BMJ Open

Enhanced CJD surveillance in the 65+ population group in Scotland: study protocol for neuropathological screening of brain tissue donations for research

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033744
Article Type:	Protocol
Date Submitted by the Author:	20-Aug-2019
Complete List of Authors:	Peden, Alexander; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences Kanguru, Lovney; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences Ritchie, Diane; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences Smith, Colin; National CJD Research and Surveillance Unit, National CJD Research and Surveillance Unit Molesworth, Anna; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences
Keywords:	prion, vCJD, brain, Neuropathology < NEUROLOGY, screening, surveillance

Enhanced CJD surveillance in the 65+ population group in Scotland: study protocol for neuropathological screening of brain tissue donations for research

Alexander H Peden[1*], Lovney Kanguru[1], Diane Ritchie[1], Colin Smith[1,2], Anna Molesworth[1]

Affiliation

1. National CJD Research & Surveillance Unit (NCJDRSU), Centre for Clinical Brain Sciences, University of Edinburgh, Western General Hospital, Edinburgh
2. Edinburgh Brain Bank (EBB), Centre for Clinical Brain Sciences, University of Edinburgh, Chancellor's Building, 49 Little France Crescent, Edinburgh

***Corresponding author**

Alexander H Peden - a.peden@ed.ac.uk, 0131 537 1980

Word count

Abstract = 272

Manuscript = 2999 (excluding title page, abstract, references, figures and tables)

ABSTRACT

Introduction:

Creutzfeldt-Jakob disease (CJD) is a human prion disease that occurs in sporadic, genetic and acquired forms. Variant CJD (vCJD) is an acquired form first identified in 1996 in the United Kingdom (UK). To date 178 cases of vCJD have been reported in the UK, most of which have been associated with dietary exposure to the bovine spongiform encephalopathy agent. Most vCJD cases have a young age of onset, with a median age at death of 28 years. In the UK, suspected cases of vCJD are reported to the UK National Creutzfeldt-Jakob Disease Research & Surveillance Unit (NCJDRSU). There is, however, a concern that the national surveillance system might be missing some cases of vCJD or other forms of human prion disease, particularly in the older population, perhaps because of atypical clinical presentation. This study aims to establish whether there is unrecognised prion disease in people aged 65 years and above in the Scottish population by screening banked brain tissue donated to the Edinburgh Brain Bank.

Methods:

Neuropathological screening of prospective and retrospective brain tissue samples are performed. This involves histopathological and immunohistochemical analysis and prion protein (PrP) biochemical analysis. During the study, descriptive statistics are used to describe the study population including the demographics, clinical, pathological and referral characteristics. Controlling for confounders, univariate and multivariate analyses will be used to compare select characteristics of newly identified suspect cases with previously confirmed cases referred to the NCJDRSU.

Ethics and dissemination:

Brain tissue donations to EBB are made voluntarily by the relatives of patients, with consent for use-in-research. The EBB has ethical approval to provide tissue samples to research projects (REC

reference 16/ES/0084). The findings of this study will be disseminated in meetings, conferences, workshops and as peer reviewed publications.

Registration details:

Edinburgh Brain Bank (Clinicaltrials.gov identifier: 10/S1402/69; 10/S1402/70)

KEYWORDS

prion, vCJD, brain, neuropathology, screening, surveillance

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study could provide valuable information on the possibility of unascertained prion disease occurring in the over 65 year age group.
- The study includes five biochemical analysis methods, which are used in research for the detection of the abnormal misfolded prion protein (PrP^{Sc}) associated with prion diseases.
- Two of these biochemical methods (Western blotting and RT-QuIC) are routinely used in the diagnosis of prion disease at NCJDRSU.
- The other three methods have been used in research, but have not been used routinely as tools for prion disease diagnosis or surveillance.
- This study is restricted to the Scottish population, but it serves as a pilot study to explore the feasibility of extended, UK-wide, enhanced CJD surveillance in the 65+ population group.

INTRODUCTION

Human prion diseases are rare, invariably fatal neurodegenerative diseases associated with an abnormal misfolded form of the prion protein, designated PrP^{Sc}. The most common human prion disease is Creutzfeldt–Jakob disease (CJD), which is mainly idiopathic in origin, occurring sporadically worldwide at a rate of 1 to 2 cases per million population per year. A variant form (vCJD) is associated with dietary exposure to bovine spongiform encephalopathy (BSE), although person-to-person transmission of vCJD infection has also occurred through both blood and possibly blood products. [1,2] In contrast to the sporadic form (sporadic CJD, or sCJD) which affects individuals mainly in the seventh decade of life, the median age at onset for vCJD in the UK is 26.5 years and the median age at death 28 years.[3] To date, 178 cases of vCJD have been reported in the United Kingdom (UK) with the first cases reported in 1996 and the most recent death occurring in 2016.[4] However, prevalence studies indicate that 1 in 2000 people in the UK may be subclinical carriers of vCJD infection.[5-7] Therefore, it is possible that future cases of vCJD may occur.[4,5,8,9]

The national surveillance system for CJD in the UK has comprehensive mechanisms in place for the ascertainment of prion disease.[3] However, it is possible that the national surveillance system could be missing some vCJD cases, particularly in older age groups, perhaps because the clinical presentation in these individuals is atypical of vCJD. For example, age-related changes in the brain may mask the magnetic resonance imaging (MRI) signal and characteristic pathology that supports the diagnosis of vCJD.[10] There is also the potential that typical cases of vCJD may simply not be recognised as such in older individuals, because vCJD patients are typically much younger. Furthermore, dementia is also relatively common among people aged 65 years and above[11] and a diagnosis of vCJD may be more difficult to recognise, or may not be considered, if the patient has been referred to non-neurology medical specialities that are less familiar with prion disease. A similar situation may also exist for sporadic CJD (sCJD), which in the UK currently occurs at a rate of 5 to 6 cases per million of the population aged 65 years and above, with mortality peaking in the 65 to 79 age group and then rapidly

declining.[3] The reasons for this rapid decline are unclear, but may, in part, be linked to under ascertainment of cases in the elderly, rather than the absence of disease.[3]

In order to enable robust and accurate clinical and epidemiological surveillance of CJD and to help protect public health from the potential iatrogenic transmission of CJD,[12] the identification and investigation of CJD cases across all age-groups is essential. This study aims to screen banked brain tissue donations for evidence of otherwise unrecognised prion disease (including vCJD and sCJD) in the 65+ age group. Specifically the study aims:

- To undertake in-depth histopathological, immunohistochemical, PrP biochemical and molecular subtype (*PRNP* codon 129) screening,
- To describe the range of clinical and pathological characteristics associated with prionopathy, in life (alternative) diagnoses and referral characteristics,
- To assess the feasibility and value of extending this approach to other research tissue banks throughout the UK.

METHODS

Study design and population

This study is part of a larger feasibility study considering approaches to determine if there is otherwise unrecognised prion disease (including vCJD, sCJD and other forms of prionopathy) in the UK population. The approach taken for this part of the study involves neuropathological screening of prospective and retrospective brain tissue donations, donated to the Edinburgh Brain Bank (EBB) from donors in the 65+ age group from throughout Scotland.[13,14] The testing methods applied include histopathological, immunohistochemical and PrP biochemical analysis, and genotyping polymorphic codon 129 of the prion protein gene (*PRNP)*.

Case inclusion definition

All brain tissue donations to the EBB from people aged 65 years and above are eligible for inclusion in the study. Donated tissue is excluded only if there is insufficient quantity for planned laboratory investigations. The number of eligible donations received at EBB is currently estimated at 30 each year. In addition, there are approximately 175 donations already banked at EBB from 2005 (referred to as retrospective samples), which are eligible for screening in this study.

Outcome

Our primary outcome of interest is evidence of prion pathology, which includes the presence of abnormal prion protein PrP^{Sc} in brain tissue following brain tissue testing. We are interested in the associated clinical, pathological and referral characteristics, and in life (alternative) diagnosis of any cases detected in this way.

Source of samples

The EBB is part of the UK population wide Brain Bank Network, providing high quality post-mortem materials for diagnosis and research into disorders of the brain and nervous system. EBB was established in 2005, and receives donations from a number of national and local research studies in

Scotland.[13,14] Currently, this includes donations made through the NCJDRSU 65+ enhanced clinical surveillance study, Alzheimer Scotland,[15] Edinburgh Procurator Fiscal, Lothian Birth Cohort 1936, the Scottish Motor Neurone Disease (MND) Register,[16] the Lothian study of IntraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN)[17] and the Multiple Sclerosis Society Tissue bank (Table 1). These form a highly select patient group with a set of neurodegenerative (non-CJD) conditions amongst which a "missed" diagnosis of prionopathy might be found. An overview of the protocol put in place including neuropathological screening of brain tissue donations is shown in Figure 1.

Donations to EBB

All donations made to the EBB are handled by a team comprising a neuropathologist(s), research nurse, laboratory technicians and a laboratory manager.[18] Neuropathologists provide cellular and molecular diagnoses from post-mortem examinations. The research nurse is responsible for obtaining authorisation for a post-mortem examination and use of brain tissue for research purposes from the families of donors. The research nurse liaises with donor families and funeral directors throughout the whole process. The laboratory technicians are responsible for collecting and storing the tissue samples and checking their quality. The laboratory manager ensures the smooth running of the laboratory, including appropriate governance on tissue sample requests from researchers in the UK and internationally.

Sample identification and preparation at EBB

Once a tissue donation is made to the EBB, staff check its eligibility for inclusion into our study. Eligible donations are flagged, and the study team at NCJDRSU is informed. For all donations made there is a standard protocol for tissue sampling that is applied during the post-mortem examination.[19] Firstly, the brain is removed and cut into coronal slices. These individual brain slices are further sub-sampled to provide a small tissue block from a wide range of specified brain regions. Each block of tissue is divided into two, with one sample immersed in formalin fixative and processed into a paraffin

embedded (FFPE) tissue block, with the second sample frozen in liquid nitrogen vapour and stored at -80◦C. Both frozen and fixed samples are stored within the EBB and made accessible for further neuropathological and biochemical investigations. From the FFPE tissue blocks, 5µm thin tissue sections are cut and mounted onto glass slides (referred to as fixed tissue), and analysed by microscopy following haematoxylin and eosin (H&E) staining or immunohistochemical probing with specific antibodies. The frozen tissue is subjected to biochemical and genotypic analyses. For our study, EBB provides both fixed and frozen tissue samples from each of the four cortical regions (frontal, temporal, occipital and parietal) as well as the thalamus and cerebellum, if available.

Sample transportation

The frozen and fixed tissue samples are anonymised before being transported to NCJDRSU, and accompanied in transit by a study tissue form containing a unique EBB donation identifier number. For the fixed tissue, no specific precautions are necessary for transportation. However, these samples are packaged appropriately in microscope slide boxes to prevent damage in transit. Frozen tissue is packaged together with dry ice in accordance with the regulations for road transport of Category B (UN3373) tissue specimens. Both frozen and fixed samples are delivered to NCJDRSU in person by the EBB laboratory manager.

Processing of samples at NCJDRSU

Due to the infectious nature of prion diseases, all personnel handling frozen tissue samples within the NCJDRSU laboratory, are required to do so in accordance with NCJDRSU Category 3 laboratory health and safety policies and national regulations.[20,21] Both frozen and fixed samples are delivered to the NCJDRSU category 3 containment laboratory,[20] where they are registered electronically and tracked within the unit using the same unique EBB donation identifier number as above. The frozen samples are stored immediately in a designated -80°C freezer, while the fixed samples are stored at room temperature in the laboratory.

Histopathology testing

For all prospective and retrospective samples, laboratory technicians at EBB conduct a standard suite of histopathological screening on the fixed tissue from all six brain regions mentioned above for the identification of pathological changes associated with common neurodegenerative diseases, including screening for spongiform change, astrogliosis, neuronal loss and plaque formation. This standard suite includes basic immunohistochemical analysis using a panel of antibodies against neurodegenerative proteins: anti-Aβ40, anti-Aβ42, anti-α-synuclein, anti-phospho-tau, anti-phospho-TDP-43 (transactive-response DNA-binding protein 43) and anti-p62.

Immunohistochemically testing for PrP

Additional immunohistochemically testing for the prion protein (PrP) in the fixed tissues are performed at NCJDRSU using two anti-PrP monoclonal antibodies: 12F10, which recognises the PrP epitope 142-160 (Bioquote Ltd, York, UK), and KG9, which recognises the PrP epitope 140-160 (TSE Resource Centre, Roslin Institute, UK). Both are used in combination with the highly sensitive Novolink™ Polymer Detection System.[22] PrP immunohistochemistry is routinely carried out on fixed tissue sections on just two of the six brain regions, namely frontal cortex and cerebellum. Subsequent analysis on the thalamus and the remaining three cortical regions (temporal, occipital and parietal) is conducted if the cases are flagged to be of interest following their histopathological and/or biochemistry investigations for prion disease.

PrP biochemical analysis

For all prospective samples, this investigation requires approximately 2-3 grams of frozen tissue each from the frontal, temporal, occipital and parietal cortical regions as well as the thalamus and cerebellum, whereas for retrospective samples, only the frontal cortex and cerebellum are analysed. We use a panel of biochemical analysis methods (Table 2), which are designed to maximise the potential for detecting low levels of prion disease PrP^{Sc}. These include:

i) Standard diagnostic Western blot (WB) for the protease-resistant core of PrP^{Sc} (PrP^{res})[22,23] with samples prepared according to the method of Parchi et al.[24]

ii) High sensitivity sodium phosphotungstic acid precipitation (NaPTA), followed by WB for PrP^{res}. [2,25,26]

iii) Conformation dependent immunoassay (CDI) analysis for PrP^{Sc}.[27] This method is highly sensitive and is able to detect both protease resistant and protease sensitive forms of PrP^{Sc} [28,29]

iv) Single round protein misfolding cyclic amplification (PMCA) for ultra-sensitive vCJD PrP^{Sc} detection [30,31]

v) Real-time quaking induced conversion (RT-QuIC) for ultra-sensitive sCJD PrP^{Sc} detection[32-34]

Sensitivity of the PrP biochemical analysis methods

Western blot is a well-established diagnostic method used in prion disease research and surveillance, but has limited sensitivity. This technique is also limited to the detection of the protease resistant form of the misfolded PrP. It may therefore be less able to detect new or atypical prion disease subtypes if a significant component of PrP^{Sc} is protease sensitive.[23] The other four biochemical analysis methods (NaPTA, CDI, PMCA, RT-QuIC) used have higher sensitivities for detecting PrP^{Sc}, and RT-QuIC detection of prion seeding activity in cerebrospinal fluid is used in the UK to assist the clinical diagnosis of CJD patients.[35] However, the effectiveness of the four tests other than western blotting as methods for brain tissue sample screening is yet to be fully established. Therefore, when using this panel of biochemical analysis methods, careful consideration is given to the process used to assign positive results and to assess anomalous findings. Accordingly, we have developed an algorithm for each test that is used to facilitate classification of cases as “negative” or “negative – anomalous” or “positive” as shown in Figure 2.

Genotyping

PRNP codon 129 genotyping is performed using a sample of frontal cortex tissue for all cases in this study, except for 65+ study patients (see Table) where the codon 129 genotype may already be known from a previous analysis of blood. The methionine(M)/valine(V) polymorphism at *PRNP* codon 129

affects prion disease clinicopathological phenotype and susceptibility to prion disease at the population level.[36] *PRNP* codon 129 genotyping is essential for classifying the different forms of prion disease. The process of genotyping involves extracting DNA from the frozen brain tissue samples (20-30mg). Thereafter, *PRNP* codon 129 genotype analysis is performed by polymerase chain reaction and restriction fragment length polymorphism analysis.[37]

Data management

All staff at NCJDRSU have a duty to maintain patient confidentiality, and procedures and relevant training are in place for data safeguarding. The University of Edinburgh has records management and information security policies, procedures and guidance on the handling of confidential information. In addition, NCJDRSU has comprehensive information governance procedures to ensure data security and protection.

All samples received from EBB (fixed and frozen) are de-identified by EBB staff, in line with EBB ethical approval prior to sharing with NCJDRSU. Samples are accompanied by a limited set of data only: The study requests the gender of the patient, their year of birth, age at death and post-mortem information such as brain weight, pH and the time between death and post-mortem. All the results are documented and recorded in the study database at NCJDRSU. Paper records are filed securely at NCJDRSU in locked filing cabinets when not in use. Electronic records are processed in a password-protected controlled secured network with access restricted to named users on a need-to-know basis. At no point in time is personal information disclosed to anybody other than the named-users; linkage of records for study analyses, and for follow-up is restricted to authorised personnel by use of a unique study number.

Action for positive cases

The outcome of investigations is shared between the NCJDRSU and EBB study teams as part of the investigation record. If there is evidence of vCJD, sCJD or other prion pathology, then further

investigations are undertaken according to standard NCJDRSU surveillance procedures.[38] A final diagnostic report would be submitted to the senior clinician, then sent to the donor's general practitioner for communication to the family.

Quality assurance

For quality assurance, and to test the sensitivity and specificity of the protocol, a blinded analysis is conducted in conjunction with the analysis of samples from EBB. Under the direction of the principle investigator, and in strict accordance with NCJDRSU Category 3* laboratory health and safety policies, the blinded approach is undertaken as follows. A panel of human prion disease cases is used as positive controls. This panel includes vCJD cases, a range of sCJD subtypes, and rarer forms such as variably protease sensitive prionopathy (VPSPr) to test the ability of the protocol to detect a range of prion disease subtypes, characterised by varying levels and isotypes of PrP^{Sc}.

The positive samples are anonymised and packaged in identical manner to the ordinary study test samples, by the EBB and the NCJDRSU laboratory managers. True data for the positive cases is not attached to the samples because it could lead to identification of the sample prior to testing. Instead, the positive samples are assigned dummy data, which is linked to their true identifiers using a coded key only known to the EBB and NCJDRSU laboratory managers who are responsible for the blinding process. Researchers conducting the analyses will not know which samples are positive or negative until the end of the planned analysis when the identities will be revealed. All results will be recorded in the study database.

Disposal of samples

All residual tissue samples are retained until the end of the study, after which NCJDRSU will handle the disposal of any remaining samples in accordance with the EBB procedures. Samples from cases that are suspected to be CJD or any other prionopathy are retained routinely in the Brain and Tissue

Bank at NCJDRSU. Any residual tissue or material linked to the positive samples (i.e. frozen tissue samples, homogenates, microscope slides, DNA) are destroyed or returned storage at NCJDRSU.

Statistical Analysis

Any case with pathological evidence of prion disease which, prior to this study, was not considered to have prion disease, is referred to as a "missed" case of prion disease. Descriptive statistics including frequency tables, cross-tabulations and graphics will be used to describe the demographics of the study population including the date of death, age, sex and provenance of the donation. Clinical and pathological characteristics of the missed cases with attention to presenting features and in life (alternative) diagnoses will also be described. In addition, description of case classification (molecular subtype) and referral characteristics will be included. Univariate and multivariable analysis adjusting for potential confounders such as age and sex will be used to compare characteristics of missed cases with previously confirmed cases referred to NCJDRSU.

Ethics and approvals

Brain tissue donations are made voluntarily by the relatives of those involved, with consent for use in research. EBB has ethical approval to provide tissue samples to research projects (REC reference 16/ES/0084), including those for pilot studies. Findings of this study will be disseminated in meetings, conferences and as peer reviewed publications.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination of our research.

Acknowledgements

We thank Tracy Millar and Chris-Anne Mckenzie, both of EBB, for their assistance in preparing this manuscript. We would like to express our gratitude to Suzanne Lowrie and Helen Yull at NCJDRSU, and

local mortuary and laboratory staff for their help in managing and undertaking tissue investigations, as well as the patients and their relatives.

Authors' contributions

AM and CS designed the study, in consultation with AHP and DR, and together with AHP and LK drafted the manuscript.

Funding statement

This work is independent research commissioned and funded by the Department of Health and Social Care Policy Research Programme (PR-ST-1214-10002). The views expressed in this publication are those of the authors and not necessarily those of the Department of Health and Social Care.

Competing interests

None declared

Figures

Figure 1: An overview of processes put in place including neuropathological screening

Figure 2: Algorithm for assessing the results of biochemical analyses

Tables

Table 1: Sources of donations to EBB

Source	Description
65+ Study	Includes donations from participants who are 65 years and older across Edinburgh and NHS Lothian including the Ann Rowling Clinic, Old Age Psychiatry, Medicine of the Elderly and Neurology services, with atypical features of dementia
Alzheimer's Scotland	Includes donations from adults diagnosed with dementia in Scotland
Edinburgh Procurator Fiscal	Includes donations from sudden or accidental death investigated by Procurator Fiscal in Scotland
Lothian Birth Cohort 1936	Includes donations from participants born in 1936 in Lothian
Motor Neurone Disease Register	Includes donations from patients with Motor Neurone Disease in Scotland
LINCHPIN - Lothian IntraCerebral Haemorrhage Pathology Imaging and Neurological outcome	Includes donations from adults in Lothian diagnosed with intracerebral haemorrhage after 1st JUNE 2010
Multiple Sclerosis Society Tissue Bank	Includes donations from patients with Multiple Sclerosis in Scotland

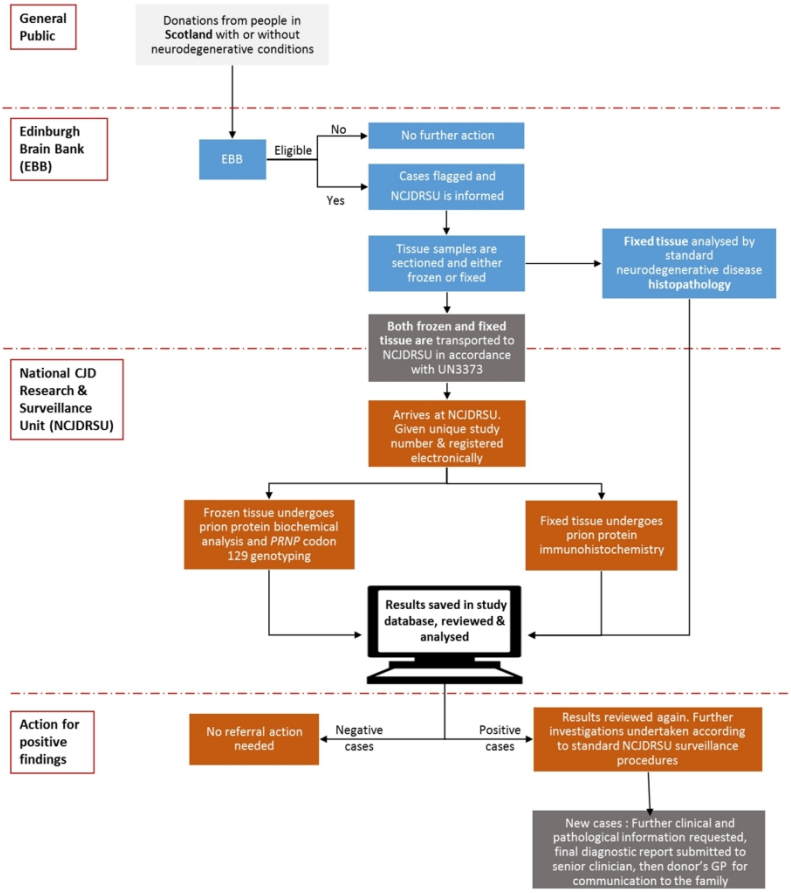
Table 2: Biochemical analysis methods

Method	Function of Test	Advantages	Disadvantages	References
1. Western blot (WB)	Detection of protease-resistant PrP^{Sc}	Standard method used in the diagnosis of prion diseases	Relatively low analytical sensitivity	22-24
2. Sodium phosphotungstic acid (NaPTA) precipitation/ western blotting	Concentration and detection of protease-resistant PrP^{Sc}	Can detect low levels of PrP^{Sc} e.g. in vCJD spleen and sCJD muscle	Not tested for use in routine diagnostics or screening	2 ,25 ,26
3. Conformation dependent immunoassay (CDI)	Detection of PrP^{Sc} based on concealed epitopes that are exposed when PrP^{Sc} is denatured.	Can detect protease sensitive forms of PrP^{Sc}	Not tested for use in routine diagnostics or screening	28 ,29
4. Real-time Quaking induced Conversion (RT-QuIC)	Uses incubation and shaking to recapitulate and accelerate prion replication *in vitro* using recombinant PrP^{C} substrate	Ultra-sensitive for detecting low levels of sCJD PrP^{Sc}	Less able to detect vCJD PrP^{Sc}	32-34
5. Protein Misfolded Cyclic Amplification (PMCA)	Uses incubation and sonication to recapitulate and accelerate prion replication *in vitro* using brain PrP^{C} substrate	Ultra-sensitive for detecting low levels of vCJD PrP^{Sc}	Less sensitive for sCJD PrP^{Sc} in our hands	30 ,31

Reference List

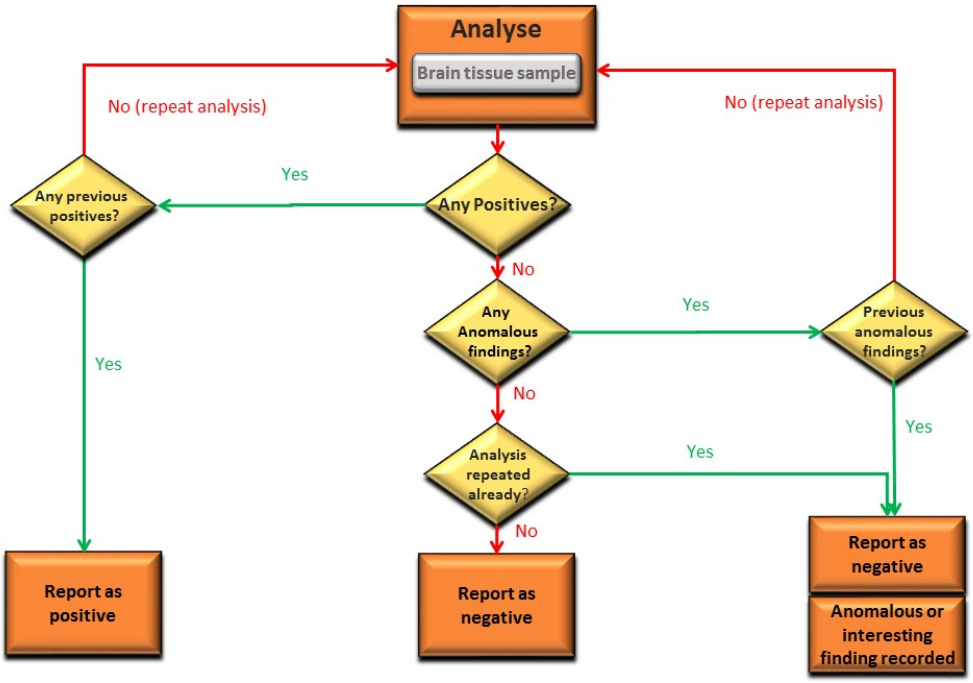
1. Peden AH, Head MW, Ironside JW. Risk of Transmission of Creutzfeldt-Jakob Disease by Blood Transfusion. In: Zou WQ, Gambetti P, eds. Prions and diseases. New York: Springer 2013:121-38.
2. Peden A, McCardle L, Head MW, et al. Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia* 2010;16(2):296-304.
3. Creutzfeldt-Jakob Surveillance in the UK: 26th Annual Report 2017, 2017.
4. Mok T, Jaunmuktane Z, Joiner S, et al. Variant Creutzfeldt-Jakob Disease in a Patient with Heterozygosity at PRNP Codon 129. *N Engl J Med* 2017;376(3):292-94. doi: 10.1056/NEJMc1610003
5. Gill ON, Spencer Y, Richard-Loendt A, et al. Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: large scale survey. *BMJ* 2013;347:f5675.
6. Clewley JP, Kelly CM, Andrews N, et al. Prevalence of disease related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey. *British Medical Journal* 2009;338
7. Hilton DA, Ghani AC, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004;203(3):733-39.
8. d'Aignaux JN, Cousens SN, Smith PG. Predictability of the UK variant Creutzfeldt-Jakob disease epidemic. *Science* 2001;294(5547):1729-31.
9. Ironside JW. Variant Creutzfeldt-Jakob disease: risk of transmission by blood transfusion and blood therapies. *Haemophilia* 2006;12 Suppl 1:8-15.
10. Collie DA, Summers DM, Sellar RJ, et al. Diagnosing variant Creutzfeldt-Jakob disease with the pulvinar sign: MR imaging findings in 86 neuropathologically confirmed cases. *AJNR Am J Neuroradiol* 2003;24(8):1560-9.
11. Prince M, Knapp, M, Guerchet, M, McCrone, P, Prina, M, Comas-Herrera, A, Wittenberg, R, Adelaja, B, Hu, B, King, D, Rehill, A and Salimkumar, D. Dementia UK: Update, 2014.
12. Bonda DJ, Manjila S, Mehndiratta P, et al. Human prion diseases: surgical lessons learned from iatrogenic prion transmission. *Neurosurg Focus* 2016;41(1):E10. doi: 10.3171/2016.5.FOCUS15126
13. About the UK Brain Banks Network - Research - Medical Research Council [Available from: https://www.mrc.ac.uk/research/facilities-and-resources-for-researchers/brain-banks/about-the-uk-brain-banks-network/ accessed 22/08/2017.
14. Samarasekera N, Al-Shahi Salman R, Huitinga I, et al. Brain banking for neurological disorders. *Lancet Neurol* 2013;12(11):1096-105. doi: 10.1016/S1474-4422(13)70202-3
15. Alzheimer Scotland [Available from: http://www.alzscot.org/ accessed 22/08/2017.
16. Scottish MND Register [Available from: http://www.mndscotland.org.uk/research/research-we-fund/scottish-mnd-register/ accessed 22/08/2017.
17. Samarasekera N, Lerpiniere C, Fonville AF, et al. Consent for Brain Tissue Donation after Intracerebral Haemorrhage: A Community-Based Study. *PLoS One* 2015;10(8):e0135043. doi: 10.1371/journal.pone.0135043
18. Millar T, Walker R, Arango JC, et al. Tissue and organ donation for research in forensic pathology: the MRC Sudden Death Brain and Tissue Bank. *J Pathol* 2007;213(4):369-75. doi: 10.1002/path.2247
19. Kovacs GG. Practical approach to diagnosis: sampling and basic stains. In: Kovacs GG, ed. Neuropathology of neurodegerative diseases: a practical guide. Cambridge: Cambridge University Press 2015:55-67.
20. Minimise transmission risk of CJD and vCJD in healthcare settings 2012 [Available from: https://www.gov.uk/government/publications/guidance-from-the-acdp-tse-risk-management-subgroup-formerly-tse-working-group accessed 3/5/19.

21. Ironside JW. Ethical, health and safety considerations. Neuropathology of Neurodegerative diseases: a practical guide. Cambridge, UK: Cambridge University Press 2015:51-54.
22. Ritchie DL, Barria MA, Peden AH, et al. UK Iatrogenic Creutzfeldt-Jakob disease: investigating human prion transmission across genotypic barriers using human tissue-based and molecular approaches. *Acta Neuropathol* 2017;133(4):579-95. doi: 10.1007/s00401-016-1638-x
23. Head MW, Yull HM, Ritchie DL, et al. Variably protease-sensitive prionopathy in the UK: a retrospective review 1991-2008. *Brain* 2013;136(Pt 4):1102-15. doi: aws366 [pii];10.1093/brain/aws366 [doi]
24. Parchi P, Strammiello R, Notari S, et al. Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrPSc types: an updated classification. *Acta Neuropathol* 2009;118(5):659-71. doi: 10.1007/s00401-009-0585-1
25. Glatzel M, Abela E, Maissen M, et al. Extraneural pathologic prion protein in sporadic Creutzfeldt-Jakob disease. *N Engl J Med* 2003;349(19):1812-20.
26. Wadsworth JD, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001;358(9277):171-80.
27. Peden AH, Sarode DP, Mulholland CR, et al. The prion protein protease sensitivity, stability and seeding activity in variably protease sensitive prionopathy brain tissue suggests molecular overlaps with sporadic Creutzfeldt-Jakob disease. *Acta Neuropathol Commun* 2014;2:152. doi: 10.1186/s40478-014-0152-4
28. Safar JG, Geschwind MD, Deering C, et al. Diagnosis of human prion disease. *Proc Natl Acad Sci U S A* 2005;102(9):3501-6. doi: 10.1073/pnas.0409651102
29. Bellon A, Seyfert-Brandt W, Lang W, et al. Improved conformation-dependent immunoassay: suitability for human prion detection with enhanced sensitivity. *J Gen Virol* 2003;84(Pt 7):1921-25.
30. Barria MA, Balachandran A, Morita M, et al. Molecular barriers to zoonotic transmission of prions. *Emerg Infect Dis* 2014;20(1):88-97. doi: 10.3201/eid2001.130858 [doi]
31. Castilla J, Saa P, Morales R, et al. Protein misfolding cyclic amplification for diagnosis and prion propagation studies. *Methods Enzymol* 2006;412:3-21.
32. Peden AH, McGuire LI, Appleford NE, et al. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. *J Gen Virol* 2012;93(Pt 2):438-49. doi: 10.1099/vir.0.033365-0
33. Atarashi R, Moore RA, Sim VL, et al. Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. *Nat Methods* 2007;4(8):645-50.
34. Wilham JM, Orru CD, Bessen RA, et al. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog* 2010;6(12):e1001217.
35. Green AJE. RT-QuIC: a new test for sporadic CJD. *Pract Neurol* 2019;19(1):49-55. doi: 10.1136/practneurol-2018-001935
36. Kobayashi A, Teruya K, Matsuura Y, et al. The influence of PRNP polymorphisms on human prion disease susceptibility: an update. *Acta Neuropathol* 2015;130(2):159-70. doi: 10.1007/s00401-015-1447-7
37. Bishop MT, Pennington C, Heath CA, et al. PRNP variation in UK sporadic and variant Creutzfeldt Jakob disease highlights genetic risk factors and a novel non-synonymous polymorphism. *BMC Med Genet* 2009;10:146. doi: 10.1186/1471-2350-10-146
38. National CJD Research & Surveillance Unit [Available from: http://www.cjd.ed.ac.uk/ accessed 22/08/2017.



An overview of processes put in place including neuropathological screening

190x275mm (300 x 300 DPI)



Algorithm for assessing the results of biochemical analyses

BMJ Open

Study protocol for enhanced CJD surveillance in the 65+ population group in Scotland: an observational neuropathological screening study of banked brain tissue donations for evidence of prion disease

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-033744.R1
Article Type:	Protocol
Date Submitted by the Author:	10-Sep-2019
Complete List of Authors:	Peden, Alexander; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences Kanguru, Lovney; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences Ritchie, Diane; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences Smith, Colin; National CJD Research and Surveillance Unit, National CJD Research and Surveillance Unit Molesworth, Anna; National CJD Research and Surveillance Unit, Centre for Clinical Brain Sciences
Primary Subject Heading:	Public health
Secondary Subject Heading:	Pathology, Infectious diseases, Epidemiology, Neurology
Keywords:	prion, vCJD, brain, Neuropathology < NEUROLOGY, screening, surveillance

Study protocol for enhanced CJD surveillance in the 65+ population group in Scotland: an observational neuropathological screening study of banked brain tissue donations for evidence of prion disease

Alexander H Peden[1*], Lovney Kanguru[1], Diane Ritchie[1], Colin Smith[1,2], Anna Molesworth[1]

Affiliation

1. National CJD Research & Surveillance Unit (NCJDRSU), Centre for Clinical Brain Sciences, University of Edinburgh, Western General Hospital, Edinburgh
2. Edinburgh Brain Bank (EBB), Centre for Clinical Brain Sciences, University of Edinburgh, Chancellor's Building, 49 Little France Crescent, Edinburgh

***Corresponding author**

Alexander H Peden - a.peden@ed.ac.uk, 0131 537 1980

Word count

Abstract = 272

Manuscript = 2999 (excluding title page, abstract, references, figures and tables)

ABSTRACT

Introduction:

Creutzfeldt-Jakob disease (CJD) is a human prion disease that occurs in sporadic, genetic and acquired forms. Variant CJD (vCJD) is an acquired form first identified in 1996 in the United Kingdom (UK). To date 178 cases of vCJD have been reported in the UK, most of which have been associated with dietary exposure to the bovine spongiform encephalopathy agent. Most vCJD cases have a young age of onset, with a median age at death of 28 years. In the UK, suspected cases of vCJD are reported to the UK National Creutzfeldt-Jakob Disease Research & Surveillance Unit (NCJDRSU). There is, however, a concern that the national surveillance system might be missing some cases of vCJD or other forms of human prion disease, particularly in the older population, perhaps because of atypical clinical presentation. This study aims to establish whether there is unrecognised prion disease in people aged 65 years and above in the Scottish population by screening banked brain tissue donated to the Edinburgh Brain Bank.

Methods:

Neuropathological screening of prospective and retrospective brain tissue samples are performed. This involves histopathological and immunohistochemical analysis and prion protein (PrP) biochemical analysis. During the study, descriptive statistics are used to describe the study population including the demographics, clinical, pathological and referral characteristics. Controlling for confounders, univariate and multivariate analyses will be used to compare select characteristics of newly identified suspect cases with previously confirmed cases referred to the NCJDRSU.

Ethics and dissemination:

Brain tissue donations to EBB are made voluntarily by the relatives of patients, with consent for use-in-research. The EBB has ethical approval to provide tissue samples to research projects (REC

reference 16/ES/0084). The findings of this study will be disseminated in meetings, conferences, workshops and as peer reviewed publications.

Registration details:

Edinburgh Brain Bank (Clinicaltrials.gov identifier: 10/S1402/69; 10/S1402/70)

KEYWORDS

prion, vCJD, brain, neuropathology, screening, surveillance

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This study could provide valuable information on the possibility of unascertained prion disease occurring in the over 65 year age group.
- The study includes five biochemical analysis methods, which are used in research for the detection of the abnormal misfolded prion protein (PrP^{Sc}) associated with prion diseases.
- Two of these biochemical methods (Western blotting and RT-QuIC) are routinely used in the diagnosis of prion disease at NCJDRSU.
- The other three methods have been used in research, but have not been used routinely as tools for prion disease diagnosis or surveillance.
- This study is restricted to the Scottish population, but the approaches used may be applicable to UK-wide enhanced CJD surveillance in the 65+ population group, in the future.

INTRODUCTION

Human prion diseases are rare, invariably fatal neurodegenerative diseases associated with an abnormal misfolded form of the prion protein, designated PrP^{Sc}. The most common human prion disease is Creutzfeldt–Jakob disease (CJD), which is mainly idiopathic in origin, occurring sporadically worldwide at a rate of 1 to 2 cases per million population per year. A variant form (vCJD) is associated with dietary exposure to bovine spongiform encephalopathy (BSE), although person-to-person transmission of vCJD infection has also occurred through both blood and possibly blood products. [1,2] In contrast to the sporadic form (sporadic CJD, or sCJD) which affects individuals mainly in the seventh decade of life, the median age at onset for vCJD in the UK is 26.5 years and the median age at death 28 years.[3] To date, 178 cases of vCJD have been reported in the United Kingdom (UK) with the first cases reported in 1996 and the most recent death occurring in 2016.[4] However, prevalence studies indicate that 1 in 2000 people in the UK may be subclinical carriers of vCJD infection.[5-7] Therefore, it is possible that future cases of vCJD may occur.[4,5,8,9]

The national surveillance system for CJD in the UK has comprehensive mechanisms in place for the ascertainment of prion disease.[3] However, it is possible that the national surveillance system could be missing some vCJD cases, particularly in older age groups, perhaps because the clinical presentation in these individuals is atypical of vCJD. For example, age-related changes in the brain may mask the magnetic resonance imaging (MRI) signal and characteristic pathology that supports the diagnosis of vCJD.[10] There is also the potential that typical cases of vCJD may simply not be recognised as such in older individuals, because vCJD patients are typically much younger. Furthermore, dementia is also relatively common among people aged 65 years and above[11] and a diagnosis of vCJD may be more difficult to recognise, or may not be considered, if the patient has been referred to non-neurology medical specialities that are less familiar with prion disease. A similar situation may also exist for sporadic CJD (sCJD), which in the UK currently occurs at a rate of 5 to 6 cases per million of the population aged 65 years and above, with mortality peaking in the 65 to 79 age group and then rapidly

declining.[3] The reasons for this rapid decline are unclear, but may, in part, be linked to under ascertainment of cases in the elderly, rather than the absence of disease.[3]

In order to enable robust and accurate clinical and epidemiological surveillance of CJD and to help protect public health from the potential iatrogenic transmission of CJD,[12] the identification and investigation of CJD cases across all age-groups is essential. This study aims to screen banked brain tissue donations for evidence of otherwise unrecognised prion disease (including vCJD and sCJD) in the 65+ age group. Specifically the study aims:

- To undertake in-depth histopathological, immunohistochemical, PrP biochemical and molecular subtype (*PRNP* codon 129) screening,
- To describe the range of clinical and pathological characteristics associated with prionopathy, in life (alternative) diagnoses and referral characteristics of any "missed" cases identified in this screen.

METHODS

Study design and population

This study aims to determine if there is otherwise unrecognised prion disease (including vCJD, sCJD and other forms of prionopathy) in the Scottish population. The approach taken for this part of the study involves neuropathological screening of prospective and retrospective brain tissue donations, donated to the Edinburgh Brain Bank (EBB) from donors in the 65+ age group from throughout Scotland.[13,14] The testing methods applied include histopathological, immunohistochemical and PrP biochemical analysis, and genotyping polymorphic codon 129 of the prion protein gene (*PRNP)*.

Case inclusion definition

All brain tissue donations to the EBB from people aged 65 years and above are eligible for inclusion in the study. Donated tissue is excluded only if there is insufficient quantity for planned laboratory investigations. The number of eligible donations received at EBB is currently estimated at 30 each year. In addition, there are approximately 175 donations already banked at EBB from 2005 (referred to as retrospective samples), which are eligible for screening in this study.

Outcome

Our primary outcome of interest is evidence of prion pathology, which includes the presence of abnormal prion protein PrP^{Sc} in brain tissue following brain tissue testing. We are interested in the associated clinical, pathological and referral characteristics, and in life (alternative) diagnosis of any cases detected in this way.

Source of samples

The EBB is part of the UK population wide Brain Bank Network, providing high quality post-mortem materials for diagnosis and research into disorders of the brain and nervous system. EBB was established in 2005, and receives donations from a number of national and local research studies in Scotland.[13,14] Currently, this includes donations made through the NCJDRSU 65+ enhanced clinical

surveillance study, Alzheimer Scotland,[15] Edinburgh Procurator Fiscal, Lothian Birth Cohort 1936, the Scottish Motor Neurone Disease (MND) Register,[16] the Lothian study of IntraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN)[17] and the Multiple Sclerosis Society Tissue bank (Table 1). These form a highly select patient group with a set of neurodegenerative (non-CJD) conditions amongst which a "missed" diagnosis of prionopathy might be found. An overview of the protocol put in place including neuropathological screening of brain tissue donations is shown in Figure 1.

Donations to EBB

All donations made to the EBB are handled by a team comprising a neuropathologist(s), research nurse, laboratory technicians and a laboratory manager.[18] Neuropathologists provide cellular and molecular diagnoses from post-mortem examinations. The research nurse is responsible for obtaining authorisation for a post-mortem examination and use of brain tissue for research purposes from the families of donors. The research nurse liaises with donor families and funeral directors throughout the whole process. The laboratory technicians are responsible for collecting and storing the tissue samples and checking their quality. The laboratory manager ensures the smooth running of the laboratory, including appropriate governance on tissue sample requests from researchers in the UK and internationally.

Sample identification and preparation at EBB

Once a tissue donation is made to the EBB, staff check its eligibility for inclusion into our study. Eligible donations are flagged, and the study team at NCJDRSU is informed. For all donations made there is a standard protocol for tissue sampling that is applied during the post-mortem examination.[19] Firstly, the brain is removed and cut into coronal slices. These individual brain slices are further sub-sampled to provide a small tissue block from a wide range of specified brain regions. Each block of tissue is divided into two, with one sample immersed in formalin fixative and processed into a paraffin embedded (FFPE) tissue block, with the second sample frozen in liquid nitrogen vapour and stored at

-80◦C. Both frozen and fixed samples are stored within the EBB and made accessible for further neuropathological and biochemical investigations. From the FFPE tissue blocks, 5µm thin tissue sections are cut and mounted onto glass slides (referred to as fixed tissue), and analysed by microscopy following haematoxylin and eosin (H&E) staining or immunohistochemical probing with specific antibodies. The frozen tissue is subjected to biochemical and genotypic analyses. For our study, EBB provides both fixed and frozen tissue samples from each of the four cortical regions (frontal, temporal, occipital and parietal) as well as the thalamus and cerebellum, if available.

Sample transportation

The frozen and fixed tissue samples are anonymised before being transported to NCJDRSU, and accompanied in transit by a study tissue form containing a unique EBB donation identifier number. For the fixed tissue, no specific precautions are necessary for transportation. However, these samples are packaged appropriately in microscope slide boxes to prevent damage in transit. Frozen tissue is packaged together with dry ice in accordance with the regulations for road transport of Category B (UN3373) tissue specimens. Both frozen and fixed samples are delivered to NCJDRSU in person by the EBB laboratory manager.

Processing of samples at NCJDRSU

Due to the infectious nature of prion diseases, all personnel handling frozen tissue samples within the NCJDRSU laboratory, are required to do so in accordance with NCJDRSU Category 3 laboratory health and safety policies and national regulations.[20,21] Both frozen and fixed samples are delivered to the NCJDRSU category 3 containment laboratory,[20] where they are registered electronically and tracked within the unit using the same unique EBB donation identifier number as above. The frozen samples are stored immediately in a designated -80°C freezer, while the fixed samples are stored at room temperature in the laboratory.

Histopathology testing

For all prospective and retrospective samples, laboratory technicians at EBB conduct a standard suite of histopathological screening on the fixed tissue from all six brain regions mentioned above for the identification of pathological changes associated with common neurodegenerative diseases, including screening for spongiform change, astrogliosis, neuronal loss and plaque formation. This standard suite includes basic immunohistochemical analysis using a panel of antibodies against neurodegenerative proteins: anti-Aβ40, anti-Aβ42, anti-α-synuclein, anti-phospho-tau, anti-phospho-TDP-43 (transactive-response DNA-binding protein 43) and anti-p62.

Immunohistochemically testing for PrP

Additional immunohistochemically testing for the prion protein (PrP) in the fixed tissues are performed at NCJDRSU using two anti-PrP monoclonal antibodies: 12F10, which recognises the PrP epitope 142-160 (Bioquote Ltd, York, UK), and KG9, which recognises the PrP epitope 140-160 (TSE Resource Centre, Roslin Institute, UK). Both are used in combination with the highly sensitive Novolink™ Polymer Detection System.[22] PrP immunohistochemistry is routinely carried out on fixed tissue sections on just two of the six brain regions, namely frontal cortex and cerebellum. Subsequent analysis on the thalamus and the remaining three cortical regions (temporal, occipital and parietal) is conducted if the cases are flagged to be of interest following their histopathological and/or biochemistry investigations for prion disease.

PrP biochemical analysis

For all prospective samples, this investigation requires approximately 2-3 grams of frozen tissue each from the frontal, temporal, occipital and parietal cortical regions as well as the thalamus and cerebellum, whereas for retrospective samples, only the frontal cortex and cerebellum are analysed. We use a panel of biochemical analysis methods (Table 2), which are designed to maximise the potential for detecting low levels of prion disease PrP^{Sc}. These include:

i) Standard diagnostic Western blot (WB) for the protease-resistant core of PrP^{Sc} (PrP^{res})[22,23] with samples prepared according to the method of Parchi et al.[24]

ii) High sensitivity sodium phosphotungstic acid precipitation (NaPTA), followed by WB for PrP^{res}. [2,25,26]

iii) Conformation dependent immunoassay (CDI) analysis for PrP^{Sc}.[27] This method is highly sensitive and is able to detect both protease resistant and protease sensitive forms of PrP^{Sc} [28,29]

iv) Single round protein misfolding cyclic amplification (PMCA) for ultra-sensitive vCJD PrP^{Sc} detection [30,31]

v) Real-time quaking induced conversion (RT-QuIC) for ultra-sensitive sCJD PrP^{Sc} detection[32-34]

Sensitivity of the PrP biochemical analysis methods

Western blot is a well-established diagnostic method used in prion disease research and surveillance, but has limited sensitivity. This technique is also limited to the detection of the protease resistant form of the misfolded PrP. It may therefore be less able to detect new or atypical prion disease subtypes if a significant component of PrP^{Sc} is protease sensitive.[23] The other four biochemical analysis methods (NaPTA, CDI, PMCA, RT-QuIC) used have higher sensitivities for detecting PrP^{Sc}, and RT-QuIC detection of prion seeding activity in cerebrospinal fluid is used in the UK to assist the clinical diagnosis of CJD patients.[35] However, the effectiveness of the four tests other than western blotting as methods for brain tissue sample screening is yet to be fully established. Therefore, when using this panel of biochemical analysis methods, careful consideration is given to the process used to assign positive results and to assess anomalous findings. Accordingly, we have developed an algorithm for each test that is used to facilitate classification of cases as “negative” or “negative – anomalous” or “positive” as shown in Figure 2.

Genotyping

PRNP codon 129 genotyping is performed using a sample of frontal cortex tissue for all cases in this study, except for 65+ study patients (see Table) where the codon 129 genotype may already be known from a previous analysis of blood. The methionine(M)/valine(V) polymorphism at *PRNP* codon 129

affects prion disease clinicopathological phenotype and susceptibility to prion disease at the population level.[36] *PRNP* codon 129 genotyping is essential for classifying the different forms of prion disease. The process of genotyping involves extracting DNA from the frozen brain tissue samples (20-30mg). Thereafter, *PRNP* codon 129 genotype analysis is performed by polymerase chain reaction and restriction fragment length polymorphism analysis.[37]

Data management

All staff at NCJDRSU have a duty to maintain patient confidentiality, and procedures and relevant training are in place for data safeguarding. The University of Edinburgh has records management and information security policies, procedures and guidance on the handling of confidential information. In addition, NCJDRSU has comprehensive information governance procedures to ensure data security and protection.

All samples received from EBB (fixed and frozen) are de-identified by EBB staff, in line with EBB ethical approval prior to sharing with NCJDRSU. Samples are accompanied by a limited set of data only: The study requests the gender of the patient, their year of birth, age at death and post-mortem information such as brain weight, pH and the time between death and post-mortem. All the results are documented and recorded in the study database at NCJDRSU. Paper records are filed securely at NCJDRSU in locked filing cabinets when not in use. Electronic records are processed in a password-protected controlled secured network with access restricted to named users on a need-to-know basis. At no point in time is personal information disclosed to anybody other than the named-users; linkage of records for study analyses, and for follow-up is restricted to authorised personnel by use of a unique study number.

Action for positive cases

The outcome of investigations is shared between the NCJDRSU and EBB study teams as part of the investigation record. If there is evidence of vCJD, sCJD or other prion pathology, then further

investigations are undertaken according to standard NCJDRSU surveillance procedures.[38] A final diagnostic report would be submitted to the senior clinician, then sent to the donor's general practitioner for communication to the family.

Quality assurance

For quality assurance, and to test the sensitivity and specificity of the protocol, a blinded analysis is conducted in conjunction with the analysis of samples from EBB. Under the direction of the principle investigator, and in strict accordance with NCJDRSU Category 3* laboratory health and safety policies, the blinded approach is undertaken as follows. A panel of human prion disease cases is used as positive controls. This panel includes vCJD cases, a range of sCJD subtypes, and rarer forms such as variably protease sensitive prionopathy (VPSPr) to test the ability of the protocol to detect a range of prion disease subtypes, characterised by varying levels and isotypes of PrP^{Sc}.

The positive samples are anonymised and packaged in identical manner to the ordinary study test samples, by the EBB and the NCJDRSU laboratory managers. True data for the positive cases is not attached to the samples because it could lead to identification of the sample prior to testing. Instead, the positive samples are assigned dummy data, which is linked to their true identifiers using a coded key only known to the EBB and NCJDRSU laboratory managers who are responsible for the blinding process. Researchers conducting the analyses will not know which samples are positive or negative until the end of the planned analysis when the identities will be revealed. All results will be recorded in the study database.

Disposal of samples

All residual tissue samples are retained until the end of the study, after which NCJDRSU will handle the disposal of any remaining samples in accordance with the EBB procedures. Samples from cases that are suspected to be CJD or any other prionopathy are retained routinely in the Brain and Tissue

Bank at NCJDRSU. Any residual tissue or material linked to the positive samples (i.e. frozen tissue samples, homogenates, microscope slides, DNA) are destroyed or returned storage at NCJDRSU.

Statistical Analysis

Any case with pathological evidence of prion disease which, prior to this study, was not considered to have prion disease, is referred to as a "missed" case of prion disease. Descriptive statistics including frequency tables, cross-tabulations and graphics will be used to describe the demographics of the study population including the date of death, age, sex and provenance of the donation. Clinical and pathological characteristics of the missed cases with attention to presenting features and in life (alternative) diagnoses will also be described. In addition, description of case classification (molecular subtype) and referral characteristics will be included. Univariate and multivariable analysis adjusting for potential confounders such as age and sex will be used to compare characteristics of missed cases with previously confirmed cases referred to NCJDRSU.

Ethics and approvals

Brain tissue donations are made voluntarily by the relatives of those involved, with consent for use in research. EBB has ethical approval to provide tissue samples to research projects (REC reference 16/ES/0084), including those for pilot studies. Findings of this study will be disseminated in meetings, conferences and as peer reviewed publications.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination of our research.

Acknowledgements

We thank Tracy Millar and Chris-Anne Mckenzie, both of EBB, for their assistance in preparing this manuscript. We would like to express our gratitude to Suzanne Lowrie and Helen Yull at NCJDRSU, and

local mortuary and laboratory staff for their help in managing and undertaking tissue investigations, as well as the patients and their relatives.

Authors' contributions

AM and CS designed the study, in consultation with AHP and DR, and together with AHP and LK drafted the manuscript.

Funding statement

This work is independent research commissioned and funded by the Department of Health and Social Care Policy Research Programme (PR-ST-1214-10002). The views expressed in this publication are those of the authors and not necessarily those of the Department of Health and Social Care.

Competing interests

None declared

Figures

Figure 1: An overview of processes put in place including neuropathological screening

Figure 2: Algorithm for assessing the results of biochemical analyses

Tables

Table 1: Sources of donations to EBB

Source	Description
65+ Study	Includes donations from participants who are 65 years and older across Edinburgh and NHS Lothian including the Ann Rowling Clinic, Old Age Psychiatry, Medicine of the Elderly and Neurology services, with atypical features of dementia
Alzheimer's Scotland	Includes donations from adults diagnosed with dementia in Scotland
Edinburgh Procurator Fiscal	Includes donations from sudden or accidental death investigated by Procurator Fiscal in Scotland
Lothian Birth Cohort 1936	Includes donations from participants born in 1936 in Lothian
Motor Neurone Disease Register	Includes donations from patients with Motor Neurone Disease in Scotland
LINCHPIN - Lothian IntraCerebral Haemorrhage Pathology Imaging and Neurological outcome	Includes donations from adults in Lothian diagnosed with intracerebral haemorrhage after 1st JUNE 2010
Multiple Sclerosis Society Tissue Bank	Includes donations from patients with Multiple Sclerosis in Scotland

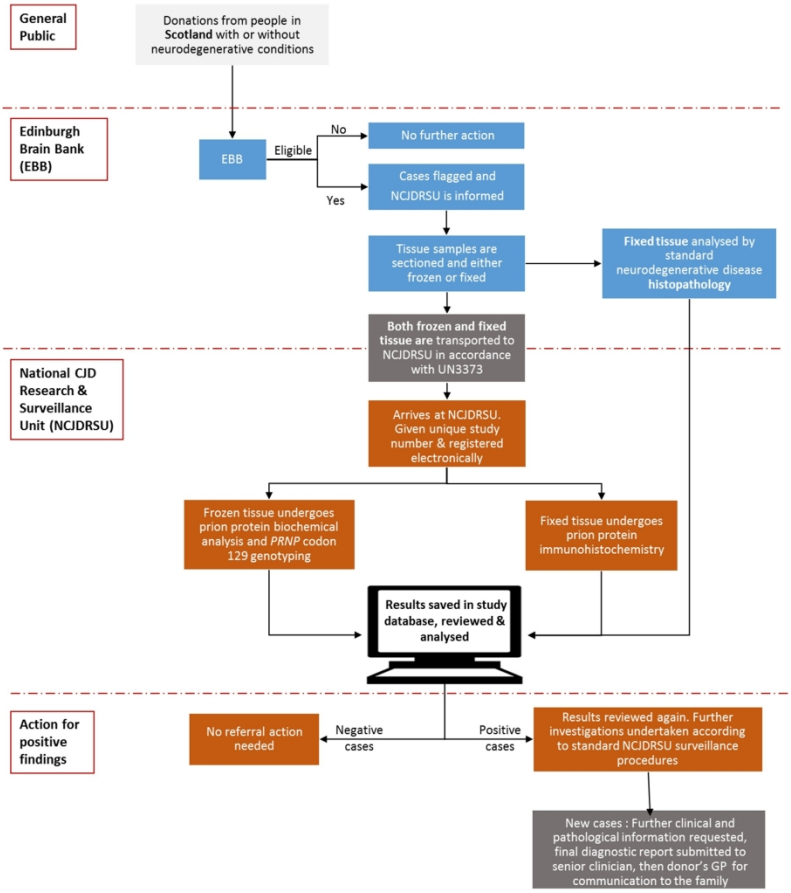
Table 2: Biochemical analysis methods

Method	Function of Test	Advantages	Disadvantages	References
1. Western blot (WB)	Detection of protease-resistant PrP^{Sc}	Standard method used in the diagnosis of prion diseases	Relatively low analytical sensitivity	22-24
2. Sodium phosphotungstic acid (NaPTA) precipitation/ western blotting	Concentration and detection of protease-resistant PrP^{Sc}	Can detect low levels of PrP^{Sc} e.g. in vCJD spleen and sCJD muscle	Not tested for use in routine diagnostics or screening	2 ,25 ,26
3. Conformation dependent immunoassay (CDI)	Detection of PrP^{Sc} based on concealed epitopes that are exposed when PrP^{Sc} is denatured.	Can detect protease sensitive forms of PrP^{Sc}	Not tested for use in routine diagnostics or screening	28 ,29
4. Real-time Quaking induced Conversion (RT-QuIC)	Uses incubation and shaking to recapitulate and accelerate prion replication *in vitro* using recombinant PrP^{C} substrate	Ultra-sensitive for detecting low levels of sCJD PrP^{Sc}	Less able to detect vCJD PrP^{Sc}	32-34
5. Protein Misfolded Cyclic Amplification (PMCA)	Uses incubation and sonication to recapitulate and accelerate prion replication *in vitro* using brain PrP^{C} substrate	Ultra-sensitive for detecting low levels of vCJD PrP^{Sc}	Less sensitive for sCJD PrP^{Sc} in our hands	30 ,31

Reference List

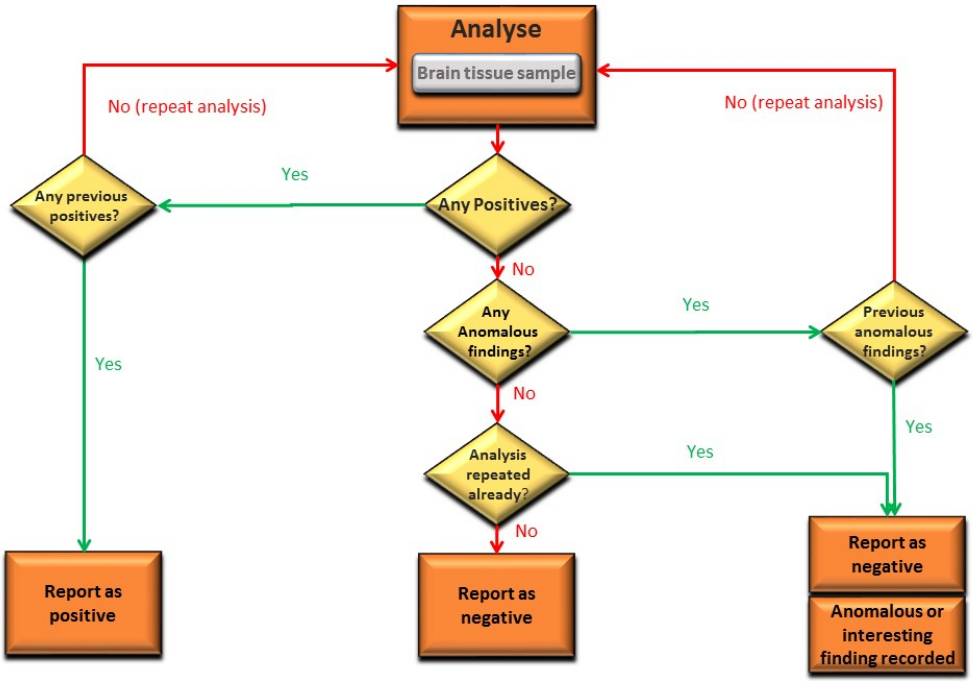
1. Peden AH, Head MW, Ironside JW. Risk of Transmission of Creutzfeldt-Jakob Disease by Blood Transfusion. In: Zou WQ, Gambetti P, eds. Prions and diseases. New York: Springer 2013:121-38.
2. Peden A, McCardle L, Head MW, et al. Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. *Haemophilia* 2010;16(2):296-304.
3. Creutzfeldt-Jakob Surveillance in the UK: 26th Annual Report 2017, 2017.
4. Mok T, Jaunmuktane Z, Joiner S, et al. Variant Creutzfeldt-Jakob Disease in a Patient with Heterozygosity at PRNP Codon 129. *N Engl J Med* 2017;376(3):292-94. doi: 10.1056/NEJMc1610003
5. Gill ON, Spencer Y, Richard-Loendt A, et al. Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: large scale survey. *BMJ* 2013;347:f5675.
6. Clewley JP, Kelly CM, Andrews N, et al. Prevalence of disease related prion protein in anonymous tonsil specimens in Britain: cross sectional opportunistic survey. *British Medical Journal* 2009;338
7. Hilton DA, Ghani AC, Conyers L, et al. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol* 2004;203(3):733-39.
8. d'Aignaux JN, Cousens SN, Smith PG. Predictability of the UK variant Creutzfeldt-Jakob disease epidemic. *Science* 2001;294(5547):1729-31.
9. Ironside JW. Variant Creutzfeldt-Jakob disease: risk of transmission by blood transfusion and blood therapies. *Haemophilia* 2006;12 Suppl 1:8-15.
10. Collie DA, Summers DM, Sellar RJ, et al. Diagnosing variant Creutzfeldt-Jakob disease with the pulvinar sign: MR imaging findings in 86 neuropathologically confirmed cases. *AJNR Am J Neuroradiol* 2003;24(8):1560-9.
11. Prince M, Knapp, M, Guerchet, M, McCrone, P, Prina, M, Comas-Herrera, A, Wittenberg, R, Adelaja, B, Hu, B, King, D, Rehill, A and Salimkumar, D. Dementia UK: Update, 2014.
12. Bonda DJ, Manjila S, Mehndiratta P, et al. Human prion diseases: surgical lessons learned from iatrogenic prion transmission. *Neurosurg Focus* 2016;41(1):E10. doi: 10.3171/2016.5.FOCUS15126
13. About the UK Brain Banks Network - Research - Medical Research Council [Available from: https://www.mrc.ac.uk/research/facilities-and-resources-for-researchers/brain-banks/about-the-uk-brain-banks-network/ accessed 22/08/2017.
14. Samarasekera N, Al-Shahi Salman R, Huitinga I, et al. Brain banking for neurological disorders. *Lancet Neurol* 2013;12(11):1096-105. doi: 10.1016/S1474-4422(13)70202-3
15. Alzheimer Scotland [Available from: http://www.alzscot.org/ accessed 22/08/2017.
16. Scottish MND Register [Available from: http://www.mndscotland.org.uk/research/research-we-fund/scottish-mnd-register/ accessed 22/08/2017.
17. Samarasekera N, Lerpiniere C, Fonville AF, et al. Consent for Brain Tissue Donation after Intracerebral Haemorrhage: A Community-Based Study. *PLoS One* 2015;10(8):e0135043. doi: 10.1371/journal.pone.0135043
18. Millar T, Walker R, Arango JC, et al. Tissue and organ donation for research in forensic pathology: the MRC Sudden Death Brain and Tissue Bank. *J Pathol* 2007;213(4):369-75. doi: 10.1002/path.2247
19. Kovacs GG. Practical approach to diagnosis: sampling and basic stains. In: Kovacs GG, ed. Neuropathology of neurodegerative diseases: a practical guide. Cambridge: Cambridge University Press 2015:55-67.
20. Minimise transmission risk of CJD and vCJD in healthcare settings 2012 [Available from: https://www.gov.uk/government/publications/guidance-from-the-acdp-tse-risk-management-subgroup-formerly-tse-working-group accessed 3/5/19.

21. Ironside JW. Ethical, health and safety considerations. Neuropathology of Neurodegerative diseases: a practical guide. Cambridge, UK: Cambridge University Press 2015:51-54.
22. Ritchie DL, Barria MA, Peden AH, et al. UK Iatrogenic Creutzfeldt-Jakob disease: investigating human prion transmission across genotypic barriers using human tissue-based and molecular approaches. *Acta Neuropathol* 2017;133(4):579-95. doi: 10.1007/s00401-016-1638-x
23. Head MW, Yull HM, Ritchie DL, et al. Variably protease-sensitive prionopathy in the UK: a retrospective review 1991-2008. *Brain* 2013;136(Pt 4):1102-15. doi: aws366 [pii];10.1093/brain/aws366 [doi]
24. Parchi P, Strammiello R, Notari S, et al. Incidence and spectrum of sporadic Creutzfeldt-Jakob disease variants with mixed phenotype and co-occurrence of PrPSc types: an updated classification. *Acta Neuropathol* 2009;118(5):659-71. doi: 10.1007/s00401-009-0585-1
25. Glatzel M, Abela E, Maissen M, et al. Extraneural pathologic prion protein in sporadic Creutzfeldt-Jakob disease. *N Engl J Med* 2003;349(19):1812-20.
26. Wadsworth JD, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001;358(9277):171-80.
27. Peden AH, Sarode DP, Mulholland CR, et al. The prion protein protease sensitivity, stability and seeding activity in variably protease sensitive prionopathy brain tissue suggests molecular overlaps with sporadic Creutzfeldt-Jakob disease. *Acta Neuropathol Commun* 2014;2:152. doi: 10.1186/s40478-014-0152-4
28. Safar JG, Geschwind MD, Deering C, et al. Diagnosis of human prion disease. *Proc Natl Acad Sci U S A* 2005;102(9):3501-6. doi: 10.1073/pnas.0409651102
29. Bellon A, Seyfert-Brandt W, Lang W, et al. Improved conformation-dependent immunoassay: suitability for human prion detection with enhanced sensitivity. *J Gen Virol* 2003;84(Pt 7):1921-25.
30. Barria MA, Balachandran A, Morita M, et al. Molecular barriers to zoonotic transmission of prions. *Emerg Infect Dis* 2014;20(1):88-97. doi: 10.3201/eid2001.130858 [doi]
31. Castilla J, Saa P, Morales R, et al. Protein misfolding cyclic amplification for diagnosis and prion propagation studies. *Methods Enzymol* 2006;412:3-21.
32. Peden AH, McGuire LI, Appleford NE, et al. Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion. *J Gen Virol* 2012;93(Pt 2):438-49. doi: 10.1099/vir.0.033365-0
33. Atarashi R, Moore RA, Sim VL, et al. Ultrasensitive detection of scrapie prion protein using seeded conversion of recombinant prion protein. *Nat Methods* 2007;4(8):645-50.
34. Wilham JM, Orru CD, Bessen RA, et al. Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. *PLoS Pathog* 2010;6(12):e1001217.
35. Green AJE. RT-QuIC: a new test for sporadic CJD. *Pract Neurol* 2019;19(1):49-55. doi: 10.1136/practneurol-2018-001935
36. Kobayashi A, Teruya K, Matsuura Y, et al. The influence of PRNP polymorphisms on human prion disease susceptibility: an update. *Acta Neuropathol* 2015;130(2):159-70. doi: 10.1007/s00401-015-1447-7
37. Bishop MT, Pennington C, Heath CA, et al. PRNP variation in UK sporadic and variant Creutzfeldt Jakob disease highlights genetic risk factors and a novel non-synonymous polymorphism. *BMC Med Genet* 2009;10:146. doi: 10.1186/1471-2350-10-146
38. National CJD Research & Surveillance Unit [Available from: http://www.cjd.ed.ac.uk/ accessed 22/08/2017.



An overview of processes put in place including neuropathological screening

190x275mm (300 x 300 DPI)



Algorithm for assessing the results of biochemical analyses