. Neuropathological analysis is performed in numerous sites across Italy.

4.1. **Demographics**

263 individuals were contributed by the Italian surveillance centres. 216 (82.1%) had sCJD, while the remaining 47 (17.9%) were non-cases with alternative neuropathological diagnoses (**table 8.1)**. 51% (n=112) of cases were male, compared to 42.5% (n=20) of non-cases. The mean age in cases was 69.5 years (SD 10.6), while non-cases were older (73.7 years, SD 8.9). Median disease duration was shorter in non-cases at 101 days (IQR 65-209.5) compared to cases (130 days, IQR 78.25-241.25).

4.2. **Diagnoses in non-cases**

While neuropathological examination in non-cases had definitively excluded prion disease, the Italian centre contributing the combined dataset from the participating centres was unable to provide information on final neuropathological diagnoses in any non-cases (**table 8.2**).

4.3. **Clinical features**

215 (99.5%) cases and 45 (95.7%) non-cases had clinical information available, as summarised in **table 8.3**. Notably, cases were more likely than non-cases to have had rapidly-progressive cognitive impairment [99.1% (n=213) vs 86.7% (n=39)], myoclonus [65.5% (n=141) vs 37.8% (n=17)], visual disturbance [46.5% (n=100) vs 11.1% (n=5)] and cerebellar features [71.2% (n=153) vs 31.1% (n=14)].

4.4. **Case classification**

Case classification via diagnostic criteria was possible in 215 (99.5%) of cases in the Italian cohort (**table 8.4 & 8.5**). By prior criteria, 172 (80.0%) fulfilled *probable* classification, rising by to 206 (95.5%) using revised criteria, a 15.8% increase. 12 (5.6%) and 1 (0.5%) of cases fulfilled *possible* diagnoses by prior and revised criteria respectively. 31 (14.4%) of cases were classified *unclear* by prior criteria, which decreased by 10.7% to 8 (3.7%) via revised criteria.

Classifications were assessed in cases who had undergone the full panel of investigations (n=132, 61.1%). The numbers of cases fulfilling *probable* sCJD diagnosis by prior and revised criteria were 102 (77.3%) and 130 (98.5%) respectively, a 21.2% increase.

In non-cases, classification was possible in 43 (91.5%). Comparing prior and revised criteria, 7 (16.3%) and 8 (18.6%, a 2.3% increase) of non-cases were classified as *probable* sCJD, while 13 (30.2%) and 12 (27.9%, 2.3% decrease) met criteria for *possible* sCJD. The number of non-cases classified as *unclear* was 23 (53.5%) by both sets of criteria, as no non-cases had positive RT-QuIC assays and this revision is not possible with any other positive diagnostic investigations. Hence the specificity was 83.7% (36 of 43) on prior criteria and 81.4% (35 of 43) on revised criteria.

The ‘all’ analysis was performed in 18 non-cases. 3 (16.7%) met prior criteria for *probable* diagnosis and 4 (22.2%) on revised criteria. 5 (27.8%) met prior criteria for *possible* diagnosis and 4 (22.2%) on revised criteria. In both sets of criteria, 10 (55.6%) met criteria for *unclear* classification. Hence the specificity on the *all* analysis was 83.3% (n=15) with prior criteria and 77.8% (n=14) with revised criteria.

As demonstrated above, 172 and 12 cases fulfilled *probable* and *possible* sCJD diagnosis using prior criteria, a total of 184 (85.6%). By definition these individuals will have had adequate clinical features present to fulfil these classifications, and thus the sensitivity of clinical features was 85.6% in the Italian cohort. Similarly, in non-cases, 7 (16.3%) and 13 (30.2%) fulfilled *probable* and *possible* classifications, yielding specificity 53.5% (23 of 43) for clinical features.

4.5. **Investigations**

The sensitivity and specificity of diagnostic investigations is demonstrated in **table 8.6**. RT-QuIC was the most sensitive (138 of 145, 95.2%) and specific (27 of 27, 100.0%) investigation. EEG was the least sensitive (102 of 213, 48.4%) investigation, with specificity 86.0% (37 of 43). Overall, MRI was 81.6% (164 of 201) sensitive and 79.4% (27 of 34) specific. The sensitivities of separate MRI abnormalities occurring individually and in combination are shown in **table 8.6**.

4.6. **Genotype & neuropathology**

PRNP c129 genotype was available in 150 (69.4%) sCJD cases. The commonest genotype was MM (n=97, 64.7%), followed by MV (n=30, 20.0%) and VV (n=23, 15.3%). PrP glycotype was available in 175 (81.0%) cases; type 1 PrP was identified in 106 (60.0%) cases, type 2A in 50 (28.6%) and co-occurrence of both types in 19 (10.9%). For the frequencies of genotype-glycotype combinations (sCJD subtypes) see **table 8.7**.

Discussion

In this **Chapter** I interrogated the features of the individual cohorts contributed by the four participating nations and found marked variations worthy of discussion. Many of these reflect contrasts in the methodology employed by the various centres.

As with the overall cohort, in all nations cases were slightly younger than non-cases. While sCJD is not associated with a biological sex preponderance[2], interestingly the German cohort featured 60.4% male sCJD cases, whereas the French cohort had a rate of 39.1% males. This may simply reflect random chance rather than cultural factors influencing the likelihood of individuals of a particular sex undergoing autopsy; however, the earlier German study by Hermann *et al* using a cohort recruited during an earlier time window (April 2014-March 2017) featured a similar preponderance of males with autopsy-confirmed sCJD[91], and perhaps an array of factors beyond the scope of this thesis influence this preponderance. All four cohorts featured shorter survival in non-cases, and the shortest duration was seen in UK non-cases (median 60 days).

The differences in case cohort sizes are likely to have reflected individual nations' autopsy rates as opposed to reflecting absolute sCJD case numbers or mortality rates in each nation. The Italian case cohort was the largest; a high proportion of individuals with suspected sCJD undergo autopsy (personal communication with Dr Anna Ladogana; see **Chapter 3**). The UK cohort was the smallest. Autopsy rates in the UK had been declining for years prior to the study period and continued to do so throughout[42].

Aetiologies in the non-case cohorts were similar in the UK, Germany and France, with neurodegenerative disorders representing the commonest category. Of these, AD with or without co-occurrence of another neurodegenerative disorder was the commonest individual aetiology. While vascular aetiologies were the second-commonest in the UK and Germany, interestingly the French cohort only featured one individual in this category, the diagnosis in whom was antiphospholipid syndrome. In contrast, a relatively high proportion of French cases had aetiologies classified as cerebral insults, most commonly anoxic brain injuries. However, the non-case cohorts in the UK and France were small, and may not truly represent practices in those nations.

Unfortunately while the Italian centre contributed 47 non-cases the study, known to have had CJD excluded through neuropathology, the centre was unable to provide neuropathological diagnoses on any of these, perhaps due to difficulties in retrieving these reports from centres performing autopsies. These may have been exacerbated by widespread healthcare disruptions due to the outbreak of COVID-19 during the period of data collection; Italy was particularly severely affected during the early stages of the European pandemic experience in spring 2020[447], the period when I had contacted the participating centres to obtain data which had not available during my earlier visits.

Missing neuropathological data in a number of non-cases was common to all cohorts. In contrast to sCJD, where specialist neuropathology services are utilised (in part due to the transmissibility risks associated with working with prion disease tissue samples, as well as dedicated diagnostic procedures being necessary), a number of non-cases are likely to have had examinations performed in regional centres after CJD has been tested for and excluded to a reasonable degree of confidence by antemortem testing. This may have impacted negatively on ability to retrieve final reports in such non-cases, most dramatically in Italy, with only partial responder rates from pathology centres not directly based in the surveillance unit.

The sensitivity and specificity of specific individual investigations varied markedly between nations. These variations are likely to reflect differences in surveillance methodology. The sensitivity of RT-QuIC was high in all nations performing the assay, and was highest in Italy (95.5%) and lowest in Germany (85.9%). In all centres performing RT-QuIC the specificity was 100%. No French individuals underwent the RT-QuIC assay, reflecting the fact that it was not yet widely in use during the study period. The French centre kindly provided me with data on RT-QuIC sensitivity among sCJD cases during the study period on request, but no assays were from individuals with tissue-confirmation of sCJD. This reflected the practice at the time of using the assay in unclear circumstances. Cases with positive 14-3-3 typically did not undergo the assay as they already fulfilled *probable* diagnosis (Jean-Philippe Brandel, personal communication; see **Chapter 3**). This is in contrast to other centres, for example the method used by the NRZ-TSE in Germany of using RT-QuIC following a positive 14-3-3 for confirmation[91].

The specificity of 14-3-3 varied substantially between nations. The lowest was in Germany (37.5%, i.e. a false-positive rate of 62.5% in non-cases). As outlined in **Chapter 3**, the 14-3-3 assay is widely used in Germany for screening purposes by regional laboratories, and a positive 14-3-3 assay is a common 'entry point' for referral to the NRZ-TSE for further evaluation of cases with suspected prion disease. This selection bias towards non-cases with positive 14-3-3 will have significantly lowered the detected specificity of the assay, and indeed this was the case in the initial validation study by Hermann *et al*[91,334]. It is likely that this contributed somewhat to the aggregate low specificity seen in the full international cohort. In addition, as discussed in **Chapter 4**, the overall specificity likely reflected a bias in the non-case cohort towards individuals with rapidly-fatal neurological diseases, as seen by the short survival in all nations' non-case cohorts. This lower specificity can be seen in other nations: in Italy the specificity was 60.5% and France 62.5%. The NCJDRSU laboratory is the only specialist CJD biomarker laboratory for the UK and employs a more selective approach to testing, only performing assays in select cases with a modest-to-high pre-test probability of CJD, which may account for the observed higher specificity of the assay among UK non-cases (84.6%). As discussed in **Chapter 4**, it is likely that the real-world cohort of non-cases tested for potential CJD are less likely to display a positive 14-3-3 and hence the specificity observed in surveillance is likely to be higher.

Variations in the sensitivity and specificity of MRI are likely to correspond to the methods employed in each nation. The highest values were in the UK cohort, where all MRI sequences in referred individuals are reviewed by an expert blinded to clinical information (David Summers). This is likely to both maximise identification of CJD-related changes in all cases[398] (including those with subtle abnormalities) as well as to appropriately distinguish CJD-like features due to alternative aetiologies such as seizures. The German cohort displayed the second-highest specificity and third-highest sensitivity. The NRZ-TSE team review many MRI sequences. Comparatively low specificities were seen in the Italian and French cohorts, where imaging is not reviewed in most circumstances, and it is possible that a proportion of non-cases with MRIs defined as positive would in fact be down-graded if reviewed by an expert. It is interesting that the sensitivity of MRI was higher in France than in Germany despite lack of routine expert reviews.

Similar patterns of MRI abnormalities were observed in all nations, albeit to lesser extents. The commonest pattern was co-occurring cortical ribboning and basal ganglia hyperintensity in most with the exception of Italy. In all nations, isolated basal ganglia hyperintensities were the least frequently observed pattern. This is in keeping with the overall distribution of c129 genotypes and strain subtypes in the cohort. While 52.2% of VV individuals display this pattern, this genotype despite being the second-commonest only accounted for 15.9% of the cohort. Likewise, 25.9% of MV cases displayed isolated basal ganglia hyperintensities, but this mostly applied to MV2 (seen in 45.5%). The dominant genotype was MM, and a minority (9%) of these cases display this pattern.

The sensitivity and specificity of EEG varied; in general the specificity was lower with higher sensitivity and vice versa. The maximal sensitivity was in France (53.4%) with the lowest specificity (70.0%), whereas in the UK cohort sensitivity was 21.8% and specificity 100.0%. A strict approach was taken in the UK series to grading EEGs, with only those displaying diffuse periodic sharp wave complexes on a background of generalised slowing being graded as positive. The French system may have graded a number of recordings as positive with less stringent criteria – for example with pseudo-periodic complexes or focal sharp waves; I did not have direct data on individual EEG reports to assess this possibility Other factors such as rates of performing EEG in cases with advanced disease might theoretically have influenced sensitivity, as sensitivity increases with disease progression.

The distribution of *PRNP* c129 genotypes in sCJD cases was similar in all nations. The German centre contributed data on only 13.1% of the cohort, owing to limited performance of *PRNP* sequencing. The excess of MM cases in the German cohort may reflect under-sampling; a larger sample would likely approach the frequency seen in the overall cohort, rather than there being an excess of MM sCJD cases in Germany. Data on PrP^{Sc} glycotyping was available in only 3.3% owing to the preference of the pathology centre performing the majority of German autopsies in cases of suspected prion disease, as outlined in the **Chapter 3**. This rate was markedly below that in other nations, where a majority of autopsied cases had biochemical data available. As a consequence it was only possible to subtype 2.2% of German cases, vastly limiting the analysis. This was also the case in the earlier study by Hermann *et al*[91]. The German centre provided morphological subtyping data, but to maximise consistency and accuracy I did not use this, despite the morphological features associate with specific subtypes being well-characterised. In the other centres the frequency of individual subtypes was similar and resembled frequencies reported in the literature[6,448], suggesting no particular bias in the cohort towards atypical subtypes (for example those with atypical features defying in-life classification and necessitating autopsy), nor an excess of 'typical' subtypes, excluding relevant atypical forms which have been valuable to evaluate in this study.

In all centres, the diagnostic criteria sensitivity rose while specificity did not change with the exception of in Italy where there was a slight decrease. The French cohort was unable to be analysed as part of the 'all' investigation approach due to no individuals having RT-QuIC assays performed. In the 'any' analyses the extent of increase in sensitivity for *probable* case classification varied. The least marked rise was in France as no *unclear* cases were able to be re-classified by RT-QuIC, so all re-classified cases transitioned from *possible* definitions. In most other centres this reclassification was less dramatic than that seen among *unclear* cases, hence the relatively low impact of novel criteria in the French cohort; it is likely that addition of RT-QuIC would have enabled reclassification in some of such cases. In the UK cohort a relatively large proportion were re-classified from *possible* to *probable* (12.7%), in contrast to others. This might reflect the detailed clinical assessments performed by the NCJDRSU: in my personal experience, a relatively high number of cases referred

would appear to have limited features (such as subacute cognitive decline) and on closer assessment would in fact display additional signs such as subtle ataxia or hyperreflexia. This might have been the case for a number of cases labelled *unclear* in other nations, where similar in-person assessments are not undertaken in the vast majority of individuals.

Summary

This sub-analysis exploring individual nations has indicated important differences in results. These can be understood in relation to the methodological differences used in surveillance systems and illustrates the subtle variation in performance of the criteria when these are present. While the revised diagnostic criteria improved sensitivity in all nations, differences in the relative impact among specific subgroups such as cases with limited clinical features varied between nations as a consequence of these differences. However, with ongoing expansion of RT-QuIC services it is likely that the highly sensitive criteria will continue to improve case recognition in all centres as well as others participating in the important global surveillance effort[1].

Chapter 9. Summary discussion

This **Chapter** provides an overarching summary of the results of this thesis, including the main implications, strengths and limitations, and scope for further work stemming from this body of research. The **Chapter** is divided into sections accordingly.

Contents

Values of the study

The values of the study in relation to its primary aims and novel insights into important subgroups and aspects of modernising surveillance are explored in the following sections:

1. **Sensitivity and specificity quantification**
2. **Superiority of criteria and investigations over ante-mortem diagnostic methods**
3. **Individual diagnostic investigations: strengths and limitations**
4. **sCJD subgroups and subtypes**
5. **False-positive investigations by aetiology**
6. **sCJD cases with specific investigation outcomes**
7. **Diagnostic test co-performance**
8. **Implications for surveillance systems**

1. Sensitivity and specificity quantification

The primary purpose of this study was to validate the diagnostic criteria. I expanded on the single-centre study by Hermann *et al*[91], demonstrating high sensitivity and specificity. I went further by evaluating important features such as age, duration and sCJD subtype, and interrogated characteristics of RT-QuIC negative cases and those with isolated cortical ribboning, and the performance of different investigation combinations. Thus, the study has demonstrated the performance of the diagnostic criteria and investigations across an array of relevant settings.

2. Superiority of criteria and investigations over other ante-mortem diagnostic methods

With the significantly improved sensitivity of the diagnostic criteria, traditional methods such as brain biopsy will have a diminishing role for ante-mortem diagnosis. This poses many advantages. The investigations used in the diagnostic criteria are less invasive and less expensive[383]. Biopsy poses infection control hazards[37]. Post-operative recovery prolongs admissions and may be poorly tolerated in individuals with dementia and behavioural symptoms.

The sensitivity of biopsy for establishing a diagnosis (including for non-prion aetiologies) in undifferentiated dementia has been reported to be only 57%[449]. One study indicated the sensitivity in individuals with acute progressive neurological decline (in the absence of mass lesions or HIV) was only 35%; only 8% of biopsies influenced management, and a mere 4% of biopsy results altered disease trajectories, while the haemorrhage rate was 4%[450]. These studies indicate that biopsy is a poor diagnostic option in comparison to less-invasive techniques employed in the diagnostic criteria, which can effectively differentiate between CJD and non-cases with greater sensitivity and avoid these limitations[349]. The subset of non-cases with non-diagnostic autopsies in the series also support its limitations.

It should be stated that I do not suggest biopsy has no role in rapidly-progressive neurological conditions. Rather, I recommend consideration of biopsy in unexplained disorders if CJD is not suggested by other sensitive biomarkers and other important treatable differentials (e.g. CNS vasculitis) are possible[451]. Concerning the possibility of CJD, the diagnosis can be effectively made or excluded using the investigations assessed in this thesis; biopsy has little role in diagnosing CJD.

3. Individual diagnostic investigations: strengths and limitations

The study provides detailed information not only on the performance of investigations for all sCJD cases as well as across a range of settings including different age, survival duration and *PRNP* codon 129 genotype groupings. I also evaluated non-case aetiologies producing false-positive investigations.

The findings yield important questions in relation to modernising CJD surveillance. For example: given how few sCJD cases were diagnosed on EEG alone, and the inferior sensitivity of EEG compared to others (with marked inter-centre heterogeneity) the study raises questions over its utility. Likewise, questions emerge regarding 14-3-3 given the superiority of RT-QuIC.

Surveillance systems might adopt responses ranging from discontinuing performing 14-3-3 altogether, or using it as an initial screening tool (the German approach), with RT-QuIC used in uncertain cases

or situations where diagnosis is not secured by 14-3-3 (e.g. a negative result, or a potential false-positive in a non-prion 'mimic').

Thus the study can be used to inform future service planning, including for nations seeking to develop programmes, particularly amid pressures such as limited funding, with investment best directed away from inferior investigations (which had utility in earlier generations of diagnostic criteria but have now been superseded).

4. sCJD subgroups and subtypes

This study is the first to address the performance of the revised criteria in important sCJD subgroups. The large, comprehensive, multicentre sample has facilitated this, in contrast to the study by Hermann *et al*[91].

The study provides information which is of great utility to clinicians assessing cases of potential sCJD. For example, I have demonstrated the impact of disease duration on the sensitivity of diagnostic criteria and investigations. When faced with potential cases of sCJD with prolonged survival (see **vignettes 5.2 & 6.1**), clinicians can use this information to better inform decision-making, including how to respond to negative investigations (e.g. 14-3-3). There was a clear improvement in criteria sensitivity among long-survivors (from 57.1% to 94.6%), indicating that this subgroup is now better recognised and classified.

5. False-positive investigations by aetiology

This study provides valuable information on false-positive investigations in non-cases, including specific associated aetiologies. This is useful for in-life assessment: clinicians may encounter positive results posing a dilemma over whether these indicate CJD or another aetiology. In particular I evaluated on the two newest investigations: while no positive RT-QuIC results were seen among non-cases, some displayed cortical ribboning, and clinicians will benefit from heightened information on causative non-prion aetiologies, recognising other conditions with the potential for mis-classification.

6. sCJD cases with specific investigation outcomes

I was able to evaluate characteristics of sCJD cases defined by specific investigation outcomes. Three important groups exist concerning the revised criteria, explored below.

i. sCJD with isolated cortical ribboning

28.1% of cases had isolated cortical ribboning (see **vignette 4.1)**. Cortical ribboning was not a component of prior criteria. Such cases could not be classified as having *probable* sCJD by MRI alone, defying classification and limiting surveillance. This analysis has evaluated characteristics of these cases, These are explored in **Chapter 7** (e.g. prevalent MM1 and MM2 cases and almost no VV2 cases).

ii. 'Clinically-limited' sCJD with positive RT-QuIC

Cases with limited features and positive RT-QuIC were not classified by prior criteria, hence I classified them as *unclear*. I quantified the extent of their re-classification via RT-QuIC. It is a clear advantage of the revised criteria that such cases can now be appropriately classified.

The many benefits of this include accurate classification for registry and epidemiological purposes (with this group accounting for 55.6% of the rise in *probable* cases), resolution of diagnostic dilemmas, and earlier diagnosis. The latter has benefits for care planning and delivery as well as recruitment of these cases into clinical trials, for whom therapeutic agents may be more likely to yield benefit (discussed later in this **Chapter**).

With 100% specificity of RT-QuIC, no clinically-limited non-cases were misclassified. This is of great reassurance for clinicians who might fear making a potentially-premature diagnosis of a rapidly-fatal and incurable disease with numerous treatable mimics.

iii. RT-QuIC-negative sCJD cases

In contrast to the above examples, RT-QuIC-negative cases (see vignettes 5.1 & 5.2) would not necessarily previously have posed challenges for surveillance. Instead they pose challenges in the modern era, where a negative outcome on a 91.6% sensitive biomarker may put the diagnosis in question.

Such individuals may have compatible phenotypes and simply be in the minority (8.4%) with false-negative RT-QuIC assays, but clinicians may be reluctant to assume this. Alternatively, with atypical phenotypes or suspicion of an alternative diagnosis), clinicians may prematurely rule out sCJD and redirect investigations and treatment efforts to alternative possibilities, which may produce harm in addition to being futile.

Thus there is an imperative for studies such as this thesis study which enhance the literature on RT-QuIC-negative sCJD cases. Their characteristics remain incompletely-understood. This study did not indicate an effect of age with RT-QuIC outcome, and the minor variations in sensitivity between

duration groups are of uncertain significance. I identified an important association with MM2 sCJD (22.2% of RT-QuIC-negative cases).

Future research can build on these results and assess features that may help distinguish between true- and false-negative RT-QuIC results, optimising in-life diagnosis and care.

7. Diagnostic test co-performance

I evaluated particular investigation combinations. This is useful to explore 'real-world' situations, and allows surveillance clinicians to efficiently investigate patients, rather than performing the full investigation battery in all individuals; for example, the combined sensitivity of MRI and RT-QuIC was 98.4%, hence additional investigations may be redundant.

Most nations delivering surveillance face challenges around ever-increasing demands on healthcare services amid financial pressures. Furthermore, some tests may be poorly tolerated, difficult to access or contraindicated. Hence clinicians frequently must opt for partial workup, and this study can guide this, as well as aiding surveillance programmes seeking to commission the optimal diagnostic pathway. This will continue to evolve as newer technologies emerge such as blood-based biomarkers and peripheral tissue RT-QuIC (discussed below) [227,228].

8. Implications for surveillance systems

This study pose a number of additional, valuable implications for international surveillance systems reflecting emerging challenges. Resourcing pressures are inevitable amid increasing demands on global healthcare systems due to population expansion and ageing, competing pressures such as those due to COVID-19 (the effects of which on CJD surveillance and care have been described in a publication I authored[293]) and challenges from known threats such as climate change[452] and currently-unknown ones. Resourcing allocated to CJD surveillance may diminish in future, despite the arguments for its continuation outlined in **Chapter 1**. Emerging public health crises may also take precedent.

Modernising surveillance is essential. This study has provided useful content to guide this endeavour, offering great value for established systems and emerging ones in terms of optimal, modern surveillance.

Strengths

There are a number of strengths in the design and data capture of this study, discussed in the following sections.

1. **Comprehensive design**
2. **Gold-standard approach: neuropathology**
3. **Multicentre cohort**
4. **Established centres with similar methods**
5. **Inclusion of cases with dual glycotypes**
6. **Diverse non-case cohort**

1. Comprehensive design

The major strength of the study is its comprehensive, population-based design.

sCJD is rare, with incidence of 1-2 per million in nations with sophisticated surveillance systems[1,81]. Such systems have the advantage of maximising case ascertainment on a population-based level as opposed to a select subset of patients (as might be the case for regional referral centres or tertiary dementia services[115]) which would limit samples and bias selection. The participating centres maintain national records on all known sCJD during the study period. In France, Italy and Germany (though only for sporadic and acquired cases in Germany) CJD is a notifiable diagnosis, further enhancing capture.

Furthermore I used a strict time period, enlisting all *definite* sCJD cases deceased between 2017-2019. This ensured a comprehensive intake of individuals, minimised the risks of selection bias which can negatively affect retrospective cohort studies[453], and was an appropriate choice as the diagnostic criteria were introduced in January 2017[385]. The earlier study by Hermann *et al* assessed the criteria during an earlier time interval (April 2014-March 2017)[91]. My method maximised the likelihood that surveillance centres would be performing RT-QuIC and including MRI images with cortical ribboning as diagnostic, providing a realistic cohort with which to effectively assess the criteria in aggregate (i.e. maximising the number individuals with the full panel of investigations) and the performance of specific investigations with maximal power.

Finally, all cases and 95.2% of non-cases were deceased (the remaining 4.8% being biopsy-evaluated), ensuring definitive diagnosis, and eliminating the risk posed by including living individuals whose final diagnosis was unresolved during the study. Numerous cases of sCJD defy in-life classification and using such individuals for evaluation of diagnostic investigations and criteria would pose numerous risks, including incorrect classification as case or non-case. However, these

individuals are important from the perspective of research to enhance surveillance and excluding such individuals would limit the utility of a diagnostic study. The method includes these individuals, allows interrogation of their characteristics, and avoids misclassification.

2. Gold-standard approach: neuropathology

I included all individuals with neuropathologically confirmed sCJD, the diagnostic gold-standard [1,68,81,242]. An alternative approach would be validating novel investigations against established ones Such investigations are not 100% specific and this approach would risk non-cases being erroneously diagnosed as *probable* sCJD, and the performance of newer investigations would be inappropriately quantified. Using neuropathologically diagnosed individuals prevents this. Despite the 100% specificity of RT-QuIC, in the literature there are rare cases of RT-QuIC positive non-cases[97], some confirmed on neuropathology[242,392,423,425] (see **Chapter 4**). I identified none, but the inclusion only of individuals with tissue diagnoses prevented this risk of misclassification, whereas it is possible that some sCJD cases might have had in-life diagnoses of alternative conditions, wrongly reducing specificity of RT-QuIC in the absence of tissue diagnosis.

The converse is true for the control group, which only included individuals with neuropathological exclusion of prion disease. Many studies employ an alternative approach including individuals with CJD excluded by ante-mortem investigations as non-cases[91,92,344,454]. While I have demonstrated high sensitivity of the diagnostic criteria it is possible that such an approach could incorrectly classify individuals with sCJD as non-cases following the diagnosis being excluded by false-negative in-life investigations, particularly with incomplete workup. One exception would be non-case individuals who made clinical recoveries, effectively excluding prion disease. Likewise, the presence of other supportive evidence of alternative aetiologies would reduce this potential misclassification risk.

3. Multicentre cohort

I ensured a robust sample by combining four nations' registries. Autopsies are increasingly uncommon in nations performing surveillance[42,81], and a single-centre approach such as that used by Hermann *et al*[91] is limited to the subset of autopsy-confirmed cases. This study was led by the NCJDRSU, but only 84 definite UK cases were available. The multicentre approach bolsters the sample to 501 cases, despite declining autopsies.

Furthermore, individual centres may have had specific approaches which introduce bias, for example selection of individuals for autopsy (e.g. targeting those with negative RT-QuIC), or widespread 14-3-3 testing in Germany [91,334]. The multicentre approach reduces the relative impact of such variations.

4. Established surveillance centres

CJD surveillance has been active for over three decades[1]. All nations contributing to the study have been world leaders in surveillance and prion disease research including diagnostics[92,100,305,326,336,349,356,360,363,389], clinicopathological features[6,63,180,181,304,308,356] and epidemiology[4,25,46,51,167,168,173,191,235,245]. This experience extends beyond sCJD. The UK and France experienced the majority of the vCJD epidemic[1], in addition to having extensive experience with c-hGH-iCJD[1,25,46], and all four nations have contributed to important studies on the ever-expanding spectrum of IPDs[4,109,120]. Thus, a major strength of this study is the collaboration between nations with advanced surveillance systems and extensive experience in CJD diagnostics.

These nations all achieve high case ascertainment, report similar incidence and mortality figures[40], and are similar in population sizes and geographical scope. Thus the study encompasses nations with broadly-homogenous surveillance methods across similar populations and with advanced national healthcare systems.

Furthermore, the contributing centres are the main CJD diagnostic services in their nations, ensuring a high degree of availability of investigation results from cases of potential CJD. Centres would have detailed results of investigations used for in-life diagnosis. Specialist investigations will have been performed by the centres themselves or their allied units (e.g. RT-QuIC and *PRNP* sequencing). This reduces the potential for missing data regarding key diagnostic investigations evaluated in this study.

Furthermore, for many of the investigations assessed, reporting of results is performed by a small number of individuals. In the NCJDRSU, all MRI results were obtained via reporting by a single neuroradiologist (David Summers), minimising the risk of inter-observer variability (the NCJDRSU method is enhanced further by the neuroradiologist's strict reporting of images blinded to clinical information and diagnoses). Likewise UK CSF biomarker assays were reported by a small team of expert biochemists. This is in contrast to approaches where results are obtained from numerous centres which may feature methodological variations, impacting on observed performance of individual investigations.

5. Inclusion of cases with dual glycotypes

I included cases with co-occurring PrP^{Sc} types 1 and 2A, accounting for 12.8% (33 of 258) of the sample with subtyping possible. These cases potentially represent up to 35% of sCJD[455] and are often excluded from studies[92,305,336,435]. Exclusion risks over-simplifying findings and limiting the result validity.

In cases with dual glycotypes, the relative quantity of types 1 and 2A PrP^{Sc} influences clinicopathological features, including presenting features, disease duration, and the extent of cortical and cerebellar disease[455]. Such cases may have specific phenotypes arising from the relative quantities of glycotypes, yet their exclusion from other studies poses a risk of overlooking additional important cases in favour of a simplified, binary approach not reflective of the real-world population. My approach provides a more detailed evaluation of investigation and criteria performance across the full range of subtypes. It also preserves sample size, and maintains the comprehensive design, with no tissue-confirmed sCJD cases excluded owing to overlapping glycotypes.

6. **Diverse non-case cohort**

By including all individuals assessed by contributing centres during the study period with neuropathological exclusion of CJD, I have provided a robust non-case control cohort, comprising a range of aetiologies encountered by surveillance systems, including neurodegenerative diseases, vascular diseases and a range of cerebral insults; for an example of a similar cohort encountered by a specialist rapidly-progressive dementia centre, see Geschwind 2016[384]. This cohort provides valuable information concerning aetiologies which may mimic CJD[287] , for example displaying cortical ribboning. The diversity of the non-case group ensures a representative sample, providing a valuable contribution to the literature.

Limitations

There are numerous limitations affecting this study.

1. **Missing data**
2. **Regional variations in methodology**
3. **Ethnicity**
4. **Investigation results excluded from analysis**
5. **Limited clinical information**
6. **Limited information on additional investigations**
7. **Impact of advanced care measures**
8. **In-life diagnosis as alternative disease**
9. **Limitations of criteria when CJD is not considered**

1. Missing data

The following sections outline areas of the analysis for which I had limited data capture, affecting the analysis. In general there were two possibilities for missing data. Firstly, situations where information existed (for example on clinical features or diagnostic test outcomes) but was unavailable to contributing centres. Secondly, where key information did not exist, for example a diagnostic investigation had not been performed. The potential effects of these are examined below.

1.1. Clinical information

Besides the UK cohort, I did not have access to detailed clinical information concerning included individuals. In other centres, the majority of individuals do not undergo direct clinical assessment by surveillance centre neurologists. Information is instead collected from referring clinicians. This can limit available information.

Data were available for most individuals (488 of 501 cases [97.4%] and 127 of 146 non-cases [87.0%]) on clinical features by category as per the diagnostic criteria (**Chapter 4**), allowing diagnostic classification . However, the absence of available clinical data in some individuals will have limited the sample. In addition, in the author's experience, reported clinical features by referring centres sometimes do not include additional features identified on direct assessment by surveillance clinicians, for example subtle clinical features not noted at the time of referral, or yet to emerge at that stage; detection of these would alter classification, affecting sensitivity and specificity figures, but this would only have been possible in certain cases (for example those undergoing detailed evaluation). In addition, clinical deficits emerge with disease progression [6,68], and longitudinal review would have enhanced classification of cases which subsequently developed additional features (e.g. myoclonus or akinetic mutism).

1.2. *PRNP* codon 129 genotype and mutations

40.0% (n=200) cases lacked *PRNP* codon 129 genotype data. Analysis was only possible in 60.0% of cases, limiting sample size and statistical power. Reasons for *PRNP* codon 129 genotyping not being performed will likely have varied between centres according to differing methodologies.

In the UK, for patients from nations other than Scotland *PRNP* codon 129 genotyping is processed by the NCJDRSU following initial venous blood sampling for *PRNP* sequencing to test for inherited mutations. Samples for this are obtained by the National Prion Clinic (NPC), a branch of the Medical

Research Council (MRC) Prion Unit, and blood is couriered to the NCJDRSU for c129 genotyping. While genetic testing is offered in all cases assessed during life, a number of individuals with suspected CJD, or more commonly their relatives (who are making the decision as surrogates due to impaired capacity), decline gene testing for a variety of reasons. In my experience of assessing over 150 cases the most frequent reason for declining was a perception in relatives that they would prefer not to know if the affected family member's form of CJD was genetic, and hence they were potentially at risk of an incurable disease with high penetrance[61], and would rather 'not think about it' than perform testing and then have to consider the ethical implications surrounding undergoing predictive testing themselves. Hence, some missing c129 data will have reflected blood sampling being declined. Furthermore, in some situations, for example in moribund patients, blood samples may not have been obtained before death.

It is possible in many cases to obtain material during autopsy for genotyping. This provides another means of identifying the *PRNP* codon 129 genotype as well as screening for inherited mutations. However, this requires fresh frozen tissue to be stored at the time of autopsy, and a number of cases undergo autopsy without this: reasons might include local preferences in the centre performing autopsy, and in some cases, autopsy being performed in cases not suspected to have had CJD during life. Furthermore, in the German centre, almost no cases undergoing autopsy also had *PRNP* codon 129 genotyping performed, owing to the preferences of the practising neuropathologists as outlined in **Chapter 3**.

Finally, it is possible that some individuals underwent *PRNP* c129 genotyping (by blood or neuropathology) yet the contributing centres were unable to provide this data (i.e. data missing due to unavailability).

21.6% (n=108) of cases were confirmed to have undergone *PRNP* sequencing, with inherited prion diseases (IPD) excluded. Of the remaining 78.4%, data were not available, but the contributing centres had confirmed these cases were diagnosed as definite sCJD; notably this category does not stipulate mandatory exclusion of IPD by genetics (**figure 1.5**). Of cases lacking *PRNP* sequencing data, some will have undergone sequencing but the contributing centre was not able to provide this (for example the Italian centre did not provide this for any cases). A remainder, for which the proportion is unknown, will not have undergone sequencing. It is hence possible that a subset of these had IPDs.

10-15% of CJD is thought to be hereditary, with the frequency of IPD and of individual mutations varying by nation and ethnicity [1,61]. Phenotypes can overlap with sCJD, particularly in common mutations (e.g. E200K[4]). Depending on the mutation, the sensitivity of investigations can differ from sCJD[4]. Thus, the presence of misclassified IPDs should be considered as this may have affected investigation outcomes. However, characteristic neuropathological abnormalities might have alerted

neuropathologists to a genetic aetiology, such as multicentric plaques in Gerstman-Straussler-Scheinker syndrome (GSS) and thalamic neuronal loss in fatal familial insomnia (FFI)[456], although this is not the case for E200K, where histological changes are indistinguishable from sCJD[61]. Thus, in the absence of genetic testing, it is possible that a minority of cases classified as *definite* sCJD might have had a genetic aetiology.

1.3. Neuropathology and PrP^{Sc} glycotyping

Not all cases underwent PrP^{Sc} glycotyping. Reasons for this might have included initial autopsies being performed by regional centres with material subsequently forwarded for further analysis; if the initial centre does not preserve fresh frozen tissue, molecular PrP^{Sc} typing is not possible. 17.9% of UK cases did not have PrP^{Sc} glycotyping data available. As outlined in **Chapter 3,** the German centre did not perform glycotyping in most cases, instead using morphological subtyping; glycotype data were only available in 3 (3.3%) cases.

As a consequence the analysis was underpowered. Some cases lacking subtyping may have had atypical Parchi subtypes such as MM2 and VV1[6], and *PRNP* c129 and PrP glycotype data might have provided further valuable information on these atypical subtypes, including clinical manifestations and diagnostic investigation outcomes[304,430]. With more complete data I might have been able to quantify in more detail the sensitivity of investigations such as RT-QuIC, where the descriptive analysis suggested reduced sensitivity in these subtypes, potentially accounting for disproportionate numbers of RT-QuIC negative cases (for example MM2 accounting for 22.2% of RT-QuIC negative cases with subtyping possible, versus 2.6% of RT-QuIC positive cases; **Chapter 7**).

While it was a strength of the study to include the 12.8% of cases with dual PrP glycotypes[455], the information merely stated the co-occurrence; I was unable to quantify the dominant strain (if any), which might have allowed us to define these subtypes in more detail

1.4. Neuropathological diagnoses in non-cases

Neuropathological diagnoses were only available in 66.4% (97 of 146) of non-cases. In those for which reports were not available, it was known that prion disease had been excluded via tissue analysis (morphology, immunohistochemistry, usually, western blot analysis), but lack of detail on final non-prion diagnoses limited analysis. In the Italian cohort no reports were available, significantly reducing sample size. This may have reflected the particular difficulties posed by COVID-19 in Italy in early 2020, the period of data collection for this study.

However, in the Italian cohort, complete and systematic absence of information perhaps poses less potential for bias than a select subset of reports missing, where numerous possibilities which might have influenced report availability in a non-random fashion. Non-cases lacking reports may have had relevant characteristics influencing where their care was located, for example features influencing provision of care outside of a tertiary neurosciences centre, such as advanced age or less marked neurological features, decreasing the likelihood of eventual communication with surveillance centres, in contrast to autopsies undertaken in neurosciences centres. Likewise, factors might have led to these individuals being lost to follow-up and termination of correspondence between surveillance centres and treating teams, for example a lower ante-mortem probability of CJD due to atypical characteristics or a clinically-evident alternative diagnosis, with the matter of diagnosis considered 'resolved' from a CJD surveillance perspective prior to autopsy. Finally, specific centres not answering written requests for data may theoretically have covered a geographical region with an excess of patients with relevant atypical characteristics, such as older age or higher proportions of ethnic minority individuals[457]. All of these examples might lead to various forms of bias affecting the cohort with available reports for the study.

Furthermore, information on neuropathological diagnoses was limited to diagnostic headings. For example, in non-cases with diagnoses I categorised as cerebral insults, summary diagnoses such as anoxia or status epilepticus were available. It might have been valuable to know more regarding the extent of neurological damage and the affected regions to enhance clinicopathological correlation, for example in terms of features overlapping with CJD, including myoclonus or cortical hyperintensities, and what might distinguish these cases during life, for example if the distribution of pathological and imaging abnormalities were atypical for CJD[401,458].

Neurodegenerative aetiologies were most frequent. However, information was frequently limited to a heading such as Alzheimer's disease (AD). It would be helpful to know more about these cases, for example if they had atypical disease distribution within brain tissue that might have influenced features, triggering suspicion of CJD. I focused data on demographics and features relevant to CJD; more detailed neuropatholoical and clinical information would have been of value. Likewise, this would have been valuable in relation to those with false-positive investigation results.

2. Regional variations in methodology

Whilst surveillance systems use the same diagnostic criteria and follow similar methodology, important differences exist which impact on available data and observed results.

2.1. MRI

Sensitivity and specificity of MRI in CJD is observer-dependent, maximal with expert reporting. Abnormalities are underreported by non-experts[391], while artefact or CJD-like features (e.g. cortical signal changes due to seizures) may be misinterpreted. However, expert review was not performed uniformly in all centres (see **Chapter 3**). These differences likely account for marked inter-centre variations in MRI sensitivity and specificity observed in this study (see **Chapter 8**). The aggregate figures reflect overall sensitivity and it should be noted that this was drawn from varying methods.

2.2. CSF testing

There are differences between centres in CSF testing methods. In the UK, all patients are referred to the NCJDRSU where testing is performed if appropriate, i.e. in individuals with suggestive features. In Germany, many laboratories perform 14-3-3, and many individuals are referred to the NRZ-TSE following positive assays. This reduces specificity as in the study, and will have reduced the composite specificity figure.

The RT-QuIC assay is relatively novel and certainly was so during the study period, hence participating centres utilised RT-QuIC in different ways. In the UK, RT-QuIC is performed in all patients undergoing CSF testing, unless there are assay-specific limitations or contraindications. In Italy, numerous centres perform RT-QuIC; olfactory mucosal RT-QuIC is also used, in contrast to the other nations. In France, during the study period RT-QuIC was only being performed in a subset of cases, for example those with inadequate evidence for sCJD diagnosis (e.g. negative investigations) but ongoing suspicion of CJD. There were 14 individuals with RT-QuIC data available (Angeline Denouel, personal communication), but none underwent neuropathological examination, hence they were not included in the study. RT-QuIC was the least-frequently performed investigation in this overall international series, limiting sample size. While the French sample contains 100% missing data for RT-QuIC, a positive element of this is that it minimises any potential for selection bias to have predisposed to non-random missing data, for example, RT-QuIC being selectively performed only in atypical cases or those with negative investigation outcomes (similar to the above discussion concerning non-case neuropathology data in Italy).

2.3. PrP^{Sc} glycotyping

Variations in frequency of available PrP^{Sc} data were seen between centres (**table 8.7**) and were discussed above. The major contrast was observed in Germany. The leading pathology centre uses morphological appearances as a surrogate for subtyping. While the morphological characteristics of subtypes are well-described[6,63], I did not use this data in order to maximise objective grading of PrP^{Sc} typing.

It is likely that PrP^{Sc} typing, when performed using standard protocols, is objective, so I would not anticipate major centre-dependent variations in sensitivity and specificity. However, missing data has reduced the sample size and hence power, precluding statistical analysis on subtype groups.

3. **Ethnicity**

I did not assess ethnicity as a demographic feature in this study. The NCJDRSU recently published a 28-year review of records assessing the role of ethnicity on sCJD diagnosis and surveillance[457]. This study did not identify significant differences in diagnostic test performance, but did identify a 30% lower autopsy rates (35% vs 65%) and younger age of onset in non-white cases; similar has been observed in the US [459]. To my knowledge, similar analyses have not been performed in the other nations participating in this study. A large analysis of surveillance data from Europe, Australia and Canada did not assess ethnicity[72].

Ethnicity-related effects may be relevant to the study. Non-white cases may comprise a disproportionate number of young-onset cases [457], and this may compound with other elements including cultural and socioeconomic factors impacting on access to and engagement with healthcare, affecting the diagnostic process and overall performance of criteria. Discrepancies in autopsy rates [457] [459] may theoretically have created a selection bias in the cohort towards white individuals.

The influence of ethnicity in sCJD is likely to be complex. Contributing factors may include those explained above and biological factors such as polymorphisms at *PRNP* c129[2] as and currently unknown sites influencing disease manifestations and duration. Ethnicity did not influence investigation performance in the study by Langlands *et al*, but this contrasts to a US study by Appleby *et al* [459]. A 2020 USA study identified a higher frequency of RT-QuIC positive Hispanic/Latino individuals, but the authors were unable to postulate regarding possible explanations for this[242]. The study was limited in terms of data capture: 97.6% (10250 of 10498 individuals) did not have available ethnicity data. Studies in the USA have indicated lower CJD incidence and mortality among African American, Native American and Alaskan individuals[2,50,460,461]. It is unknown whether these disparities reflect differences in disease susceptibility versus under-ascertainment, the latter due to factors including limited healthcare access and varying disease manifestations.

The demographic makeup of the contributing nations has changed over decades and will continue evolving. Thus the effects of ethnicity warrant exploration in order to deliver effective surveillance and optimal care for all affected individuals. This includes for other nations: as discussed in the **Chapter 1,** many lack CJD surveillance programmes[1], and the incompletely-understood interactions between ethnicity and sCJD may influence multiple dimensions of delivery for such programmes. Further studies exploring these interactions would be valuable for global surveillance efforts.

4. **Investigation results excluded from analysis**

As outlined in **Chapter 3**, I excluded investigations from the analysis which could not be performed, or could not be interpreted, owing to technical reasons. The first group consisted of CSF analyses which were not performed due to sampling issues such as contamination with blood, improper handling or insufficient volume[97]. The second consisted of uninterpretable investigations, (e.g.MRI sequences degraded by artefact). I did not count either group as negative; thus these were not included in the denominator for sensitivity and specificity analyses, as the outcome in each group was unknown.

In the real-world of surveillance, attempted investigations will sometimes be negatively affected by sampling issues, patient intolerance, and distortions to signal degrading final sequences[462]. For example, myoclonus is frequent in advanced sCJD[439,440,463], and produces motion artefact. Likewise, agitated behaviour is a challenging symptom[17,464] which can impact on ability to comply with investigations. A subset of individuals are managed in the ICU setting[324,465-467], and in many centres intubated individuals cannot undergo MRI; the use of sedatives may also preclude or confound EEG usage.

It is worth noting that the subset of cases undergoing the full complement of investigations, and for whom the investigations were able to be performed and interpreted to an adequate standard, are a minority within the cohort (44.9%; see **figure 4.5**) and the real-world population. Thus, it is important to acknowledge real-world barriers to assessment. However, as discussed in **Chapter 4**, it is also important to note that for many individuals, full workup is unnecessary given overlapping positive investigations.

5. Limited clinical information

I limited clinical information to the presence/absence of cardinal features in the diagnostic criteria.. The study was not designed to explore the clinical features of sCJD cases or the non-case cohort. The analysis was thus somewhat simplistic compared to the in-depth process employed in real-world clinical surveillance.

Relevant factors I did not explore are discussed below.

5.1. Clinical features pointing away from CJD

Many features strongly indicate non-prion aetiologies. Prominent fluctuation can suggest DLB[468], while stepwise deterioration characterises vascular dementia[469]. Seizures are uncommon in sCJD[287,314] and may indicate autoimmune or infective encephalitis[432,470]. Sudden and subacute onset can suggest a vascular insult or autoimmune aetiology. Prominent autonomic disturbance can indicate multiple system atrophy (MSA)[471] or autoimmune conditions[472]. Peripheral nervous system involvement can

indicate alternative disorders, for example motor neuron disease (MND) [473], or neuropathy due to toxic, metabolic, hereditary or autoimmune conditions [474].

5.2. Antecedent and provocative features

Provocative clinical factors were not evaluated, but provide diagnostic information in surveillance. Anoxic brain injuries accounted for 8.3% of non-cases. In my two-year experience, this was a frequent aetiology among referrals, but individuals typically had an antecedent history of cardiac arrest or severe cardio-respiratory insults[475], and rarely required detailed evaluation for potential CJD by NCJDRSU clinicians. Likewise individuals with hypoglycaemic brain injuries usually had an evident antecedent history[476]. In reality, such provoking factors can usually be elicited, making the diagnosis obvious.

5.3. Simplistic, categorical approach to clinical feature evaluation

I did not assess recorded clinical features in detail, and thus cannot comment in depth on the different clinical manifestations affecting individuals. In some cases, reported cerebellar features may have represented severe, fulminant cerebellar syndromes while in others these may have been subtle and potentially asymptomatic, incidental findings on examination. There are major differences between the two in terms of diagnosis and care; more detail would have allowed intensive clinicopathological typing.

5.4. Non-chronological approach

I did not explore chronicity and evolution of features. It would have been useful to model the latency from first symptom to the time of fulfilling classification for a *probable* case for the entire cohort as well as among subgroups for an indication of real-world criteria performance and limitations.

5.5. Severity and impact of clinical features

I did not grade features according to severity or functional impact. Such an analysis would have allowed more detailed clinicopathological correlation as well as enhancing established literature on the progression and care requirements seen among sCJD subtypes, of relevance for diagnosis as well as prognostication and management across the spectrum of sCJD cases. This was a study to evaluate the diagnostic criteria performance, so clinical features were limited to their presence or absence in line with criteria requirements.

6. Additional, ‘non-CJD’ investigations

Individuals assessed for CJD undergo numerous investigations for alternative conditions, including reversible aetiologies[287,384]. Biomarker assays (e.g. serology) may indicate non-prion diagnoses such as autoimmune encephalitis. Imaging may demonstrate features of Alzheimer's disease (mesial temporal atrophy on MRI[477]), dementia with Lewy bodies (reduced ligand uptake in basal ganglia on DaTscan[478]), cerebrovascular disease[479] or mass lesions. Biochemical analysis may indicate metabolic conditions (e.g. hyperammonaemia).

I did not quantify in-life evidence for alternative aetiologies. This would likely have been present in many non-cases. Including this as a condition of the diagnostic criteria – perhaps worded as 'AND no clinical, biomarker or imaging evidence for an alternative aetiology', similar to the suggestion in the study by Hermann *et al*[91] – would likely improve specificity, and this approach is used by clinicians in the real-world of surveillance.

However, the evidence for alternative conditions does not necessarily refute the possibility of CJD. Some cases have dual pathology, for example coinciding cerebral infarction or a co-morbid neurodegenerative disorder, and judicious assessment is necessary to determine whether identified abnormalities account for an observed clinical syndrome (**vignette 9.1**). This poses additional challenges where non-prion pathology may account for positive outcomes in biomarkers such as 14-3-3.

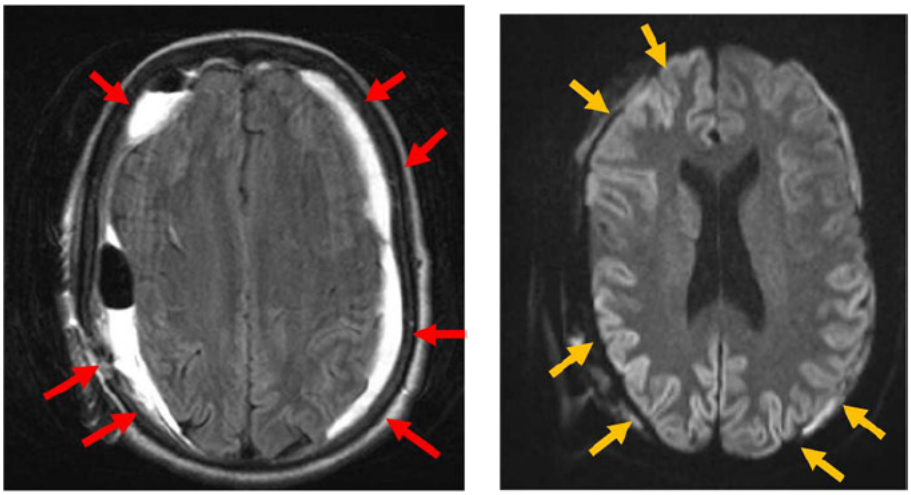
Vignette 9.1. Transmission hazards in sCJD

A 68-year-old woman presented with several months of progressive confusion and altered behaviour. She developed dressing apraxia and delusions. She developed falls, one of which led to a strike to the head, and was admitted to hospital after a CT brain showed bilateral subdural haematomas. These were initially managed conservatively.

While an inpatient her condition deteriorated with impaired mobility and cognition, incoordination and myoclonus. A decision was made to evacuate the right haematoma. However, her condition did not improve. An MRI was performed demonstrating bilateral subdural haematomas on FLAIR (red arrows, left image) and cortical ribboning in underlying brain on b=1000 DWI (yellow arrows, right image).

During the NCJDRSU assessment she was disorientated, apraxic and ataxic, had bilateral grasp reflexes and brisk right-sided reflexes. EEG showed diffuse slowing. 14-3-3 was weakly positive and RT-QuIC was positive. Following diagnosis the patient was referred on to public health and infection control teams to manage transmission risks.

The patient died 24 months after onset without undergoing post-mortem. The case illustrates both the transmission risks in sCJD and the potential for the diagnosis to be masked by comorbid pathology.



7. Impact of advanced care measures

In addition to biological factors influencing survival in sCJD, survival is prolonged with enteral feeding and artificial ventilation. These do not enhance quality of life[18] and are not routinely recommended in the contributing nations in this study, but are comparatively frequent in Japan, where duration is extended substantially[2,442].

I did not assess for these advanced measures. These may have influenced duration and clinical features (e.g. the presence of late-emerging features, such as akinetic mutism) and investigation sensitivity (e.g. 14-3-3 or EEG). It is possible that certain subgroups, such as individuals with younger onset, may have been more likely to receive life-prolonging measures, contributing to observed differences in relevant outcomes. Likewise these interventions may have been over-represented in the subgroup with prolonged duration; not recording these may have wrongly implicated possible biological reasons for between-group differences.

8. In-life diagnosis as an alternative disease

Cases of sCJD are often diagnosed with an alternative disorder during life. In some cases this applies to an earlier stage of the disease prior to the CJD diagnosis emerging during life; examples in my experience frequently included stroke, depression and vestibulopathy. In others this misdiagnosis remains in place until autopsy.

It would have been interesting to explore in-life diagnoses, quantifying how many cases received incorrect diagnoses (and what these were), as well as whether subsets of these were more likely to have atypical features or negative investigations, and thus represented a population in whom the diagnostic criteria were less effective than those with 'classical' CJD manifestations. Another relevant factor would be whether these individuals received care in centres without dedicated neurology input and imaging expertise [398].

9. Limitations of diagnostic criteria when CJD is not considered

Surveillance for prion diseases is limited to situations when clinicians refer suspected cases or when prion disease is identified on neuropathology, most commonly at autopsy as a 'late' case, as is the case for 2% of UK cases (John Centola, NCJDRSU, provisional results from an ongoing study; see below). Case ascertainment has dramatically risen over several decades[40]. Nevertheless it remains a challenge to improve ascertainment in under-recognised groups.

Thus the in-life effectiveness of the criteria is limited to circumstances where sCJD is considered a potential diagnosis. MRI is frequently under-reported[398], and clinicians not suspecting CJD are unlikely to request dedicated biomarker testing. The analysis does demonstrate that the revised criteria are vastly more sensitive, but this relies on the necessary investigations being performed, and for MRI, reviewed by an expert.

Despite efforts to promote awareness of CJD in the international literature[1], a number of currently-unrecognised cases of sCJD may continue to be so. With declining autopsy services and competing demands of the COVID-19 pandemic this may be difficult to address.

Further work

This study poses a number of questions for further exploration by the international surveillance community. This section details some of the pressing areas for future study.

1. **Comparison with other CJD subtypes**
2. **Novel biomarkers: CSF, blood and Imaging**
3. **RT-QuIC on non-CNS tissues**
4. **Earlier in-life diagnosis**
5. ***In-vivo* subtyping, prognostication and modelling**
6. **Clinicopathological features: the influence of pathology on sCJD manifestations**
7. **Demographic outliers: age and duration**
8. **'Late case' analysis: sCJD not diagnosed until post-mortem**
9. **Clinical trial facilitation**
10. **International development**
11. **2020 and beyond: CJD surveillance amid COVID-19**
12. **Application of diagnostic criteria using telehealth**
13. **Other protein misfolding disorders**

1. Comparison with other CJD subtypes

I demonstrated that the criteria are highly sensitive and specific when applied to a cohort of sCJD cases and non-case 'mimics'. This is of immense value in surveillance, where many referrals will not have prion disease, and among those which do the commonest form is sCJD.

However, a major challenge for surveillance units is recognising atypical prion diseases, in particular variant CJD[1]. BSE and vCJD drove the development of widespread international surveillance which continues to this day[1]. The unique features of vCJD led to its recognition and distinction from other forms[132,133] . A critical question for surveillance programmes is not merely whether diagnostic criteria can adequately distinguish prion vs non-prion aetiologies, but whether they allow recognition of atypical forms, including vCJD cases with extensive incubation [55] and hypothetical cases related to emerging forms of acquired prion disease[1].

It would be useful to retrospectively validate the diagnostic criteria against a cohort of vCJD cases. In addition, certain sCJD subtypes are associated with features which may make distinction from vCJD challenging such as atypical presentations (e.g. ataxic-predominant or neuropsychiatric), younger onset and prolonged survival[6,63]. A recently published example of a 34-year-old with VV1 sCJD and features overlapping with vCJD illustrates this challenge[480]. It would be particularly useful to compare such a cohort to the vCJD cohort.

A limitation of this would be that this would only validate the criteria against vCJD cases without the extensive incubation which would define modern-day cases, likewise those postulated to emerge with atypical genotypes. Furthermore, many cases emerged pre-RT-QuIC and many did not undergo MRI with DWI (in a 2003 study, only 2 of 86 vCJD cases had undergone DWI[136]). It isn't certain whether historical vCJD cases would have displayed cortical signal changes using modern sequences. The pathological basis for DWI signal changes is incompletely understood; while this may reflect restricted movement of water molecules due to vacuolation[343,481], in vCJD vacuolation was extensive in the cerebral cortex[138], and it is interesting that affected cases did not display cortical ribboning.

Ongoing surveillance work remains an essential public health activity [1]. Identification of future vCJD cases will enable such a validation exercise, which may lead to further upgrading of the criteria for sCJD and vCJD.

2. Novel biomarkers: CSF, blood and imaging

2.1 CSF biomarkers

In addition to 14-3-3 and RT-QuIC, numerous other CSF biomarkers have been explored over 3 decades of surveillance, including tau [95,96,356,482,483], S100b[95,96,327,349] and neuron-specific enolase (NSE)[96,327,334]. Other biomarkers include neurofilament light (NfL)[484,485], alpha-synuclein (αS)[486,487], YKL-40[488] and neurogranin[489,490].

Questions for ongoing studies include optimal cut-offs for positive results (which varies with intended usage, e.g. for screening versus confirmation of sCJD), the co-performance of assays (for example in

a multi-step diagnostic algorithm), and the specificity for a given cut-off against a variety of non-cases encountered in surveillance. National surveillance centres are ideally placed to perform such studies, and have made great contributions to the established biomarker literature.

Given the excellent diagnostic performance of RT-QuIC (sensitivity 91.6%, specificity 100%) outlined in this thesis and many other studies, one might question the ongoing need to explore pre-existing and novel biomarkers and whether services should simply expand usage of RT-QuIC. One study cited limitations of RT-QuIC including costs and limited availability, and sought to explore other biomarkers as screening tests in conjunction with RT-QuIC[484]. This study found particularly high sensitivity (91.3%) and specificity (78.9%) for CSF t-tau at a cut off of 1147pg/mL; adopting a lower cut-off of 757pg/mL for screening pre-RT-QuIC yielded a combined sensitivity of 93.6% and specificity 100%. Thus it is important not to abandon further biomarker development: there are many potential roles for other biomarkers including utility for screening and prognostication.

Regarding prognostication, future studies might combine emerging biomarkers (including blood-based) with clinical features, investigation results, demographics, *PRNP* codon 129 genotype and other variables to develop multivariable models with utility in predicting survival duration. A German study indicated good accuracy of a model incorporating tau[434], though did not include the wider complement of sCJD diagnostics. Clinicians currently have few evidence-based tools for prognostication, and rely on clinical parameters such as bedbound status and myoclonus as indicators of advanced disease, in addition to the preceding rate of progression. In early disease, where diagnosis is increasingly possible via RT-QuIC clinical features have limited utility for prognostication, and clinicians cannot generally comment on prognosis in terms more specific than quoted averages, which poses a source of concern for patients and relatives. *PRNP* codon 129 genotype affects survival duration [3,434], but was rarely used for prognostication in the author's surveillance experience . There is a major need for validated prognostication tools to optimise care-planning and holistic support for affected individuals.

2.2. Blood-based biomarkers

No validated blood-based sCJD biomarkers are currently available. Studies have demonstrated the potential for such biomarkers[334]. The development of a highly sensitive and specific blood assay would have tremendous value as a screening test, for example in cognitive disorder clinics and for patients with subacute ataxia. While the current diagnostic criteria are highly sensitive and specific, there are caveats to their delivery, including the need for specialist MRI reporting[398], and the invasiveness of LP and MRI. Blood biomarker assays might bypass some of these.

Blood-based assays could potentially serve a multitude of roles, including diagnosis [485], prognostication[296,491,492], and as a marker of disease progression, including for therapeutic response in trials[493]. Numerous biomarkers have been explored including including S100b[494], tau[296,485,493,495,496], and neurofilament light (NfL)[296,485,493,495,496] and YKL-40[497]. An additional role concerns pre-symptomatic IPD-causing mutation carriers[13], in whom biomarkers might demonstrate subclinical disease activity.

Future studies might explore both diagnosis and prognosis. Key diagnostic questions would include the i) time-dependent profile of biomarkers including the latency to diagnosis ii) evolution with disease progression, iii) sensitivity across sCJD subtypes, iv) associations with other investigations (including specific pattern of MRI abnormalities, e.g. reflecting cortical-predominant damage), and v) specificity against non-cases (individuals with alternative diseases as well as healthy controls). Prognostic questions might include the ability to predict total survival, time-to-diagnosis and hospitalization, and functional parameters relevant to care delivery such as loss of mobility or swallowing function.

2.3. Imaging

Imaging techniques pose exciting possibilities for sCJD. While MRI is a powerful diagnostic investigation (sensitivity 86.8%, specificity 82.0%), it is currently only employed in a binary capacity concerning sCJD diagnosis. Studies have established the longitudinal evolution of hyperintensities with disease progression[350], but the extent of changes on individual scans is not used for prognostication or to guide subtyping attempts. Some studies have explored the evolution of regional atrophy in sCJD[498,499], which may provide utility for prognostication and monitoring progression. The novel options of imaging-based in-life sCJD subtyping[435] and single-region hyperintensity to facilitate early diagnosis[419] were explored in **Chapter 4**. Diffusion tensor imaging (DTI) is a technique which assesses structural brain tissue integrity[500]. Some studies have explored DTI in sCJD[501,502], in some cases indicating a complex non-linear relationship between abnormalities and progression[502]. Future work may provide further information on progression of structural brain abnormalities in sCJD.

Nuclear imaging including positron emission tomography (PET) has been explored in sCJD both as a diagnostic and research tool[299,503-506]. The role of PET for diagnosis is potentially limited[299] given the excellent combined sensitivity of other investigations, and there are no characteristic abnormalities on PET imaging used for formal diagnosis. If such abnormalities were to be characterised, PET may still hold a role in some situations, for example individuals with contraindications to MRI or inability to lie still. As a research tool, PET offers a valuable means of functional imaging, correlating regional metabolic abnormalities to clinical features such as apraxia [299], as well as potentially being used for longitudinal assessment of progression. Such clincopathological correlation might yield insights into

characteristic patterns of disease manifestations such as the Heidenhain (visual) variant[90,507] and ataxic-predominant presentations, as well as correlating metabolic changes to abnormalities on MRI. PET has not been extensively studied in sCJD; several publications arose from case reports[508-511].

3. RT-QuIC in non-CNS tissues

Olfactory mucosa RT-QuIC is an established test for sCJD and is included in the diagnostic criteria[307,359,421]. Skin RT-QuIC testing poses an interesting possibility for minimally-invasive diagnosis[227,228] (see **Chapter 1**). Published studies involved small numbers of individuals and large-scale replication is necessary for validation . Given longitudinal increases in PrP^{Sc} levels with disease progression[228], validation studies might explore RT-QuIC positivity across disease stages and from different skin sites.

Skin is an ideal site for minimally-invasive diagnosis, and punch biopsy procedures are likely to carry advantages of better patient tolerance and ease-of-learning by medical practitioners compared to CSF or olfactory mucosa sampling[228]. The latter two may be inappropriate in individuals with advanced disease, whereas skin biopsy may be acceptable in these.

4. Earlier in-life diagnosis

A major challenge for CJD surveillance and care is the long latency to diagnosis[288]. This has a variety of negative effects, including prolongation of expensive, invasive and poorly tolerated investigations and empirical treatment measures for alternative disorders[288], delays to holistic care delivery and essential public health activities to mitigate transmission, distress for relatives, and finally, clinical trial delivery (see below).

Overcoming this latency would require comprehensive national surveillance in addition to measures to improve in-life recognition of CJD by regional clinicians and referral to national centres, and the ability to make a robust, early diagnosis. This is a major benefit of the revised criteria, which substantially improved classification of cases with clinically-limited disease.

Further work to explore the effects of the revised criteria on diagnostic latency is essential. An optimal study would prospectively evaluate all cases assessed for potential CJD in a time period, assessing time from onset to key outcomes including recognition of potential sCJD, referral to a national centre and fulfilment of criteria definitions for *probable* diagnosis . This could be combined with measures to assess the performance of biomarkers at various disease stages, as well as the work of Bizzi *et al* on the performance of MRI when limited to single region involvement[419], in a real-world

prospective cohort. Identification of cases who do not initially fulfil criteria and subsequently do so on follow-up could help better characterise individuals with a high probability of having sCJD.

5. *In-vivo* subtyping, prognostication and modelling

The subtype of sCJD, as defined by the Parchi classification (*PRNP* codon129 genotype and PrP^{Sc} glycotype combinations), is major determinant of a multitude of disease manifestations[6,179-181,304,305,308,356,430], and reliable in-life subtyping (e.g. via MRI [435]) would be valuable, for example for prognostication and therapeutic trials.

Antemortem subtyping is only possible via biopsy. There are no biomarkers enabling PrP^{Sc} typing, and the proposed use of MRI for subtyping is exciting but requires more work[435]. This, along with parameters such as *PRNP* c129[3], novel biomarker assays and clinical manifestations, would allow a level of prognostication currently unavailable to clinicians.

Therapeutic compounds have been tested in pre-clinical studies and clinical trials[8,9]. Despite encouraging preclinical results, no agent has yielded conclusive benefit in human subjects. Anti-PrP antibodies have been developed, and a clinical trial of one agent (PRN100) in a small (n=6) cohort demonstrated feasibility the agent in larger-scale trials[512] . However, perhaps unsurprisingly given the early stage of development, no anti-PrP agents have explicitly targeted the specific sCJD subtype including particular PrP^{Sc} glycotypes. Should anti-PrP agents prove effective as therapy for sCJD, this avenue may perhaps be explored in future, facilitated by in-life subtyping.

6. Clinicopathological features: the influence of pathology on sCJD manifestations

I explored subtype-dependent variations in factors such as duration and investigation sensitivity in this thesis, adding to established studies [113,179-181,304,305,308,430,435,455]. Neuropathological variations are well-described in the literature[1,448]. For example, MM1 and MV1 feature small vacuoles in the cortex, striatum and thalamus, with fine synaptic staining for PrP^{Sc} on immunohistochemistry (IHC). MM2 cases are classified MM2-C when large cortical vacuoles are present, which are often confluent and display peri-vacuolar coarse PrP^{Sc} staining on IHC, and MM2-T when thalamic atrophy is prominent. VV2 cases display vacuolation in deep neocortical layers with perineuronal staining on IHC. MV2 is characterised by Kuru-type amyloid plaques particularly prominent in the cerebellum.

These subtypes all differ greatly in in-life features The biological basis for phenotypical variations is poorly understood. Improved understanding of this would be invaluable. Better understanding of subtype-specific loci of disease involvement (e.g. deep nuclei, cerebellum or cortex) and spread through brain regions would better characterise subtypes and improve understanding of the basis for

specific manifestations, as well as disease evolution and the specific challenges affected individuals experience throughout their illness. The biological basis for different MRI lesion profiles remains incompletely understood[481] and could be better-characterised, enhancing proposed in-life subtyping algorithms[435]. The influence of pathology on investigations would be useful to understand, including lower RT-QuIC sensitivity in MM2[97,242,356,363]. Better clinicopathological understanding would support trials stratified to individual subtypes.

Further advantages would include better knowledge of the factors underlying transmission. Transgenic mice studies have indicated influences of the recipient *PRNP* c129 genotype in addition to the transmitted subtype on features such as susceptibility, incubation duration, clinical features and lesion profiles[513]. The different predominant *PRNP* c129 genotypes seen in British and French c-hGH-iCJD epidemics[25,514] likely reflected differences in PrP^{Sc} strains from cadaveric sources between nations[1,33], with certain genotypes being accordingly more susceptible to infection. sCJD has an idiopathic aetiology, with hypothetical causes including transmission via unknown exposures [1]. Better understanding of factors influencing transmission of sCJD and infection susceptibility in exposed hosts would be useful for public health work, and might yield interesting testable hypotheses around disease acquisition through transmission

7. Demographic outliers: age and duration

7.1. sCJD cases with atypical age

This study provides valuable insights into age-dependent variations in diagnostic criteria and investigation performance in sCJD. Data indicate an age-related (rather than ageing-related) onset of sCJD[42], and young- and older-onset cases are uncommon. The large cohort allowed evaluation of these cases.

It is uncertain whether lower incidence among younger and older adults reflects biological factors or under-ascertainment. Under-ascertainment seems less likely in the young population given widespread notoriety of CJD following the vCJD epidemic, a disease affecting younger adults. Referrals to the NCJDRSU have increased over several decades; these peaked during vCJD epidemic, with many referred individuals receiving non-prion diagnoses (reflecting high vigilance for the condition among undifferentiated patients), and subsequently declined, more recently rising to similar levels as 2000-2001, but with higher proportions of CJD cases[42] (indicating a tendency to refer cases with higher pre-test probability). Furthermore, with enhanced medical infrastructure and diagnostic technologies it seems unlikely that younger adults with CJD would be under-investigated and that the diagnosis would be missed.

Under-ascertainment is plausible among the elderly. The NCJDRSU is currently investigating cases identified at post-mortem. Provisional data indicate that these individuals are older, and overlap phenotypically with ageing-related neurodegenerative disorders such as Alzheimer's disease, dementia with Lewy bodies or vascular dementia, which may lead to in-life misdiagnosis[81]. Other factors impeding ascertainment in the elderly may include comorbidities masking or modifying clinical manifestations, or leading to CJD manifestations being attributed to these (e.g. basal ganglia hyperintensities being misdiagnosed as infarction).

There is a need for further characterisation of the effects of age on sCJD manifestations, as well as impacts in assessment, diagnosis and care. Younger individuals may be more likely to receive empirical therapies (e.g. immunosuppression) and advanced measures such as enteral feeding and ventilation, whereas older individuals may be less likely to undergo investigations such as lumbar puncture and to receive specialist neurology input or care in neurosciences centres. These factors may further complicate the process of diagnosis in addition to any biological effects of age.

Further clincopathological work would better characterise the biological basis for age-related variations, including effects of *PRNP* c129 genotype and PrP glycoypes[6], brain plasticity, glial and neuronal responses to damage, and co-occurring pathology[515].

The NCJDRSU is currently studying young-onset sCJD cases (onset ≤50 years) using a ten-year cohort from 2011-2021. This study is exploring differences in clinical features, investigation sensitivity, disease subtypes and neuropathological profiles among young-onset cases. Provisional results have indicated that ii) 4% of cases are young-onset, ii) cases are more likely to present with psychiatric/behavioural symptoms and experience longer diagnostic latency, and iii) RT-QuIC is less sensitive (Johnny Tam, Suvankar Pal, NCJDRSU, personal communication). Further analysis will explore how these findings relate to underlying neuropathological characteristics; for example, lower RT-QuIC sensitivity may reflect frequent atypical subtypes (e.g. VV1 and MM2).

It would be interesting to see similar studies from other nations. Likewise, a study formally assessing characteristics of older-onset cases would be invaluable.

7.2. Duration

Assessing sCJD cases stratified by disease duration yielded a number of interesting outcomes, including the enhanced classification of long-survivors with new criteria. Clinical discrepancies existed between disease duration groups, which warrant further exploration.

'Late' cases appear to feature prolonged duration and atypical presentations, including extrapyramidal phenotypes (see below). These may have consequences, including in-life misdiagnoses, prolonged

diagnostic latency (sometimes requiring post-mortem) and the likelihood that some cases are never diagnosed and evade detection by surveillance systems. The most significant consequences of this would concern public health risks via transmission.

Further studies exploring the spectrum of features associated with prolonged disease duration would be useful, including clinical features, underlying neuropathology (e.g. atypical subtypes) and iatrogenic factors influencing duration such as enteral feeding and ventilation[18,442]. As with outlying age groups, this subset is likely to be uncommon: 73 cases (14.6%) in the cohort survived > 1 year, 21 cases (4.2%) survived >2 years, and only 6 cases (1.2%) >3 years. A large sample and long study period would be necessary to investigate further. Such a study would also yield valuable insights for prognostication.

Two recent studies explored variables which influence prognosis. In the first, an array of variables including clinical phenotype, investigation results and c129 genotype were used in models which showed high accuracy for predicting survival probability as well as levels of dependency at various time points[516]. The second assessed demographic and investigation outcomes, and found that older age of onset, female sex and the presence of seizures were associated with poorer prognosis[517]. The development of prognostic models would be of great use to clinicians assessing individuals with sCJD, as well as for care planning – in my experience of surveillance, many patients and their relatives would frequently want an estimate of anticipated survival duration, and this was difficult to provide in the absence of any robust prediction models. More work exploring factors influencing duration is necessary.

8. 'Late' case analysis: sCJD not diagnosed until post-mortem

Most sCJD cases are diagnosed in-life via surveillance, but a small minority are not referred during life and are diagnosed at autopsy and referred as a 'late' case. This poses difficulties: the benefits of in-life diagnosis are lost, including prompt interventions to prevent transmission, and the diagnostic uncertainty and requirement for autopsy can pose distress for relatives. Autopsies in suspected CJD should be performed in specialist centres to minimize occupational hazards, but without in-life suspicion these may take place in non-specialist sites. Finally, 10-15% of CJD is genetic[4]; while genetic testing can be performed via autopsy, when CJD has not been considered the opportunity may be lost (e.g. lack of storage of fresh frozen tissue).

'Late' cases pose questions concerning CJD diagnosis and their characteristics warrant characterisation, hence the NCJDRSU study mentioned above. It is important to explore this subgroup given that they defy the current diagnostic criteria I have validated in this study. While these criteria are highly sensitive (97.8%) for in-life diagnosis of sCJD, this applies when the investigations are

performed. In cases where CJD is not suspected, specialist investigations such as RT-QuIC may not be performed.

'Late' cases may differ from those identified in life. Possibilities may include older age, atypical clinical features and, absent MRI abnormalities (or present but subtle and overlooked), and cases may be more likely to receive care in regional centres and not access dedicated neurology input. It would be valuable to explore common in-life misdiagnoses to promote better recognition of these cases.

A study exploring this subgroup is currently being undertaken by the NCJDRSU, led by Dr John Centola and Suvankar Pal, NCJDRSU. Provisional findings of 15 'late' cases over a 5-year window (representing 2% of all cases) indicated relatively frequent early psychiatric and extrapyramidal presentations and less frequent cognitive impairment at onset in comparison to the majority of cases. Disease duration was prolonged (median 15 months). 3 cases had VPSPr, an uncommon sCJD variant associated with longer survival and atypical features[518]. 12 of 13 cases had MRI abnormalities typical for sCJD, indicating underascertainment of relevant imaging changes in life; sCJD would likely have been diagnosed had these abnormalities been recognised and the cases referred for specialist evaluation. Finally, most had co-occurring additional neuropathology including tau, amyloid and cerebrovascular disease, which may have influenced phenotypes and confounded diagnosis.

These findings shed light on factors which might yield non-recognition of these cases during life. Further analysis will explore clinical features as well as locations of care and access to specialist neurology input. There are likely to be other individuals with sCJD who present similarly, are not suspected to have sCJD, and do not undergo post-mortem analysis, and hence evade diagnostic capture by surveillance systems. This study will help to expand the recognition of these individuals, enhancing case ascertainment. It would be interesting to see similar work conducted in other nations.

9. Clinical trial facilitation

There is a major need for clinical trials in sCJD. Trials have been performed but faced numerous challenges[9,11,12,14,15]. sCJD is rare, limiting sample sizes[10,15]. sCJD emerges sporadically, with no geographical foci[42,81] and no pre-clinical predictive factors available to identify individuals at risk (in contrast to iCJD-related exposures and carriers of pathogenic mutations). The short prognosis[6] and long diagnostic latency[288] (and hence short post-diagnostic survival) has consequences: cases may not survive long enough to be recruited and undergo effective treatment. Neurodegeneration may be too extensive for therapeutics to take effect, leading to disappointing results with agents which were effective in pre-clinical phases[9,10]. Cognitive impairment can pose ethical challenges regarding consent[519]. Trials may experience selection bias favouring atypical subtypes of sCJD[11], enrolling long survivors or those with ataxic- or neuropsychiatric onset, affecting generalizability[289].

Robust diagnostic criteria capable of identifying the full spectrum of sCJD cases, including at clinically-limited stages, is essential for trial delivery. This thesis demonstrated high sensitivity and specificity of the 2017 diagnostic criteria and particular improvements among long survivors and clinically-limited cases, and has enhanced the literature on specific disease subtypes, aiding their recognition and diagnosis. The criteria are well-placed to facilitate large-scale diagnosis of sCJD, and ongoing work to enhance earlier diagnosis[419] and in-life subtyping[435] will further improve recruitment of robust samples for therapeutic trials.

As above, the heterogenous clinical and epidemiological characteristics of sCJD[1,42,81], challenge recruitment. This is in contrast to inherited[13] and iatrogenic forms, where at-risk individuals can be identified at pre-clinical stages. IPD differs additionally due to the aetiology, posing exciting possibilities for trials[13] in line with other inherited neurological disorders such as spinal muscular atrophy (SMA)[520] and muscular dystrophy[521]. Only large-scale collaboration can identify and recruit new cases of sCJD into trials, best conducted through international collaboration[7]. Ongoing international surveillance is critical to facilitate this[1].

10. International development

sCJD has been identified in many nations[1]. Prevalence is impossible to quantify in many for reasons including limited healthcare infrastructure and absent surveillance systems. It is possible that the incidence will rise as nations develop, living standards improve and life expectancies increase, with associated public health implications[37]. In the absence of dedicated surveillance programmes this will be difficult to monitor and manage.

Nations with established systems can support other nations developing their own programmes[1]. This may include guidance on establishing registries and diagnostic services as well as offering services such as imaging reviews and biomarker and neuropathology services, and advising on public health actions following identified cases.

11. 2020 and beyond: CJD surveillance amid COVID-19

The study period assessed concluded in December 2019. The COVID-19 pandemic was beginning at that stage and had not yet reached the authors' nations. Within months it exerted devastating effects on medical care in all four nations, in addition to many others globally. These circumstances formed a 'stress test' for surveillance, and a question arising from this study is how well the criteria have performed amid the challenges imposed by COVID-19. Relevant aspects would include changes in referral numbers, the impact of widespread service disruptions, whether case ascertainment decreased,

and the impact of COVID-19 infection in CJD patients, including on overall duration of survival

I published a study assessing these questions in the European Journal of Neurology[522]. The results are beyond the scope of this thesis, but the reader is directed to the appropriate reference.

12. Application of the diagnostic criteria using telehealth

The COVID-19 pandemic led to an immediate shift of the NCJDRSU method from in-person to remote assessments via telehealth. In addition to the direct challenges of the pandemic outlined above, this shift to a novel means of delivering timely surveillance was a major change. I published a study in the Journal of the Neurological Sciences exploring the NCJDRSU experience[293]. The reader is directed to the study for the full results, but we experienced numerous advantages including shorter delays between referral and assessment, as well as major reductions in financial costs and environmental impacts. It was entirely possible to deliver the modern surveillance model using the diagnostic criteria studied in this thesis via a telehealth-based service, and the NJCDRSU continues to use telehealth for a high proportion of assessments even as unrestricted travel has resumed in the UK.

The study abstract was as follows:

> Creutzfeldt-Jakob disease (CJD) is a fatal human prion disease. Surveillance systems operate globally with the goals of accurate in-life case ascertainment, appropriate public health interventions to minimise secondary transmission, and monitoring trends in disease epidemiology. The UK experienced the highest incidence of variant CJD (vCJD) in the world following widespread population exposure to bovine spongiform encephalopathy (BSE). 178 cases of vCJD have been identified in the UK by the National CJD Research & Surveillance Unit (NCJDRSU), including three cases of secondary transmission via blood transfusion. The NCJDRSU performs high-fidelity surveillance, assessing all cases of suspected CJD referred to the unit. COVID-19 has caused widespread disruption to healthcare and poses a threat to services. The NCJDRSU converted to telehealth-based surveillance in March 2020. We report the results of the application of telehealth for comprehensive CJD surveillance during the first four months of the pandemic. 59 cases were assessed for suspected CJD. In 52 cases the relatives were interviewed for an informant history, by video conference or telephone call. 34 patients underwent video examination; 1 case was examined in-person. MRI images were assessed in all cases and 46 underwent CSF testing. Feedback was obtained from interviewees and the NCJDRSU team on their experiences. 50 cases were diagnosed with sporadic CJD; 5 received an alternative diagnosis, and the remaining 4 remained unresolved, with further investigations underway. Telehealth significantly reduced time taken to assessment compared to in-person assessments in

2019. Telehealth is an effective way to provide comprehensive CJD surveillance at a national level.

13. Other protein-misfolding disorders: is there evidence for transmissibility?

Recent studies have demonstrated that misfolded proteins in other disorders such as Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA), Parkinson's disease (PD) and motor neuron disease (MND) may have prion-like characteristics, namely protein-induced misfolding, cell-to-cell transmission, and potential inter-subject transmissibility[523-530]. Amyloid β pathology has been detected in recipients of c-hGH [523,531], hDM grafting[525] and childhood neurosurgery[524], raising questions over transmissibility of amyloid β, which has been demonstrated in transgenic mice[526,527]. Additional evidence from preclinical studies and recipients of fetal mesencephalic neuronal grafts suggests that α-synuclein may be induced to misfold in the presence of its abnormally misfolded form[528]. SOD1-linked MND is transmissible in mouse models by injection of spinal homogenates into sciatic nerves[529]. Transgenic studies also demonstrate transmissibility of tau pathology[530].

In light of this emerging evidence, questions have arisen over whether other protein-misfolding disorders are transmissible between humans in a manner similar to CJD. Whilst this is a highly interesting hypothesis, and one which has generated considerable interest in scientific news coverage, in my opinion caution is advised before drawing conclusions. It is important to note that the human studies have small sample sizes, and the public health implications of these studies, if any, are currently unclear. More evidence is required to explore whether these disorders may harbour risks of transmission comparable to prion diseases. If such a mechanism did exist it would yield very interesting novel questions around the epidemiology and aetiology of these disorders.

Chapter conclusions

I have provided a detailed discussion of the study methods and results, including its major novel features and implications for surveillance, and its strengths and limitations, including challenges posed by missing data and heterogenous surveillance systems. I concluded by providing an overview of proposed topics for further research, including dedicated studies into prognostication and the effects of atypical age on disease manifestations, and lastly the interesting body of evidence indicating prion-like behaviour in other, commoner neurodegenerative diseases. Together, the study results should enhance international surveillance efforts, and provide evidence to guide in modernisation of surveillance, which remains a public health priority.

Chapter 10. Conclusions

This thesis began by exploring the justifications for continued prion disease surveillance in the modern era. I then validated the 2017 International CJD Surveillance Network diagnostic criteria for sporadic Creutzfeldt-Jakob disease using a robust clinicopathological international cohort while also providing novel insights into criteria performance across important subgroups which had previously been unexplored, including *PRNP* c129 genotypes and different age groupings. I have also compared similarities and differences in methods used in individual nations and explored how these impact on aggregate outcomes.

The results indicate that the current diagnostic criteria enjoy high diagnostic performance across a range of settings and have greatly improved on the previous iteration in terms of case ascertainment, with a 21.5% rise in sensitivity for *probable* case classification from 74.2% to 97.8% when all investigations are performed. The improvement in ascertainment includes gains among outliers with atypical disease duration and in cases with limited clinical features previously defying classification. I have also demonstrated that some of the older diagnostic investigations such as EEG may now have limited utility in the modern era and have suggested ways in which the criteria can be further evolved.

The major hurdle for prion disease now exists in relation to clinical trials. I have addressed some of the myriad challenges these face in sCJD. Despite these, the results indicate that the current diagnostic criteria are well placed to maximise case ascertainment and recruitment into trials, for which there is a desperate need if the disease is ever to receive a disease-modifying therapy. The international community should not lose sight of this important goal amid other pressing urgencies.

In conclusion, the current diagnostic criteria are highly sensitive and specific, have improved diagnostic capacity for sCJD across a range of subtypes, and are well-placed to serve the international community in important efforts to improve the clinical diagnosis and care of individuals with sCJD, as well as epidemiological monitoring and clinical trial delivery.

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