National CJD Research & Surveillance Unit
Western General Hospital,
Edinburgh, EH4 2XU

Enhanced CJD surveillance in the older population (Scotland A REC ref: 15/SS/0196, NHS Lothian R&D ref: 2015/0391]
Study Protocol version 3 (30/10/2017)

Study Principal Investigator: Dr Anna Molesworth, PhD
Telephone: 0131 537 1980
Email: anna.molesworth@ed.ac.uk

National CJD Research & Surveillance Unit
Enhanced CJD Surveillance in the Older Population

THE 65+ DEMENTIA STUDY
STUDY PROTOCOL

Version 3.0 (30/10/2017)

Anna Molesworth with (in alphabetical order) Mark Head, Guy Holloway, James Ironside, Richard Knight, Adriana Libori, Gemma Logan, Suvankar Pal, Alexander Peden, Diane Ritchie, Susan Shenkin, Colin Smith, David Summers, Briony Waddell, Helen Yull.

Version	Date	Changes made by:	Reason(s) for change(s)
1.0	08/10/2015	Anna Molesworth (CI)	(first version of new protocol)
2.0	30/05/2017	Gemma Logan	Substantial amendment (1): changes to sections 6.7 B and D to reflect the introduction of a study introduction sheet; sections 6.8 B and 13 to reflect changes to the cognitive assessments completed; and sections 6.7 I, 6.10 L, M and O, 6.13 B and 9 E to reflect the introduction of buccal swab testing.
3.0	30/10/2017	Gemma Logan	Non-substantial amendment: change to section 12 to reflect a two year study extension

CONTENTS

ABBREVIATIONS

1	ACDP	Advisory Committee on Dangerous Pathogens
2	ACE-III	Addenbrooke's cognitive examination
3	AWI	adults with incapacity
4	BRIC	Brain Research Imaging Centre
5	BSE	Bovine Spongiform Encephalopathy
6	CDC-DART	Cognitive Disorders Clinic Diagnosis, Audit, Research & Treatment
7	CDI	conformation dependent immunoassay
8	CJD	Creutzfeldt-Jakob Disease
9	CSF	cerebrospinal fluid
10	DNA	deoxyribonucleic acid
11	DWI	diffusion weighted imaging
12	EMAS	Edinburgh motor assessment scale
13	FAB	frontal assessment battery
14	FLAIR	fluid-attenuated inversion recovery
15	GP	general practitioner
16	HADS	hospital anxiety and depression scale
17	MRC	Medical Research Council
18	MRI	magnetic resonance image
19	NaPTA	sodium phosphotungstic acid precipitation
20	NCJDRSU	National CJD Research & Surveillance Unit
21	NHS	National Health Service
22	PII	personally identifying information
23	PM	post-mortem
24	PMCA	protein misfolding cyclic amplification
25	PrP	prion protein
26	R&D	Research & Development Office
27	REC	Research Ethics Committee
28	RT-QuIC	real-time quaking induced conversion
29	sCJD	sporadic CJD
30	SIB-S	Severe Impairment Battery – Short Form
31	UK	United Kingdom
32	UoE	University of Edinburgh
33	vCJD	variant CJD
34	VPSPr	variably protease sensitive prionopathy
35	WB	western blot

SUMMARY

Background: To date 177 cases of variant Creutzfeldt - Jakob disease (vCJD) have been reported in the United Kingdom (UK), although the prevalence of detectable abnormal prion protein in the general population is estimated at 1 in 2000 people. This apparent inconsistency is poorly understood, but the possibility exists that national surveillance mechanisms could be missing some cases, particularly in older age groups, perhaps because the clinical presentation and progression of disease in these patients is atypical of vCJD or otherwise not recognised as a prion disease. A similar situation may also exist for the sporadic form of CJD (sCJD) and perhaps other forms of prion disease.

Aims: This study seeks determine whether there is otherwise unrecognised CJD (including vCJD, sCJD and other prionopathies) in the older population, and to investigate their clinical and pathological features, risk factors and whether these differ from observed clinical cases. It will do this through neuropathological screening of locally banked brain tissue donations and through the clinical-pathological investigation of patients presenting with atypical features of dementia (aged 65 and above) accessing local psycho-geriatric services. Although focussed on Edinburgh and the Lothians, the study will determine if it is possible to establish routine systems for the diagnosis and surveillance of CJD amongst older patients with atypical features of dementia that could be extended to the rest of the UK.

Research method: The study will involve the neuropathological screening of Edinburgh Brain & Tissue Bank donations from patients in the 65+ age-group for evidence of prion disease. Specimens will undergo standard neurological disease histopathology, prion protein immunocytochemistry, biochemistry and genotyping for evidence of prion disease. The second approach will involve local neurology and psycho-geriatric services. Patients 65+ with atypical features of dementia will be offered clinical examination and review, including magnetic resonance imaging (MRI) investigation, and prion protein genotyping, with an epidemiological risk factor questionnaire also undertaken. Further follow up will be undertaken for all patients to assess progress and post-mortem investigations if a patient dies. Findings will be disseminated through meetings and peer-reviewed publication.

Research team: The National CJD Research & Surveillance Unit (NCJDRSU) is an internationally recognised centre for the diagnosis of human prion disease. The Edinburgh Brain & Tissue Bank is part of the UK Brain Bank Network, providing high quality post-mortem materials for diagnosis and research into disorders of the brain and nervous system. The research team brings together leading members of staff from both institutions, with substantial expertise in prion disease surveillance and clinical and laboratory research in neurology, neuropathology and prion protein biochemistry in relation to dementing illness. Researchers also contribute to local clinic-based neurology services for patients as part of the University of Edinburgh's Division of Clinical Neurosciences. Together with NHS psychogeriatric services the staff diagnose, treat and manage patients with dementia, across southern Scotland.

Potential impact: This study will identify CJD in the study population, and pilot a method by which enhanced methods of case ascertainment can be integrated into routine diagnostic and surveillance practice. In doing so the study will raise local awareness of CJD in older patients, with implications for the diagnosis of atypical dementias, their treatment and care, and, as the older population has a relatively high frequency of medical interventions compared to the young, for informing CJD-specific public health interventions in this age group. The study will also help generate hypothesis on the relationship between prion and other neuro-degenerative diseases that could be tested in other research cohorts and service settings.

Keywords: vCJD, prion disease, dementia, old-age, surveillance, neuropathology, biochemistry, pyschogeriatrics, neurology, brain banking, public health

1. INTRODUCTION

Variant Creutzfeldt-Jakob Disease (vCJD) is a very rare neurodegenerative disease, and one of a group of diseases called prion diseases, all of which are associated with an abnormal form of a naturally occurring protein (the prion protein), the presence of which can be detected in certain body tissues. Most cases of vCJD are associated with dietary exposure to bovine-spongiform encephalopathy (BSE) contaminated meat, although secondary transmission has occurred through blood transfusion and may be possible in the wider medical setting, for instance through surgery.

To date 177 cases of vCJD have been reported in the United Kingdom (UK) in a single epidemic wave between 1994 (the first onset) and the most recent death in 2013 [NCJDRSU 2014]. However, the prevalence of detectable abnormal prion protein in the general population, indicating vCJD carrier status in the absence of disease, is estimated at 1 in 2000 people [Gill et al. 2013]. All cases who have been tested have been methionine homozygous at codon-129 of the prion protein gene, however all codon-129 genotypes are thought to be susceptible to infection and have been identified amongst the asymptomatic carriers of vCJD.

2. RATIONALE

The apparent inconsistency between few observed vCJD cases and the relatively high prevalence of abnormal prion protein in the UK general population is poorly understood. vCJD is a disease of predominantly younger ages (median age at death 28 years) and there have been only three deaths in vCJD patients aged 65 and above. However the possibility exists of future cases of vCJD in people exposed to BSE during the BSE epidemic but with different genotypic susceptibility [d'Aignaux et al. 1999] or as a result of secondary transmission through blood [Hewitt et al. 2006], and these might disproportionately affect the older population. (The asymptomatic carrier state, which has been observed in those born between 1941-1969, includes methionine-valine and valine homozygote genotypes, which may further extend the incubation period. Moreover two of the three vCJD deaths in patients aged 65 and above, acquired their infection through blood transfusion.)

In addition, there is the possibility that national surveillance mechanisms could be missing some cases, particularly in older age groups, perhaps because their clinical presentation is atypical of vCJD and/or not recognised as a prion disease. Age-related changes in the brain may mask the magnetic resonance imaging (MRI) signal and characteristic pathology that supports the diagnosis of vCJD [Collie et al. 2003]. The median duration of illness (several months) can also frequently be much shorter with increasing age, with the result that prion disease may not be considered until late in disease progression, if at all [Pocchiari et al. 2004]. There is also the real possibility that typical cases of disease may not be recognised as such, as evidenced by one referral from the Parkinson's UK Brain Bank, of an older patient whose clinical presentation was in fact typical of vCJD in life but misdiagnosed [el Tawil et al. 2015]. A theoretical possibility also exists, as reported in unconfirmed experiments in cynomolgus monkeys, that vCJD infection at low doses (for example acquired through blood products) might present as an illness distinct from currently recognised human prion diseases [Comoy et al. 2011]. With dementia relatively common in older patients, with about 7% affected in the UK over the age of 65 [Alzheimer's Society, 2014], in older age groups a vCJD diagnosis may be more difficult to recognise, particularly if the patient is referred to non-neurology specialities less familiar with prion disease, and instead lost amongst the dementias of the elderly with which the clinical presentation is most likely to be confused.

A similar situation may also exist for sporadic CJD (sCJD), which is a disease of predominantly older age groups, in the UK occurring at a rate of 5-6 cases annually per million population aged 65+, with median age at death of 68 years, and mortality peaking in the 65-79 year group and then rapidly declining. This decline is puzzling but might be attributed to under-reporting of cases rather than the absence of disease in these age groups [NCJDRSU 2014]. Moreover, the newly described prionopathy, variably protease

sensitive prionopathy (VPSPr) has also recently been reported in the UK, with atypical clinical and pathological characteristics [Head et al. 2013]. It is possible that this presents a very rare phenotype within human prion disease, with a late onset and longer duration, or it may be far more common, both as an undiagnosed cause of dementia [Cannon et al. 2014] and occurring in cognitive normal individuals [Ghoshal et al. 2014].

It is important to identify and investigate cases of CJD across all age-groups, including the older population, to enable robust and accurate clinical and epidemiological surveillance and to help protect public health. The potential gap in CJD surveillance in older people is a concern, and raised as such previously by the Advisory Committee for Dangerous Pathogens and CJD Incidents Panel, and most recently the UK House of Commons Science and Technology Select Committee public inquiry into UK blood safety and the risk of CJD [House of Commons 2014].

3. STUDY DESIGN, CONTEXT & SETTING

This is an epidemiological study piloting a method to determine if there is otherwise unrecognised prion disease in the older UK population. It will do this through two complementary local approaches targeting people in the 65+ age group. The first involves the neuropathological screening of banked brain material from donors to the Edinburgh Brain Bank. The second approach will involve clinical and pathological investigation of patients with atypical features of dementia referred to psychogeriatric services in Edinburgh and the Lothians.

Previously a study such as the one proposed has proved a major challenge because of the large numbers of cases and professionals involved in the investigation of dementia in the older population, with multiple referral pathways for patients and a significant proportion without specialist referral. More recently changes in infrastructure in the UK make this type of enquiry more feasible. In particular, local clinical networks are being developed in which patients with dementia can more easily access diagnostic, management and treatment services. The population wide UK Brain Bank Network has been established and several large cohorts of elderly patients have also come together as part of the UK Dementia Platform.

The study will be conducted in Edinburgh and the Lothians, where there is considerable expertise in prion disease surveillance and clinical and laboratory research in relation to dementing illness. The NCJDRSU is an internationally recognised World Health Organisation reference centre and European Centre for Disease Control hub for diagnosis of all forms of human prion disease and has substantial expertise in prion disease surveillance and clinical and laboratory research in neurology, neuropathology, brain imaging and biochemical investigations in relation to dementing illness (see www.cjd.ed.ac.uk). Staff at NCJDRSU contribute to clinic-based neurology services for patients as part of the University of Edinburgh Division of Clinical Neurosciences, which include neurology outpatient and cognitive clinics centred on the catchment of the two major teaching hospitals Royal Infirmary of Edinburgh and the Western General Hospital. Together with local NHS psychogeriatric services the teams diagnose, treat and manage patients with dementia across southern Scotland, including Edinburgh and the Lothians. The Edinburgh Brain Bank 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. Collaborative links with the Brain Bank Network and MRC Dementia Platform co-investigators also provide a basis to inform this and future research.

4. AIMS & OBJECTIVES

4.1. To pilot a method to determine whether there are cases of otherwise unrecognised CJD (including vCJD, sCJD and other prionopathies, such as VPSPr) in the older population, and if so to describe their clinical and pathological features and risk factors. To assess how these differ from known clinical cases of prion disease. Specifically:

- ☐ To undertake neuropathological screening of brain tissue donated to Edinburgh Brain & Tissue Bank for evidence of prionopathy in the 65+ age group.

- ☐ To undertake clinical and pathological investigation of patients with atypical features of dementia (aged 65+) accessing local psychogeriatric services in the Lothian population.

4.2 To assess the feasibility of employing these methods out-with the study population, in Scotland and the rest of the UK through the relevant clinical and pathology networks, as part of routine systems for the ascertainment of prion disease.

5 NEUROPATHOLOGICAL SCREENING OF BANKED BRAIN TISSUE DONATIONS FOR EVIDENCE OF PRIONOPATHY

5.1 BACKGROUND

In CJD and other forms of prionopathy, post mortem investigations are part of the internationally-agreed diagnostic criteria for CJD. Pathological investigation of brain tissue may detect the underlying changes which are associated with neurodegeneration, including the presence of abnormal prion protein even when someone has no clinical signs of CJD and/or with symptoms of an entirely different disease. Brain banks are a rich source of human brain tissue, providing high quality post-mortem materials for diagnosis and facilitating access to tissue for research into disorders of the brain and nervous system.

5.2 AIMS & OBJECTIVES

This part of the study aims to screen banked brain tissue donations for evidence of otherwise unrecognised prionopathy (including vCJD, sCJD and other forms of prionopathy) in the 65+ age group, with the following objectives:

- ☐ to undertake histopathological, biochemical and molecular subtype screening of brain tissue for evidence of prionopathy, using the full range of techniques available;

- ☐ to describe the range of clinical and pathological characteristics associated with prionopathy, with attention to presenting features, in life (alternative) diagnosis and reasons for non-referral;

- ☐ to assess the utility of this approach and inform standard procedures for the routine screening for prionopathy, which could be extended to other tissue banks and pathology laboratories, and used in surveillance practice. This may include planning for logistical and other problems that might impact extension to the rest of the UK.

It is possible that no such cases will be detected in the study population, however the implications of a positive finding are significant for surveillance and public health. Moreover should this occur this part of the study will help generate hypotheses on the relationship between prion and other neuro-degenerative diseases that could be tested in other cohorts/service settings.

5.3 STUDY DESIGN, POPULATION & SETTING

The Edinburgh Brain & Tissue Bank is part of the UK Brain Bank Network, and receives donations from all over Scotland from people involved in a number of different Scottish national and local research studies [see http://www.mrc.ac.uk/research/facilities/brain-banks/].The study will involve the screening of brain tissue donations, banked with the Edinburgh Brain Bank, from patients in the 65+ age-group. Currently, these mainly include donations made through the Alzheimer Scotland, the Scottish Motor Neurone

Disease (MND) Register and Lothian study of IntraCerebral Haemorrhage Pathology, Imaging and Neurological outcome (LINCHPIN). These form a highly selected patient group with a particular suite of neurodegenerative (non-CJD) conditions amongst which a "missed" diagnosis of prionopathy might be found; the Sudden Death Register will also be included representing controls. Numbers of these donations annually are currently estimated at about 40 of approximately 100 received in total.

The study will include all eligible donations made to the Edinburgh Brain Bank. Numbers therefore reflect the totality of what is available to researchers.

5.4 CASE INCLUSION DEFINITION & ASCERTAINMENT

A. Any donation made to the Edinburgh Brain Bank from an individual aged 65+ at death will be eligible for inclusion in this study. Donations will only be excluded if tissue cannot be made available for use in research, for example for reasons of poor quality.

B. Prior to processing, all donations will be registered as they are received by the Edinburgh Brain Bank and flagged for entry into the study. Each donation will be assigned a unique identifier that will allow the linking of samples to the same individual.

C. The Edinburgh Brain Bank staff will inform NCJDRSU of all newly flagged donations, as they are registered and through regular updates between the NCJDRSU and Edinburgh Brain Bank study teams.

5.5 CONSENT

Brain tissue donations are made voluntarily by the relatives of those involved, with consent for use in research. The Edinburgh Brain & Tissue Bank has ethical approval to provide tissue samples to research projects, including those for pilot studies, based on their scientific merit and whether the proposal is likely to raise ethical issues if peer review and ethics approval has not yet been obtained (REC reference 11/ES/0022).

5.6 INITIAL APPROACH TO THE BRAIN BANK

A. The NCJDRSU lead investigator will approach the Director, Edinburgh Brain Bank requesting the use of tissue in this research.

B. Mechanisms will then be put in place between NCJDRSU and Edinburgh Brain Bank to enable the identification and subsequent testing of tissue, which would normally be coordinated by the respective laboratory managers or team members on their behalf.

5.7 INITIAL PROCESSING (EDINBURGH BRAIN BANK)

Samples are likely to arrive at the Edinburgh Brain Bank every 1-2 weeks and will be registered and flagged as they come in. As soon as a new donation is flagged at the Edinburgh Brain Bank, they will inform the NCJDRSU team of the donation and arrangements will be made for the safe secure transport of tissue between sites.

Tissue samples will then be processed for a range of histopathological and biochemical investigations, which will be conducted at the Edinburgh Brain Bank and at NCJDRSU, as detailed below. Appropriate safety precautions will be employed by all personnel in the handling of samples from the time of tissue sampling, in accordance with local health & safety policy and national regulations.

5.8 SAMPLE PROCESSING & TESTING

A. STANDARD NEUROLOGICAL DISEASE HISTOPATHOLOGY (Edinburgh Brain Bank). As each brain tissue donation is received at the Brain Bank, sections of tissue from each of the four cortical regions (frontal, temporal, occipital, parietal), the thalamus and the cerebellum are cut in the brain bank laboratory, formalin fixed/embedded as paraffin blocks. Each of the six samples will then be subjected to a standard suite of investigations for neurodegenerative disease, using a panel of markers for neurodegenerative proteins but also including screening for spongiform change, gliosis/astrocytosis, neuronal loss and plaque formation.

B. PRION PROTEIN IMMUNOCYTOCHEMISTRY (NCJDRSU). These techniques are more sensitive and complex than standard histopathology. An unstained section will be taken from the frontal cortex and cerebellum from blocks cut at the Edinburgh Brain Bank. Slides from the two regions will then be transported to NCJDRSU for immunostaining and screening for abnormal prion protein. Further analysis on all six regions will be conducted if samples are flagged to be of interest through histopathological and/or biochemical investigations.

C. PRION PROTEIN BIOCHEMISTRY (NCJDRSU). These investigations will require the use of approximately 2-3g of frozen tissue from each of the frontal, temporal, occipital and parietal regions, the thalamus and the cerebellum. Investigations will include the most sensitive abnormal prion protein detection methods currently available, which might offer advantages over routine diagnostic techniques in a presumed negative population. They will be conducted in series on all samples as follows:

   a) Standard diagnostic Western blot (WB) for protease-resistant prion protein (PrP^{res}) [Head et al.2013]

   b) High sensitivity sodium phosphotungstic acid precipitation(NaPTA) WB for PrP^{res} [Peden et al.2010]

   c) Conformation dependent immunoassay (CDI) analysis for PrP^{Sc} [Peden et al.2014]

   d) Single round protein misfolding cyclic amplification (PMCA) for ultra-sensitive vCJD PrP^{Sc} detection [Barria et al 2014] and real-time quaking induced conversion (RT-QuIC) for ultra-sensitive sCJD PrP^{Sc} detection [Peden et al 2012]

D. GENOTYPING (NCJDRSU). The codon-129 genotype (MM, MV,VV) is a risk factor for developing CJD at the population level and may help to classify the different forms of disease, but does not indicate a specific genetic risk for CJD at the individual level. Codon-129 genotyping will therefore be used on the frozen tissue sent for biochemical investigations in order to help assist case classification. Codon-129 genotyping does not require specific consent for genetic analysis.

5.9 SAMPLE TRANSPORT & STORAGE

A. Formalin fixed paraffin embedded tissue blocks (histopathological investigations), and frozen tissue (biochemical investigations) will be tested. The tissue samples will be stored securely at the Edinburgh Brain Bank ready for transport to NCJDRSU. The tissue samples should be anonymised before leaving the Brain Bank, and accompanied by a study tissue form containing the unique donation brain bank identifier.

B. For fixed tissue no specific precautions are necessary for transportation, however the samples will be packaged appropriately to prevent damage to the samples in transit. If frozen tissue is available, this will be stored at -80C until ready for transport to NCJDRSU in accordance with UN3373 procedures for diagnostic specimens (biological material, category B).

C. Once received at the NCJDRSU, the samples will be brought to the laboratory reception area for computer-registration of the sample details. They will be flagged as belonging to the study and assigned a unique study number to enable tracking within the Unit of all samples associated with that tissue referral. The original brain bank identifier will be retained as it allows the linking of samples to the same individuals. The samples will then be stored, ready for processing.

5.10 RESIDUAL TISSUE

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

5.11INFORMATION COLLECTED

A. All donations will be assigned a unique brain-bank identifier, as well as a unique NCJDRSU study number for data management purposes. Within the respective organisations, these numbers enable tracking of all samples associated with that donation and linking of these samples to the same individual in the laboratory, and between relevant study databases. Donations will be identified by use of their unique study number. Under the conditions of Brain Bank approval, patient identifiable information will not be made available to the study team.

B. The sample details and investigation results will be documented and recorded in the study database at NCJDRSU. These will include the outcome of standard neurodegenerative histopathology (e.g.spongiform change/ vacuolation, gliosis/neuronal loss, plaque type) and immunohistochemistry (pattern of PrP^{res} staining), results of biochemical investigations and codon-129 analysis. In this way, evidence of vCJD, sCJD or other prion-associated pathology will also be documented.

C. Basic clinical-pathological and demographic information accompanying the donation, including the date of death, age, sex and provenance of the donation, will be recorded alongside the sample details in the study database at NCJDRSU. Cause of death and final diagnosis can also be made available when investigations have been completed.

D. Study data will be held in electronic and paper format at NCJDRSU. All source data will be checked and validated before electronic data-entry and verification. Paper records will be filed securely at NCJDRSU; electronic data will be held in a study database with sub-tables (see section 7).

E. The outcome of investigations will be shared between the respective NCJDRSU and brain bank study teams as part of the investigation record.

5.12ANALYSIS

A. Results of investigations will be reviewed and a final report would be submitted by the study lead to the Director Edinburgh Brain Bank.

B. If there is evidence of vCJD, sCJD or other prion pathology, then the case would be referred to NCJDRSU as a suspect case and further investigations would then be undertaken according to standard NCJDRSU surveillance procedures (see section 9.). A final diagnostic report would be submitted by the study lead pathologist to the Director Edinburgh Brain Bank. Results would usually then be sent to the local clinician/pathologist, with a copy to the GP for communication to the family, according to standard Brain Bank procedures.

C. Progress in the study will continually be assessed against the study objectives (section10). The final analysis will include a description of clinical-pathological variation associated with positive findings, and an assessment of how this might contribute to local surveillance practice as well as out with the local setting.

6 CLINICAL-PATHOLOGICAL INVESTIGATION OF ATYPICAL PSYCHO-GERIATRIC REFERRALS

6.1 BACKGROUND

The variant form of CJD is characterised clinically by progressive cognitive impairment and additional neuro-psychiatric features including early psychiatric symptoms, ataxia, involuntary movements and painful sensory symptoms. The sporadic form of CJD is clinically distinct from vCJD, and noted by the rapidity of disease progression (median duration 4 months for sCJD, compared to 14 for vCJD), which in many cases may raise the suspicion of CJD. The majority of referrals of suspect CJD cases to the UK national CJD surveillance system are through neurologists and neuropathologists.

Around 850,000 people in the UK suffer from dementia, however it is more common in people over 65 (7% affected, or 1 in 14) with the likelihood of developing dementia increasing significantly with age (Alzheimer's Society, 2014). Most commonly the diagnosis will be of Alzheimer's Disease (62% of dementia cases) and/or vascular dementia, together accounting for nearly 90% of dementia cases. More rarely the diagnosis will be of dementia with Lewy bodies, frontotemporal dementia, Parkinson's disease or other neurodegenerative diseases. The majority of these cases can be diagnosed with confidence, however about 10% of patients may have atypical features of dementia and some of these might be CJD.

Unfortunately, obtaining a diagnosis in these patients may be difficult. Whereas a younger patient with symptoms suggestive of CJD would be unusual and normally be referred to neurologists for assessment; in older patients amongst whom other forms of dementia are far more common, a CJD diagnosis may be more difficult to recognise. This is particularly so if the patient has an atypical presentation and may also be the case if he/she has been referred to psychiatric and/or geriatric services, which in turn may be less likely to consider a diagnosis of vCJD than would specialist teams such as neurologists. Moreover comprehensive autopsy in the elderly demented, which enables a definitive diagnosis of CJD, rarely occurs.

6.2 AIMS & OBJECTIVES

This part of the study involves the clinical and pathological investigation of patients with atypical features of dementia (aged 65+) accessing local psycho-geriatric services, for evidence of otherwise unrecognised prion disease (including vCJD, sCJD and other forms of prion disease). Its objectives are:

- ☐ to describe the clinical and pathological features and referral characteristics of existing UK cases of prion disease UK in older patients and/or those referred late in disease progression; assess whether these differ from younger cases and/or those referred closer to symptom onset, and how this may affect case ascertainment and reporting practice (based on existing national CJD surveillance data);

- ☐ to investigate NHS Lothian patients with atypical features of dementia for clinical and pathological evidence of prionopathy, informed in part by those characteristics (identified above) that are associated with older patients and the underreporting of cases;

- ☐ where such evidence is found, to describe the associated clinical, pathological and epidemiological features and referral characteristics in the Lothian population, and how these compare with other cases of prion disease;

- ☐ to describe the clinical, pathological and epidemiological characteristics of patients with atypical features of dementia in the Lothian population;

- ☐ to assess the methodology used and the feasibility of employing these methods to help inform procedures for the enhanced surveillance of prion disease in other parts of Scotland and the rest of the UK.

While it is possible that no cases will be detected in the study population, the study will raise local awareness of the possibility of CJD in older age groups and of case definitions. The implications of a positive finding in a patient in whom prion disease would otherwise not have been considered are significant for surveillance and public health, and will help generate hypothesis on the relationship between prion and other neuro-degenerative diseases that could be tested in research cohorts and service settings.

6.3 STUDY DESIGN, POPULATION & SETTING

In Edinburgh and the Lothians, dementia services are provided through local neurology and cognitive clinics; these form part of a network of neurology and psychogeriatric services throughout the south of Scotland including Fife, Forth Valley, Borders, Dumfries and Galloway.The study will involve selected patients aged 65+ accessing the clinics in Edinburgh and the Lothians (East, West and Mid Lothian). Subjects will be recruited into the study with consent, and investigated for evidence of prion disease. If CJD is suspected, then the patient will be referred to NCJDRSU for further assessment and confirmation of diagnosis, in line with UK standard referral procedures for surveillance.

The size of the Lothian population aged 65+ is approximately 125,000 (based on 2011 census estimates). Of these annually around 1% or slightly lower may be expected to develop dementia [Matthews et al., 2013, Alzheimer's Society, 2014], of which around 10% might be considered clinically atypical and therefore eligible for inclusion in the study. In this study we propose to focus on recruiting new and existing patients. We consider numbers to be in the region of 100 per year (plus up to an estimated 50 existing patients in the first year), with just over 300 recruited into this study in total. It is, however, difficult to estimate what proportion of older dementia patients would become known to dementia services out-with primary care and be identified as eligible to join the study. We note that while these figures represent the totality of potential participants eligible to join the research, they are likely to overestimate those who actually agree to participate.

6.4 CASE INCLUSION DEFINITION & ASCERTAINMENT

Potentially eligible patients will be identified by the local clinician at their respective clinics, including new and existing patients as they attend the clinic and/or are visited in the community. The clinic staff will briefly discuss the research study with the patient/representative and ask if they would agree to referral to the study research team at NCJDRSU.

The NCJDRSU neurologist / research nurse will discuss the patient's eligibility with the local clinician, and then contact the patient/ representative with further information. If appropriate they will arrange a meeting in a patient-preferred location (likely to be at home, see section 6.7) for a full discussion on what participation may involve and the implications for the patient and their relatives. Consent will not be taken until this full discussion has taken place.

A. INCLUSION

Any patient aged 65+ accessing NHS Lothian neurology and psychogeriatric services, who has features atypical for the recognised forms of dementia [McKhann et al., 2011; Rascouvsky et al., 2011; Matthew et al., 2012; McKeith et al., 2005;Gorno-Tempini et al., 2011; Collins et al., 1995; Emre et al., 2007; Neary et al., 1998; Román et al., 1993] will be eligible for inclusion in the study with appropriately informed consent.

In assessing eligibility the NCJDRSU neurologist / research nurse will apply clinical information provided by the local clinician against standard diagnostic criteria for recognised forms of dementia, in order to assess patient eligibility for inclusion. These are a selected patient group from the wider population of service users amongst whom we believe a "missed" diagnosis of prion disease might be more likely to be found. The majority will be residents of Edinburgh and the Lothians although some patients will be resident out-with this area. All will be included in the study investigations, although the main analyses will be focussed on residents.

B. EXCLUSION

Any patient diagnosed with a clear alternative demonstrable pathology will be excluded from the study. Conditions include positive diagnostic genetic tests for a known inherited dementia (excluding prion disease); a clear psychiatric diagnosis; space occupying lesions; neuroinflammatory or neuroinfectious conditions; a history and documented radiological evidence of a cerebral insult temporally related to the onset of symptoms.

C. ADULTS WITH INCAPACITY

Given that by definition this study involves patients with dementing illness, we anticipate that a significant proportion of patients will lack the capacity to consent to participate. These patients are still considered eligible for inclusion in the study under standard consent arrangements for adults with incapacity in Scotland (see below).

6.5 CONSENT

Patients may only be recruited into the study with informed consent. Capacity is defined by the ability to understand and retain the information relevant to the decision in question and to weigh that information in the balance to arrive at a choice; capacity will be assessed at the beginning of all meetings with the patient, using standard procedures according to legal requirements and medical practice guidelines [General Medical Council, 2013].

If an adult has capacity, then no-one can consent on their behalf. For adults with incapacity this may be given by the patient's legal representative (welfare attorney or welfare guardian) or, if none, their nearest relative (AWI). If the participant loses capacity to consent during the study, then the wishes of the participant whilst with capacity should be respected and the study can continue under existing consent arrangements on the basis that the wishes of the individual holds. If a participant regains capacity they will be asked to give their consent to continue.

All potential participants will be asked if they wish to join the study. Consent can only be taken at the recruitment interview, attended by the patient, their representative and the NCJDRSU research staff. Before the meeting the patient/representative will have been given information about the study and what participation involves. The interview itself provides the opportunity to discuss the study with the researchers. This ensures the potential participants are adequately informed about the project, its relevance to them and the consent process.

6.6 INITIAL APPROACH TO CLINICS

Local neurology and psycho-geriatric services in Edinburgh and the Lothians will be identified as potential research sites, and approached through the local clinicians' fora. Although the emphasis will be on

involving old age psychiatrists and geriatricians running specialist cognitive clinics, neurologists will similarly be informed in case potential subjects present in general neurology outpatient clinics. All centres should be approached with a medical consultant identified as the local investigation point of contact for the study (referred to as the local clinician).

6.7 RECRUITMENT OF PARTICIPANTS

Patient contact cannot begin until the patient/representative has agreed to referral to the study research team at NCJDRSU.

A. The study neurologist/research nurse will discuss the study with the local clinician and check they are confident with the information required for patients.

B. Potentially eligible patients will be identified by the local clinician at their respective clinics, including patients as they attend the clinic and/or are visited in the community (by clinicians or community mental health nurses). The clinic staff will briefly discuss the research study with the patient or, for adults with incapacity, their representative. A study introduction sheet for patients and their representatives will be made available to assist in this process. The clinic staff will ask the patient/representative if they would agree to referral to the study research team at NCJDRSU.

C. Representatives are defined as the welfare attorney/guardian, or if none the nearest relative (as defined under the Adults with Incapacity (Scotland) Act 2000).

D. If the patient is then considered eligible, the NCJDRSU will then contact the patient/ representative with further information about the study and ask if they can arrange a meeting in a patient-preferred location (likely to be at home)) for a full discussion on what participation may involve and the implications for the patient and their relatives. Patient information sheets will be made available to assist in this process. Consent will not be taken until this full discussion has taken place.

E. A special situation arises when recruiting those patients from the Anne Rowling Clinic, which provides clinical care and is also a research facility. When patients first attend the clinic they are asked by clinic staff to provide written consent regarding whether or not they agree to be approached for future research studies. A register is kept at the research facility with personal information about patients who have agreed to be approached for future research (the Edinburgh Cognitive Disorders Clinic Diagnosis, Audit, Research and Treatment Register (CDC-DART), REC reference 12/SS/0196). This database will be screened by the local clinician at the research facility to identify any potential participants for the study. The potential participants will then be contacted in writing by the Anne Rowling Clinic with further information about the study and asked to complete and send a reply slip back to the 65+ dementia study team, indicating whether or not they would like to find out more about the study, with no obligation to participate in the research. If the patient/representative replies saying they may be interested, then a follow-up telephone call will be made by the study research nurse/neurologist to see if the patient/representative would be happy meeting to discuss the study in further detail and answer any questions the patient may have.

F. Consent to participate in the study can only be taken after the patient/representative has had the opportunity to read the information sheet and ask any questions and reflect on whether or not they wish to participate.

G. The meeting will be in a patient preferred location arranged. It is envisaged this will usually be in the patient's own home, with the patient, representative and the study research nurse/neurologist present. Those with capacity will be asked to involve their family and nominated representative (if they have one).

H. Consent to participate in the study can only be taken at the meeting with the study neurologist/research nurse, after the patient/representative has had the opportunity to read the information sheet and ask any questions and reflect on whether or not they wish to participate. One hour should be set aside, although it is possible the patient/representative will not wish to use all their allocated time. During this time the patient/representative will have the opportunity discuss any aspects of their illness and raise any further questions.

I. All potential participants will be asked if they wish to join the study, with consent asked separately for the different elements of the study:

- ☐ for study registration, clinical examination and review of the patient's medical notes
- ☐ for epidemiological investigation
- ☐ for blood donation or buccal swab for codon-129 polymorphism typing and use in future research
- ☐ for follow-up (including clinical assessment/investigations) for evidence of prionopathy
- ☐ for brain tissue donation for post-mortem investigations for evidence of prionopathy/research

For consent to be valid, all individuals who agree to participate will be required to sign a Consent Form, or for adults with incapacity their legal representative/nearest relative on their behalf.

J. Patients/representatives declining participation should be thanked for their time and their refusal recorded in their clinic notes.

K. If a patient/representative gives consent for the study, they will be given a unique study number, which will be recorded in the patient's notes, and a copy of the consent form to keep. The original will be kept in the patient's notes and copies in the main and local site study files. For patients who decide to take part, permission will also be sought from the patient for an outline of the study to be sent to their GP, for information.

L. Opportunities for brain tissue donation and post mortem investigation for evidence of prionopathy will be discussed with the patient/representative. The participant's wishes will be recorded if a decision is made. It will be suggested to the patient/representative that they discuss the patient's wishes with the relatives, so that the relatives are more prepared in the event of the participant's death.

6.8 PARTICIPATION, INCLUDING REGISTRATION OF PERSONAL DETAILS, CLINICAL EXAMINATION AND INVESTIGATIONS AND REVIEW OF MEDICAL NOTES

A. If the patient/representative gives consent for the study, this information will be recorded on the consent form, along with the unique study number, clinic and GP contact details to enable patient follow-up. This information, together with information relating to study status (alive, died, left) and actions for follow-up will be registered in a patient database.

B. The study team member will take a history of the present illness including specific illness details (symptoms), and the patient will then undergo a detailed neurological examination. This will include general appearance, full cognitive assessment (comprising the Addenbrooke's Cognitive Examination (ACE-III) [Mioshi et al., 2006], the Severe Impairment Battery - Short Form (SIB-S) [Saxton et al., 2005], the MRC Scale [Thompson et al., 2013], a Frontal Assessment Battery (FAB) [Dubois, 2000], Hospital Anxiety and Depression Scale (HADS) [Zigmond and Snaith, 1983], and a physical examination (comprising the Edinburgh Motor Assessment (EMAS) Scale [Bak, 2013] supplemented by tests of vision, reflexes and a sensory examination). The outcome of this initial assessment will be recorded on a clinical examination form. The assessment would normally take an hour, although there may be some variation from person to person. If the patient agrees, the

patient's health-related records may also be requested and subjected to scrutiny to look for features that might indicate prion disease.

C. Before the interview the research neurologist/nurse will check with the local clinician if an ACE-III has been undertaken within the last 6-months, in which event a further ACE-III will not be undertaken, in order to avoid any learning effect. The recent scores for the prior test will be taken instead

D. As part of the clinical assessment process, MRI brain scan will normally be made through the local clinician as part of the diagnostic work-up, if not done already, but if none if forthcoming then arrangements will be made for a research MRI brain scan at the University of Edinburgh Brain Research Imaging Centre (BRIC, up to 40% of patients) with the aim of identifying features that might clarify the diagnosis. The MRI scan will take approximately 30 minutes to complete; the patient's representative or family member will be able to accompany the patient if reassurance is needed.

E. A 'core' MRI series for prion disease would include DWI and FLAIR (2D or 3D) as part of a standard brain protocol. Where possible the same senior neuroradiologist will review the MRI scans (diagnostic and research).

F. Results of the examinations and investigations will not routinely be fed back to the local clinician or patient (but see below), but will be included in the overall research outputs. However as a matter of courtesy a letter will be sent to the local clinician after the initial meeting thanking them for referring their patient, confirming whether or not the patient agreed to participate and summarising any plans for follow-up.

G. For all participants, a letter will also be sent to their GP providing an outline of the study and updating the GP of any relevant changes to protocol, for information.

H. If there is evidence of prion disease or incidental findings that could have an impact on patient management and/or require further investigation, then the study neurologist will inform the local clinician in writing with a copy to the patient's GP and through them the results fed back to the patient as appropriate, as the findings may be relevant to the patient's treatment and care.

6.9 EPIDEMIOLOGICAL INVESTIGATION AND REVIEW

I. This is to help generate hypothesis as to risk factors for disease. It may also help identify the most likely route of infection in the event of a positive finding and inform any related public health actions should the need arise.

J. If the patient consents, at the recruitment visit the participant/their relative will be asked questions about the participant's past medical/dental histories, including their donation and receipt of blood/blood products, any surgical procedures and where they have lived and worked and any family history of neurodegenerative disease, the answers to which will be recorded on a questionnaire form. The questionnaire will normally take half an hour.

6.10 PRION PROTEIN GENE CODON-129 GENOTYPING AND STORAGE FOR FUTURE GENETIC TESTING

K. If the patient consents a 2ml blood sample (normally citrate or EDTA) will be taken by the research nurse/neurologist and transported back to NCJDRSU for registration, processing and testing.

L. If it is not possible to obtain a blood sample, if the patient consents a buccal swab will be obtained by the research nurse/neurologist and transported back to NCJDRSU for registration, processing and testing.

M. The blood sample or buccal swab will be processed, with DNA extracted and tested for the codon-129 polymorphism and the result should be recorded in a samples database. The codon-129 typing genotype helps to classify the type of infection, but does not indicate a specific genetic risk for CJD at the individual level.

N. Genetic risk for prion disease can only be determined through genetic sequencing, which is outside the remit of this study. However, if the patient/representative agrees, NCJDRSU will arrange for the long term storage of residual genetic material from the codon-129 testing for use in future research into neurological conditions, which may, for example, include prion disease.

O. Any remaining blood, the buccal swab and residual genetic material that is not destined for storage for future genetic testing, will be retained until after the study has ended and all investigations have been completed and then lawfully disposed of.

6.11PATIENT FOLLOW-UP

P. Patient review will then be undertaken on a regular basis to look for evidence of prion disease. Patients will be tracked by the study research nurse on the patient management systems and in consultation with the care teams, and the patients followed up with a telephone call from the research nurse at appropriate intervals to the nature and progression of illness to assess progression and answer questions the participant may have. This is likely to be within 1 month of joining the study and every 3 months thereafter.

Q. In patients whose illness is consistent with CJD (for example someone with progressive cognitive impairment, ataxia/ involuntary movements and/or a more rapid course or acute terminal deterioration, [el Tawil et al. 2015] a home visit by the neurologist would be arranged for further clinical assessment and repeat (research) MRI undertaken if necessary.

R. Before starting the study it is not known to how many participants a follow-up visit and second MRI scan would apply; this may be in up to one half of patients, although likely to be an overestimate.

S. Lumbar puncture for CSF tests would not be part of research-led investigations, but would be undertaken if part of local clinical practice, including if there was a strong suspicion of CJD.

T. If at any stage there is a suspicion of CJD, then the treating clinician will be informed and the patient will be referred immediately to NCJDRSU for further assessment and confirmation of diagnosis, in line with UK standard referral procedures for prion disease (see section 9). Subject to availability of funding there may be the possibility of providing additional care and support through the National CJD Care Package.

U. Before any follow-up meeting the study neurologist and research nurse should consider if there is new information that patient/representative and local clinician may need to receive.

V. A note should be made of the meeting and kept on file. Results of clinical examination and investigations will be given to the senior study clinician, and this information fed back to the patient and their family via the local clinician, as this may be relevant to their treatment and care.

W. Follow-up is likely to be from recruitment until resolution of symptoms or death. It is not known for how long this will be, and at the same time it is important to maximise the number of study participants. Therefore we recognise that for some later recruits follow-up may occur out-with the

initial study period. Rather than exclude these patients from further follow up, a review of the patient's progression will be undertaken and if extension to the study is considered necessary, this will be sought.

6.12AFTER DEATH (BRAIN TISSUE DONATION FOR RESEARCH)

X. Patients and families will be made aware at the initial meeting that the diagnosis of prion disease and other forms of dementia can often only be through post-mortem examination and tissue investigations.

Y. The possibility of brain tissue donation for research purposes will have been raised in-life with the patient/representative. If not discussed at the initial meeting the possibility of post-mortem will be raised again, most likely by the research nurse or neurologist, at an appropriate time as early as possible in the follow-up process.

Z. Patients will be asked if they would consider donating brain tissue to the Edinburgh Brain Bank for research purposes, including investigations for evidence of prionopathy (the primary purpose of PM examination being for this study) and to discuss the patient's wishes with their relatives.

AA. The participant or, if the participant lacks capacity, their representative/nearest relative, may express their wishes if they are ready to make this decision. However, authorisation for the post-mortem examination will still always be sought from the patient's representative/nearest relative after death - at which point the relative will be given a hospital post-mortem authorisation form, which they will be asked to consider and complete.

BB. If a participant's condition rapidly declines / develops terminal stage illness, the representatives or local carers will be asked to inform the study research nurse/neurologist. Further clinical assessment can be undertaken with agreement of the patient/their representative (section 6.11). This also allows the research team to establish where the patient is likely to die and prepare for any PM investigations in line with the participant's consent arrangements.

CC. In the event of a participant's death, the families and local clinicians will be asked to contact the NCJDRSU directly, and will have been given a phone number to call as soon as possible after the death has occurred so that post-mortem can be arranged with the pathology staff.

DD. Establish whether a Fiscal post mortem will be required, in which case the local office of the Procurator Fiscal should be informed that consent has been given the research study. It may still be possible to collect tissue samples for the research investigations.

EE. If a Fiscal post-mortem is not required, authorisation should still be sought from the representative/nearest relative using the standard hospital post-mortem authorisation form. This should be sought by the study team, in liaison with the local pathologist.

FF. Authorisation will be requested for a limited, head only examination, including the removal, retention and use of brain tissue for research, education/training and audit. If the relatives wish for brain tissue investigations to be restricted to the prion research project (65+ study) they can express this wish on the post-mortem authorisation form. Peripheral tissue will not be requested – although this limits our investigations for vCJD infection, requests for head-only post-mortems are more likely to be successful, and overall are of greater benefit to the study objectives. Based on experience we know that not every family will agree to post-mortem, provision is made for up to 40% of patients.

GG. Normally the post mortem will be conducted in the local hospital (most likely the Edinburgh Royal Infirmary or Western General Hospital) by a pathologist between one and four days after death. After this the body will be returned to the undertakers so as not to delay the funeral arrangements.

HH. The tissue samples will then be investigated for evidence of CJD, including standard histopathology, immunohistochemical and full biochemical investigations (see section 6.13). Due to the type of tests that need to be performed to investigate evidence of CJD, it usually takes two to three months to obtain to obtain the full final results.

II. It will be possible to withdraw post-mortem consent and tissue analysis up until the time the tissue is tested; in this event we will arrange for the respectful disposal of any remaining tissue.

JJ. Where post-mortem investigations have been completed, a final report would be submitted by the study lead to the Director Edinburgh Brain Bank. This report would summarise clinical findings and the outcome of the brain tissue investigations, as well as the prion-protein codon-129 genotype. Results would usually then be sent to the local clinician with a copy usually sent to the GP, according to standard Brain Bank procedures, and the results will be conveyed by the study neurologist/research nurse or the participant's doctor, to the family members involved as appropriate.

KK. The cause of death and results of any PM investigations will be recorded on the study database.

LL. If CJD is suspected, then the patient will be referred to NCJDRSU for further assessment and confirmation of diagnosis, in line with UK standard referral procedures for surveillance.

6.13. SAMPLE TRANSPORT, PROCESSING AND TESTING

Appropriate safety precautions will be employed by all personnel in the handling of samples from the time of tissue sampling, in accordance with local health & safety policy and national regulations.

A. BLOOD FOR CODON-129 GENOTYPING.

A 2 ml blood sample should be taken, labelled with the patient's study number date of birth and date of collection only and the sample, double bagged and transported at ambient temperature back to NCJDRSU for registration in the laboratory the following working day. Samples will then be stored in a -80C freezer at NCJDRSU prior to testing.

Molecular codon-129 subtyping will be undertaken using DNA extracted from the blood sample; the codon-129 polymorphism result should be recorded in a samples database. The NCJDRSU will arrange for any remaining blood to be disposed of at the end of the study.

If the patient has consented, the NCJDRSU will arrange for the storage of any residual genetic material for use in future research into neurological conditions, which may, for example, include prion disease. Any residual genetic material that is not destined for storage for future genetic testing, will be retained until after the study has ended and all investigations have been completed.

B. BUCCAL SWAB FOR CODON-129 GENOTYPING

If a blood sample is deemed unobtainable, a buccal swab should be taken. The tip of the swab will be placed into a sterile 2ml safe-lock tube and sealed. The tube will be labelled with the patient's study number, date of birth and date of collection only, and the sample double bagged and transported at ambient temperature back to NCJDRSU for registration in the laboratory the following working day. Samples will then be stored in a fridge at NCJDRSU prior to testing.

Molecular codon-129 subtyping will be undertaken using DNA extracted from the buccal swab; the codon-129 polymorphism result should be recorded in a samples database. The NCJDRSU will arrange for any remaining material to be disposed of at the end of the study.

If the patient has consented, the NCJDRSU will arrange for the storage of any residual genetic material for use in future research into neurological conditions, which may, for example, include prion disease. Any residual genetic material that is not destined for storage for future genetic testing, will be retained until after the study has ended and all investigations have been completed.

C. AUTOPSY SAMPLES

Samples will be registered and flagged as they are received from the autopsy suite. Tissue samples will then be processed for a range of histopathological, immunocytochemical and biochemical investigations, which will be conducted at the Edinburgh Brain Bank and at NCJDRSU as per arrangements for the neuropathological screening of any banked brain tissue donation (see sections 5.7-5.10)

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

6.14 INFORMATION COLLECTED

A. For each eligible subject, consent status is recorded on a standard consent form and signed by the patient, or their representative.

B. All participants recruited into the study will be assigned a unique study identifier for data management purposes including pseudonymisation and the association of data between relevant data forms. These identifiers, together with patient details and consent status, GP and contact details should be recorded in the study database at NCJDRSU.

C. A clinical and epidemiological review form will record detail on the case onset, presentation and duration of illness, and features associated with disease progression; together with details of clinical investigations and epidemiological review undertaken. Any significant past medical history (as recorded in the medical notes), and the outcome of initial and prospective clinical assessments and pathological (post-mortem) investigations should be recorded on this form.

F. Study data will be held in electronic and paper format at NCJDRSU, Edinburgh Brain & Tissue Bank and the BRIC. All source data will be checked and validated before electronic data-entry and verification. Papers records will be filed securely; electronic data will be held in a study database with sub-tables (see section 7 below).

G. The outcome of post-mortem investigations will be shared between the respective NCJDRSU and brain bank study teams as part of the investigation record.

6.15ANALYSIS

A. Results of investigations will be reviewed. If there is evidence of vCJD, sCJD or other prion disease pathology, then the participant would be referred to NCJDRSU as from a suspect case. Further investigations would then be undertaken according to standard NCJDRSU surveillance procedures (see section 9.)

B. Progress in the study will continually be assessed against the study objectives (section 10). The final analysis will include a description of clinical-pathological variation associated with positive

findings, and an assessment of how this might contribute to local surveillance practice as well as out with the local setting.

6.16WITHDRAWAL FROM THE STUDY

A. Consent to participate in all or part of the study can be withdrawn by the patient at any time, without compromising their care.

B. If a participant does not wish to continue in the study ask if this applies to some or all of the study components and identify which ones. It will be possible to withdraw post-mortem tissue up until the time it is tested and NCJDRSU will arrange for the respectful disposal of any remaining tissue in line with standard practice. Data can be withdrawn on request by deletion from the study database, leaving the minimum required for audit purposes and where relevant to the medical record.

C. The withdrawal from the study and details of actions required and undertaken, with dates, should be recorded by the local investigator and research nurse in their respective study notes.

7 DATA MANAGEMENT

7.1 DATA PROTECTION AND CONFIDENTIALITY

A. All staff have a duty to maintain patient confidentiality, and procedures and relevant training are in place to help safeguard. The UoE has records management and information security policies, procedures and guidance on the handling of confidential information. In addition, a NCJDRSU Data Protection and Security Code of Practice, to which all NCJDRSU data users are signatories, provides guidance to staff on the handling of personal identifiable information, to safeguard confidentiality and help ensure data protection and security requirements are being met.

B. The processing of personal information as part of this study will be registered with the Office of the Information Commissioner (http://www.ico.org.uk) as part of the UoE (reference: Z6426984).

7.2 INFORMATION SECURITY ASSURANCE

A. All the information collected during the course of the research study will be kept confidential. For study purposes, all electronic data is held on a password protected secure database at NCJDRSU; all paper records are locked in secure cabinets; access to personal information is restricted to authorised personnel (the research team members and those with IT responsibilities) and at no point in time will personal information be disclosed to anybody outside these personnel; linkage of records for study analyses, and for follow-up is restricted to authorised personnel and by use of a unique study number.

B. Backups are made on a regular basis in line with NCJDRSU and University of Edinburgh protocol, no other copies of the database will be made. Where clinical review/patient follow-up is undertaken it will be done as far as possible by the same research team member and local clinician.

C. Procedures are in place to ensure the secure transfer of personal information. Email is NOT considered secure and emailing of personal identifiable information via the internet or transfer by via any electronic media (eg. memory stick) is not permitted, including emails between NHS and University accounts, unless it is encrypted.

8 INDIVIDUAL ASSESSMENT & FEEDBACK OF RESULTS

A. The study is conducted on the participants' understanding that they/their families would not routinely be informed of the outcome of the research investigations, unless there is evidence of prion disease or incidental findings that could have an impact on patient management and care.

B. In this event then the senior doctor who had been looking after the patient would be informed in writing by the study neurologist directly and through them the results conveyed to the participant and the family members involved. A copy will also be sent to the GP. If there is evidence of prion disease pathology, the participant would be referred to NCJDRSU as for a new or suspect case of CJD. Further investigations would then be undertaken according to standard NCJDRSU surveillance protocol (see section 9.)

   If post-mortem investigations have been undertaken as part of either the neuropathological screening or clinical surveillance parts of this study, a final neuropathology report will be issued by the Director, Edinburgh Brain Bank, which will have a final diagnosis. These reports go out to the senior doctor who had been looking after the patient according to standard Brain Bank procedures; the relatives have an opportunity to meet with the senior doctor (or GP) who can discuss the final diagnosis with them. If there is post-mortem evidence of prion disease pathology, the participant would be referred to NCJDRSU as for a new or suspect case of CJD.

9 ACTION IN CASE OF POSITIVE FINDINGS FOR PRION DISEASE/ PRIONOPATHY

A. In the event of clinical and/or pathological evidence of prion disease/prionopathy during the study, the Chief Investigator will speak to the person responsible for the investigations, consult with colleagues and confirm the level of certainty.

B. The Chief Investigator would then consult with the Brain Bank Manager/ participant's local clinician and request the participant is referred to the NCJDRSU surveillance team as a suspect case of prion disease. Further follow-up and investigations would then be undertaken according to standard surveillance procedures (see http://www.cjd.ed.ac.uk/surveillance.htm).

C. As part of this process further clinical and/or pathological information would be requested to support the possible diagnosis of prion disease. The Brain Bank manager and/or local clinicians/pathologists may be contacted for any further information they can provide. If necessary additional tissue samples will also be requested.

D. A clinical review of the medical notes would be undertaken, detailing the case medical history, onset, presentation, duration of illness, features associated with disease progression and final diagnosis, with particular attention to the development of cognitive and neurological features associated with the prionopathy and any co-pathology. For brain donors consent to access medical records normally accompanies the donation, however if consent is needed the review can be deferred until consent is obtained from the family involved (see below).

E. The family would also normally be contacted, with consent of the local clinicians involved, by the NCJDRSU neurologist to arrange a chance to meet. At this interview the family will have the further opportunity to discuss CJD and the implications of this as a possible diagnosis. Any further clinical assessments and a more detailed epidemiological review would be undertaken for the patient. Information and support would be given to the family as appropriate.

Should a prionopathy be suspected, genetic CJD will be considered as part of the standard process for referral of new suspect cases in line with standard diagnostic practice. Appropriate counselling and consent for sequencing will be sought. If brain tissue is available or if a blood sample or buccal swab was taken for codon-129 typing and genetic material subsequently stored, these may be made available as material for genetic testing if the family agree.

F. A final diagnostic report would be submitted to a senior clinician and then sent to the local clinician (participants) or GP (donors) for communication to the family. This report would summarise clinical findings and, where post-mortem investigations have been undertaken, the outcome of both macro-examination of the brain and microscopic examination. If the patient to whom the investigations relate was a research participant, then the results, will be conveyed where possible by the research neurologist to the family involved.

G. The new or suspect case may also be referred to public health professionals, including the national blood services and the local public health team, so they can follow national guidance on public health action to be taken in order to prevent any possible spread of CJD between people.

If necessary advice will be taken from relevant expert panels (ACDP) as to the handling of unusual presentation or novel forms of prion disease.

10 ANALYSIS

10.1 PRIMARY OUTCOME MEASURES

Evidence of prion disease in the Lothian population, a description of the associated clinical, pathological and epidemiological features and referral characteristics and how this compares with other cases of prion disease.

10.2 SECONDARY OUTCOME MEASURES

A. A description of the clinical, pathological and epidemiological characteristics of patients with atypical features of dementia in the Lothian population.

B. Assessment of the suitability of methods to support an extended system of enhanced CJD surveillance in the rest of the UK. This includes the suitability of existing case definitions for referral, ability to define cases, willingness of clinicians and patients to participate, response rates to different elements of the study, availability of data and limitations of data collection methods.

10.3METHODS OF ANALYSIS

A. Any research participant with clinical and/or pathological evidence of prion disease who, prior to the research team's clinical assessment or further pathological investigation, was not considered by the senior clinician in charge of that patient's care to have prion disease, will be referred to as a "missed" case of prion disease.

B. The numbers of missed cases identified and their clinical, pathological, epidemiological and referral characteristics will be described in terms of simple descriptive statistics (eg. frequency tables, cross-tabulations and graphics).

C. These characteristics will then be compared with those of UK cases of prion disease referred independently of this study to the UK national surveillance system over the same study period,

through univariate and multivariable analysis to look for characteristics that are associated with missed cases, adjusting for potential confounders (age, speciality).

D. A descriptive analysis will similarly be undertaken of the features of atypical dementia patients.

E. The success of the study and suitability of methods to support an extended system of enhanced CJD surveillance in the rest of the UK, will be assessed in terms of the ability to meet the primary outcomes, with reference to clinic and patient participation rates and uptake of the different elements of the study. It will also be informed by feedback received by the clinicians and patients over the course of the study and the experiences of the research team in conducting the study.

11 MANAGEMENT

11.1 KEY STAFFING

A. Chief Investigator
B. Lead neurologist NCJDRSU (co-applicant)
C. Lead neuropathologist NCJDRSU (co-applicant)
D. Lead neuropathologist Edinburgh Brain & Tissue Bank (co-applicant)
E. Lead biochemist NCJDRSU (co-applicant)
F. Research nurse
G. Research neurologist
H. Research fellow (biochemistry)
I. Research technician (biochemistry)
J. Research fellow (immunohistochemistry)
K. Research database manager
L. Research administrative assistant
M. Laboratory manager (NCJDSRU)
N. Laboratory manager (Edinburgh Brain Bank)
O. Consultant neurologist
P. Consultant neuroradiologist
Q. Consultant old-age psychiatrist
R. Consultant geriatrician

11.2 MANAGEMENT AND OVERSIGHT

A. The Chief Investigator takes overall responsibility for the overall conduct of the study, coordination of investigators, reporting of research progress and dissemination of results. Supervision and oversight of clinical activities and prion disease diagnosis will be by the study lead Neurologist. Management, supervision and oversight of laboratory activities, including testing, diagnosis and reporting, will be by the study lead Neuropathologists (Edinburgh Brain Bank and NCJDRSU laboratory), and the study lead protein biochemist as appropriate.

B. There will be an Edinburgh-based Study Team who will meet every 6 months to evaluate progress and to ensure the smooth running of the project. They may also meet when required to discuss cases of interest/other relevant information. This team will include the study Chief and co-investigators, together with the research neurologist and nurse, database manager, neuroradiologist and laboratory managers and associated research staff (NCJDRSU and Edinburgh Brain Bank).

C. Oversight of the study will be provided by a Steering Group. The Steering Group will consist of representatives from clinical specialities, public health and patient groups, and specialist advisors (statistics, UK Dementia Platform). The Steering Group will meet on a regular basis with a working

group of the Study Team to review progress and provide intellectual input, practical advice and support to the Study Team when needed.

D. At each clinic there will be an on-site lead investigator who is responsible for the conduct of the study at the local site. S/he will ensure the smooth running of the project locally and the safety and wellbeing of participants.

11.3 ADVERSE EVENTS

All investigators and team members will comply with the sponsors' (UoE and NHS Lothian) standard requirements on serious breaches of GCP (SOP reference CR003) and on identifying and reporting adverse events (SOP reference CR005) and on deviations and violations (SOP reference CR010), available at http://www.accord.ed.ac.uk/standardopprocs/CRSOPs.html

11.4 STUDY OUTPUT

A. Regular feedback on study progress will be provided to clinicians and participants throughout the study. The Management team will produce an annual information sheet for participants conveying thanks, an update on participation in the study as well as general knowledge of prion diseases, and research findings. In addition, summary information for the general public will be available through the NCJDRSU website.

B. Progress in the study will be assessed against the study objectives namely:

   i. Identifying cases of prion disease/prionopathy in the study population;
   ii. Describing their clinical, pathological, epidemiological and referral characteristics;
   iii. Describing how these differ with observed cases to date
   iv. Describing the clinical, pathological, epidemiological and characteristics of patients with atypical features of dementia in the Lothian population;
   v. Assessing how the methods used might contribute to the enhanced surveillance of prion disease in other parts of Scotland and the rest of the UK.

   Results will be presented to the Department of Health and Scottish Government Health Department, national public health agencies (Health Protection Scotland and Public Health England) and advisory panels (The Advisory Committee on Dangerous Pathogens). The content of these reports will vary but is likely to include progress in recruitment and investigations, a summary of the basic demographic, clinical and pathological findings, and assessment of the implications, if any, to diagnostic practice, disease surveillance and public health.

C. Intermediate findings and final results will also be presented at national and international meetings and conferences (Prion, UK Dementia Platform and Brain Bank Network, Alzheimer's Scotland, CJD Support Network) and submitted for publication as peer reviewed (open access) articles in medical journals.

D. While these reports are likely to contain a descriptive analysis of the data they will not contain either PII or information from which the identity of the patient could be inferred.

12 END OF STUDY

The study will run for 5 years between 01/04/2015 and 31/03/2020. The end of the study will the completion of investigations following the last follow-up visit or date of tissue donation for the final participant/donor. Banked brain tissue donations will be included from the outset until two months prior to the study end to allow time for screening and final reporting. Participants will be recruited until three

months prior to the study end date to allow some time for follow-up/onward referral if appropriate, although further follow-up and post-mortem investigations may be undertaken subsequently if this is what the relatives wish (see section 6.11).

Tissue will be retained until the end of the study and then disposed of or stored for future research in line with consent arrangements; data will be retained for a minimum of 5 years past the end of the study, at which point it will be reviewed. If considered of value it will be archived for use in future research, with appropriate ethical approval.

13 REFERENCES

1. Alzheimer's Society (2014). Dementia UK. 2nd edition. http://www.alzheimers.org.uk/site/scripts/documents_info.php?documentID=2759

2. Barria MA, Balachandran A, Morita M, Kitamoto T, Barron R, Manson J, Knight R, Ironside JW, Head MW.(2014) Molecular barriers to zoonotic transmission of prions. Emerg Infect Dis. Jan;20(1):88-97. doi: 10.3201/eid2001.130858.

3. Bak, TH (2013) Edinburgh Motor Assessment (EMAS) Accessed 4th May 2015 from https://www.era.lib.ed.ac.uk/bitstream/handle/1842/8225/EMAS%201%20Dec%202013.pdf;jsessionid=8929B5D6519981336AD4C3443812907A?sequence=1

4. Cannon A, Bieniek KF, Lin WL, Notari S, Zou WQ, Gambetti P, Pedraza O, Graff-Radford NR, Ferman TJ, Dickson DW (2014) . Concurrent variably protease-sensitive prionopathy and amyotrophic lateral sclerosis. Acta Neuropathol. 2014 Aug;128(2):313-5. doi: 10.1007/s00401-014-1309-8. Epub 2014 Jun 14.

5. Collie DA, Summers DM, Sellar RJ, Ironside JW, Cooper S, Zeidler M, Knight R, Will RG. 2003. Diagnosing variant Creutzfeldt-Jakob disease with the pulvinar sign: MR imaging findings in 86 neuropathologically confirmed cases. Am J Neuroradiol 24: 1560-1569.

6. Collins SJ, Ahlskog JE, Parisi JE, Maraganore DM. Progressive supranuclear palsy: neuropathologically based diagnostic clinical Criteria. Journal of Neurology, Neurosurgery, and Psychiatry 1995;58:167-173

7. Comoy E, Jaffre N, Mikol J, Durand V, Freire S, Correia E, Pocchiari M, Hills B, Brown P and Deslys J-P (2011). Risk Analysis of Low-Dose Prion Exposures in Cynomolgus Macaque. PRION 2011, May 16th-19th 2011. Montreal (Canada). [Abstract no:bio.039:]

8. Dubois B, Slachevsky A, Litvan I, Pillon B; The FAB: A frontal assessment battery at the beside; Neurology (2000);55:1621-1626Emre M, Aarsland D, Brown R, Burn DJ, Duyckaerts C, Mizuno Y, Broe GA et alClinical Diagnostic Criteria for Dementia Associated with Parkinson Parkinsoe. Movement Disorders 2007; 22:1689-1707

9. El Tawil S, Mackay G, Davidson L, Summers D, Knight R, Will R (2015). Variant Creutzfeldt-Jakob disease in older patients. Journal of Neurology Neurosurgery and Psychiatry NP 2015 Jan 21. pii: jnnp-2014-309397. doi: 10.1136/jnnp-2014-309397. [Epub ahead of print].

10. General Medical Council (2013). Explanatory Guidance: Consent Guidance: Part 3: Capacity Issues. Accessed 4th May 2015, from: http://www.gmc-uk.org/guidance/ethical_guidance/consent_guidance_part3_capacity_issues.asp

11. Ghoshal N, Perry A, McKeel D, Schmidt RE, Carter D, Norton J, Zou WQ, Xiao X, Puoti G, Notari S, Gambetti P, Morris JC, Cairns NJ. (2014) Variably Protease-sensitive Prionopathy in an Apparent Cognitively Normal 93-Year-Old. Alzheimer Dis Assoc Disord. 2014 May 19. [Epub ahead of print].

12. Gill ON, Spencer Y, Richard-Loendt A, Kelly C, Dabaghian R, Boyes L, Linehan J, Simmons M, Webb P, Bellerby P, Andrews N, Hilton DA, Ironside JW, Beck J, Poulter M, Mead S, Brandner S. (2013) Prevalent abnormal prion protein in human appendixes after bovine spongiform encephalopathy epizootic: large scale survey. British Medical Journal 2013; 347: f5675

13. Gorno-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, Ogar JM et al. Classification of primary progressive aphasia and its variants. Neurology 2011;76:1006–1014

14. Head, M. W., Yull, Helen M, Ritchie, Diane L, Langeveld, Jan P, Fletcher, Nicholas A, Knight, Richard S, Ironside, James W. Variably protease-sensitive prionopathy in the UK: a retrospective review 1991–2008. Brain 136(4): 1102-1115.

15. Hewitt PE, Llewelyn CA, Mackenzie J, Will RG (2006). Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. Vox Sanguinis; 91(3): 221-30.

16. House of Commons Science and Technology Committee (2014). After the Storm? UK Blood Safety and the Risk of Variant Creutzfeldt-Jakob Disease http://www.publications.parliament.uk/pa/cm201415/cmselect/cmsctech/327/327.pdf

17. Huillard d'Aignaux J, Costagliola D, Maccario J, Billette de Villemeur T, Brandel J-P, Deslys J-P, Hauw J-J, Chaussain J-L, Agid Y, Dormont D, Alperovitch A. 1999. Incubation period of Creutzfeldt-Jakob disease in human growth hormone recipients in France. Neurology 53: 1197-1201.

18. Matthew R, Bak TH, Hodges JR. Diagnostic criteria for corticobasal syndrome: a comparative study. J Neurol Neurosurg Psychiatry 2012;83:405-410

19. Matthews, F. E., Arthur A, Barnes LE, Bond J, Jagger C, Robinson L, Brayne C (2013). A two-decade comparison of prevalence of dementia in individuals aged 65 years and older from three geographical areas of England: results of the Cognitive Function and Ageing Study I and II. The Lancet 382(9902): 1405-1412.

20. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE *et al*. Diagnosis and management of dementia with Lewy bodies (Third report of the DLB consortium). Neurology 2005;65:1863–1872

21. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011; 7:263-269.

22. Mioshi E, Dawson K, Mitchell J, Arnold R, Hodges JR; The Addenbrooke's Cognitive Examintion Revised (ACE-R): A brief cognitive battery test for dementia screening; International Journal of Geriatric Psychiatry (2006); 21:1078-1085

23. NCJDRSU (2014). Creutzfeldt-Jakob Disease Surveillance in the UK. 22nd Annual Report 2013. http://www.cjd.ed.ac.uk/documents/report22.pdf

24. Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, Freedman M, Kertesz A, Robert PH, Albert M, Boone K, Miller BL, Cummings J, Benson DF. Frontotemporal lobar degeneration: A consensus on clinical diagnostic criteria. Neurology 1998;5 1: 1546-1554

25. Peden A, McCardle L, Head MW, Love S, Ward HJ, Cousens SN, Keeling DM, Millar CM, Hill FG, Ironside JW.(2010) Peden Variant CJD infection in the spleen of a neurologically asymptomatic UK adult patient with haemophilia. Haemophilia. 16(2):296-304. doi: 10.1111/j.1365-2516.2009.02181.x. Epub 2010 Jan 12.

26. Peden AH, McGuire LI, Appleford NE, Mallinson G, Wilham JM, Orrú CD, Caughey B, Ironside JW, Knight RS, Will RG, Green AJ, Head MW.(2012) Sensitive and specific detection of sporadic Creutzfeldt-Jakob disease brain prion protein using real-time quaking-induced conversion.J Gen Virol.Feb;93(Pt 2):438-49. doi: 10.1099/vir.0.033365-0. Epub 2011 Oct 26

27. Peden AH, Sarode DP, Mulholland CR, Barria MA, Ritchie DL, Ironside JW, Head MW. (2014).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. Oct 21;2:152. doi: 10.1186/s40478-014-0152-4

28. Pocchiari M, Puopolo M, Croes EA, Budka H, Gelpi E, Collins S, Lewis V, Sutcliffe T, Giulivi A, Delasnerie-Laupretre N, Brandel J-P, Alperovitch A, Zerr I, Poser S, Kretzschmar HA, Ladogana A, Rietvald I, Mitrova E, Martinez-Martin P, de Pedro Cuesta J, Glatzel M, Aguzzi A, Cooper S, Mackenzie J, van Duijn CM, Will RG. 2004. Predictors of survival in sporadic Creutzfeldt-Jakob disease and other human transmissible spongiform encephalopathies. Brain 127: 2348-2359.

29. Rascouvsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, van Swieten JC, Seelaar H, Dopper EGP *et al*. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 2011: 134; 2456–2477

30. Román GC[1], Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo JM, Brun A, Hofman A, *et al* Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop Neurology. 1993;43:250-60

31. Saxton J, Kastango KB, Hugonot-Diener L, Boller F, Verny M, Sarles CE, Girgis RR, Devouche E, Mecocci P, Pollock BG, DeKosky ST (2005) Development of a short form of the Severe Impairment Battery. The American Journal of Geriatric Psychiatry 13 (11): 999-1005

32. Thompson AGB, Lowe J, Fox Z, Lukic A, Porter M, Ford L, Gorham M, Gopalakrishnan GS, Rudge P, Walker AS, Collinge J, Mead S (2013) The Medical Research Council Prion Disease rating scale: a new outcome measure for prion disease therapeutic trials developed and validated using systematic observational studies. Brain 136: 1116-1127

33. Zigmond AS, Snaith RP; The Hospital Anxiety and depression scale; Acta Psychiatrica Scandinavica (1983); 67:361-370

14 APPENDIX

A. INSTRUMENTS

1) Protocol
2) Participant's Information Sheet and Consent Form
3) Representative's Information Sheet and Assent Form
4) GP letter

5) Patient Assessment and Epidemiological Review Form
6) Participant's Information Sheet and Consent Form for post-mortem examination
7) Representative's Information Sheet and Assent Form for post-mortem examination
8) Letter of invitation (Anne Rowling CDC-DART registered patients)