MRC Prion Unit

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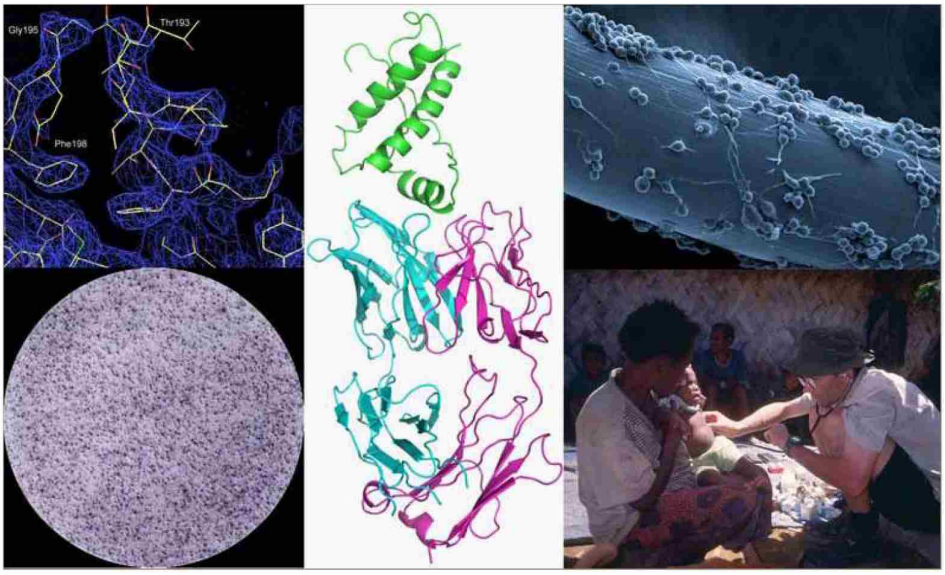
2nd Quinquennial Report & Proposals

April 2004 - March 2010



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SECTION 1: DIRECTOR'S OVERVIEW

Unit formation and philosophy

The MRC Prion Unit was established in 1998 at Imperial College and completed its recruitment following relocation in 2001 to purpose built facilities at the UCL Institute of Neurology (ION). This was funded by a major Joint Infrastructure Fund award to form the Department of Neurodegenerative Disease, within which the Unit was, and continues to be, embedded (headed by the Unit Director). The Unit was formed to provide a national centre of excellence with all necessary facilities to pursue a major, and inherently long-term, research strategy in prion research.

While prion diseases are an area of intense research interest because of their extraordinary biology and the wider implications that flow from this, there was a strategic imperative to establish a national centre with an overtly translational as well as basic science agenda to tackle the problems posed by bovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD). Such a programme needed to be highly multidisciplinary and focus both on immediate areas of public health concern and a long term approach to the understanding of prion disease. The wider relevance of prion-like molecular mechanisms in pathobiology is clear, not least in other neurodegenerative diseases in which accumulation of aggregates of misfolded host proteins is a key pathological feature.

Our research philosophy is to seamlessly combine basic and clinical research. Many of the key contributions towards understanding the basic biology of these diseases have come from clinical and neuropathological observations. Efficient translation of these basic studies to the clinic is crucial. A key part of the Unit's mission was also to develop the infrastructure and patient base, in partnership with the NHS and MRC Clinical Trials Unit, to progress methods for early diagnosis of prion infection and to permit effective therapeutic trials. A UK-wide tertiary referral service, designated in 2001 the National Prion Clinic (NPC), at the adjacent National Hospital for Neurology and Neurosurgery, is centrally funded by the Department of Health and closely integrated with the Unit.

The Unit provides a key training resource and centre of specialist expertise in this unique area of biology and medicine and plays a central role in supporting the development of neurodegenerative disease research more widely at the Institute and UCL. The Unit has many local, national, European and other international links and collaborations in prion and other neurodegenerative disease research, and is seen as the most authoritative independent source of advice and opinion by policy makers and media in what continues to be an area of major scientific, public health, animal health, political and economic importance.

Unit mission

This can be considered in four areas:

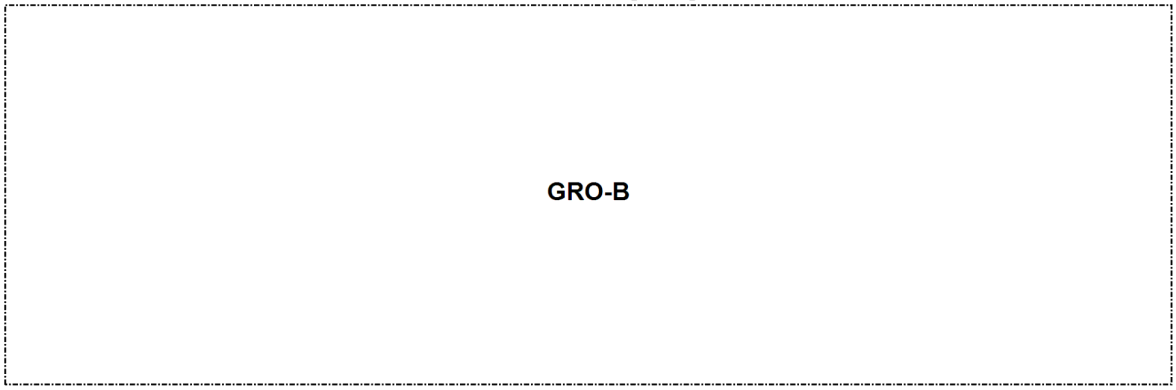
(1) To study the *fundamental aspects of prion biology*. It is often not fully realised outside the field how revolutionary these concepts are. Prions not only constitute infectious agents capable of entering and colonising a host while being composed essentially of a single polypeptide, but the existence of multiple prion strains that can be serially passaged in a range of hosts constitutes protein-based inheritance, both aspects raising challenging evolutionary questions. Similar protein-based inheritance is now well characterised with several distinct yeast and fungal proteins.

(2) These *molecular processes are clearly of far wider relevance in human disease*, and the emerging and rapidly developing field of "protein-misfolding diseases" has prion disease as a key paradigm. The commonest neurodegenerative diseases can be considered in this category, notably Alzheimer's disease, and these processes also appear to be a significant component of normal brain aging.

(3) Tackling the *specific issues posed by BSE/vCJD in the UK* and leading in the development of effective means of prion sterilisation, early diagnosis, and effective therapy and post-exposure prophylaxis. It also provides a national specialist centre to safely handle and characterise existing and emerging human and zoonotic prion pathogens with appropriate biosecurity and expertise. Prion diseases remain a strategic priority area for both MRC and Department of Health and major ongoing challenges and risks continue.

(4) The Unit plays a key role in *training, capacity building and collaboration to help development of neurodegenerative disease research* at the ION/UCL and more widely. The Unit has a key programme in frontotemporal dementia and is developing other projects in neurodegenerative disease, where there are fundamental mechanisms in common with our core research, as well as in areas relevant to differential diagnosis of prion disease. In addition we have ongoing collaborative projects supporting the research of others across the neurodegenerative spectrum at UCL and much more widely.

This mission is inherently long term, ambitious and demanding, and remains essentially the same for the forthcoming quinquennium where we hope solutions for the key public heath issues and effective therapeutics will now be developed built on the work of the past decade. The Unit, while maintaining the tight strategic focus on prion disease needed for real success, sees prion disease as both a paradigm and test bed for other neurodegenerative diseases involving accumulation of misfolded host proteins, notably Alzheimer's, where there are clear interactions and overlapping mechanisms at several levels, and in which new collaborations are being forged.



Research strategy, its evolution and planned developments

Our research programmes at the previous quinquennial review were as follows:

1. Molecular genetic studies of human prion disease susceptibility
2. Quantitative trait studies of genes that modify prion incubation time in the mouse
3. Transgenic modelling of human prion diseases and intermammalian species barriers to prion transmission
4. Normal cellular function of PrP: study of PrP null mice and conditional gene expression studies
5. Molecular and phenotypic analysis of human prion strains
6. Structure of normal and mutant prion proteins
7. Clinical research: diagnostics, therapeutics and kuru field studies

8. PrP in health and disease: Weissmann group

9. Experimental neuropathology: Brandner group

Professor Weissmann retired, as he had planned, from the Unit at the start of the current quinquennium. It was proposed to utilise the resource from his group to establish a Prion immunotherapeutics programme to capitalise on the Unit's success in this area and to develop humanised versions of monoclonal antibodies that had successfully halted peripheral prion pathogenesis in mouse models. The rationale in particular was to be able to provide secondary prophylaxis by passive immunisation should iatrogenic vCJD, for example from blood or blood products, occur. This was not supported at that time. However, following the subsequent recognition of blood transfusion-associated vCJD, MRC asked that we submit an application for an external award to the Unit to develop such antibodies. A progress report on that award is given at Appendix 1.

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The arrival of blood transfusion-associated vCJD during this quinquennium highlighted the need to advance blood-based diagnostics for prion infection. In the absence of such a screening test current risk reduction measures costing the NHS several hundred £M annually, of uncertain necessity and efficacy, continue, as does periodic disruption and anxiety to patients and vulnerable notified at-risk groups numbering thousands. Further, the advances in therapeutics research in the Unit and our major investment to take forward development of both conventional drugs and biopharmaceuticals necessitates parallel advances in early diagnostics if such interventions are to be made before advanced and irreversible neurodegeneration has occurred. CJD is rapidly progressive and patients are always severely impaired, and frequently moribund, at the stage diagnosis is currently reached.

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Unit's competitiveness and distinctive contribution

The Unit transformed the MRC research portfolio in this strategically important field. The breadth as well as depth of our highly multidisciplinary integrated research programmes - spanning structural biology, human and mouse genomics, proteomics, cellular and animal models through to patient-based research including neuroimaging, kuru field studies and national level clinical trials (with Unit staff leading a national NHS clinical facility) and close collaboration with some of the world's leading biotechnology and pharmaceutical companies is unique in the field. While other groups in Europe and the US overlap with parts of our research portfolio, our particular focus on human disease, including our specialist clinic, trials infrastructure and human molecular and population genetics (where the Unit has made many of the key contributions on genetic susceptibility and diagnosis) is unparalleled; our recent work on kuru – the key example of an epidemic prion disease of humans - is unique. The Unit has, and continues to play a wider role in catalysing and underpinning development and excellence in neurodegenerative disease research at UCL.

Achievements during current quinquennium

These are described in detail in section 2. Notable advances in the current quinquennium against our objectives include the following:

- Completion of a successful genome-wide association study in vCJD and the identification of multiple human prion susceptibility genes

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- Proof of principle of blood-based detection of PrP^{Sc} using mouse model

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- Molecular explanation of why vCJD has occurred to date exclusively in *PRNP* codon 129 methionine homozygotes: human PrP V129 incompatible with vCJD prion strain conformation

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- Successful knockdown of PrP expression in polyploid PK1 cells and their reconstitution with other PrP to generate lines propagating hamster and vCJD

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- First antemortem diagnosis of secondary vCJD (related to blood transfusion) and characterisation of pathogenesis and diagnostic features

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Strategic plans for forthcoming quinquennium

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Background

Prions have attracted immense research interest for many years because of their unique composition and properties - being apparently devoid of significant nucleic acid. According to the widely accepted 'protein-only' hypothesis, an abnormal isoform of host-encoded cellular prion protein (PrP^{C}) is converted to an alternative form designated PrP^{Sc}. It is proposed that PrP^{Sc} acts as a template which promotes the conversion of PrP^{C} to PrP^{Sc} and that the difference between these isoforms lies purely in the monomer conformation and its state of aggregation.

In addition to ongoing public health concerns (see below), prions have assumed much wider relevance in understanding neurodegenerative and other diseases involving accumulation of misfolded host proteins ("protein-folding diseases") and analogous processes are described in yeast and fungi involving distinct proteins with prion-like properties. Central to understanding prion propagation remains the conundrum of prion strains - how a protein-only infectious agent can encode information required to specify distinct disease phenotypes – and also the so-called species barrier effect which limits cross species infection. While considered different aspects of the prion problem it is now clear that species barriers and strains are intimately related by "conformational selection", a model we proposed in 1999[2].

The principles of protein conformation-based inheritance that emerged from study of mammalian prions are now strongly supported by elegant studies with analogous systems in yeast models[3]. While at least some yeast and fungal prions may, in certain environments, confer advantage to their hosts, mammalian prions cause massive cell death in the central nervous system. The basis of this profound neurotoxicity has been poorly understood and, intriguingly, it appears to relate neither to PrP^{C} loss of function nor to direct neurotoxicity of extracellular PrP^{Sc}. Understanding these interlinked phenomena will not only facilitate development of diagnostics and therapeutics but will also illuminate processes involving protein misfolding and aggregation, and protein-based inheritance, which clearly have far-reaching implications in pathobiology, ageing and the evolution of cellular processes.

We have recently summarised the Unit's thinking on prion strains and neurotoxicity, and proposed a *general model* of prion propagation in *Science* which also outlines the wider relevance of these emerging concepts in neurodegeneration[4]. While it is clear that some other amyloidotic processes, including features of Alzheimer pathology, are transmissible in an appropriate experimental paradigm, the relevance of prion-like seeded aggregation processes to other neurodegenerative diseases may be fundamental in understanding what triggers disease onset, how the protein-aggregation process spreads in the CNS, and how toxic species are generated in this process, rather than simply whether other neurodegenerative diseases might be infectious. In this regard, prion disease not only provides the archetypal paradigm, but also supplies a body of experience and specialised techniques. Not least these include animal models which are without parallel in terms of their faithful recapitulation of human pathology. Indeed, it could be argued that these are not really models at all, since laboratory mammals are naturally susceptible to these diseases. The remarkably consistent pathogenesis and incubation periods seen in prion transmission in inbred mice (with all animals succumbing to disease over a 2-3 day period after a silent incubation period that may be over a year) provides an extraordinary tool for assessment of disease modifiers. Indeed, the utility of these models for assessment of putative generic treatments for neurodegenerative processes (for example by stimulating cellular response to misfolded proteins) is clear.

Owing to our fundamental understanding of prion diseases, there is a real possibility that they will be the first neurodegenerative disorders for which effective treatments become available. Such studies may provide a key paradigm for studying the pathways

of late onset neurodegeneration and the ability of the brain to recover function following therapeutic intervention: much will be learnt of far wider relevance in neurodegenerative disease.

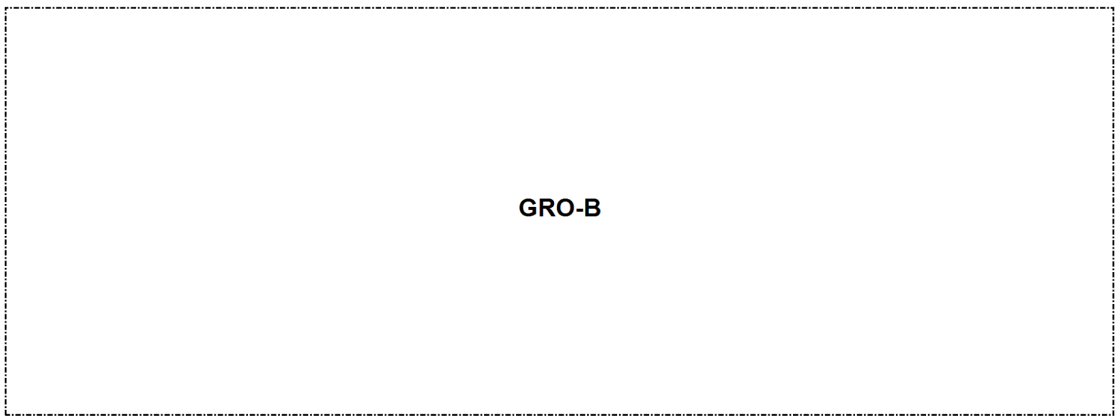
The aims of the Unit remain the achievement of a comprehensive understanding of the molecular basis of prion propagation, strain diversity and neurotoxicity with effective translation to public health and clinical care.

We will develop and validate early diagnosis of, and curative treatment for, prion infection. The Unit has now developed an unparalleled range of closely integrated and interdependent teams spanning basic molecular biology, through cellular and animal models to clinical research and a full (and proven) national clinical trials capability in a neurological disease. This has been built on nearly twenty years of the Director's experience in this field and in partnership with superb collaborators and industrial colleagues. Given the substantial advances and successes at the Unit, and the critical mass and necessary collaborations and industrial support now fully established, we consider this achievable within a decade. This will essentially eradicate the public health and economic risks posed by prions, and provide crucial insights more broadly in neurodegeneration.

Rationale for Unit level support

Why is a Unit essential to achieve these goals? The success of a highly multidisciplinary research objective such as this is critically dependent on all the key elements being supported on a stable long term basis to retain and support the necessary staff and the highly specialised facilities needed. A full Unit structure and firm leadership are essential to ensure sustained long-term coordinated focus on a common agreed mission. The added scientific value and synergy generated is obvious to all who visit the Unit, and is also very relevant to our proven ability to translate basic advances to clinical practice. All our groups are highly interdependent: few publications now come from a single programme. A significant critical mass and an efficient integrated administrative and governance infrastructure is needed to be effective in this regard.

Our mission is disease-focussed and highly translational. Success requires effective management of the interface between basic research and clinical practice. The Prion Unit is led by a clinician scientist and Unit clinical staff lead the specialist national referral service for prion disease. The Unit is therefore exceptionally well placed to perform effective translational research, and the success and reputation of the Unit's basic research has allowed it to attract substantial Department of Health funding to support our clinical, translational and therapeutics research adding enormous value to MRC investment. The major awards in therapeutics research (both in monoclonal antibodies and small molecules) were, and are, only possible in the context of an established Unit with the long-term resource, infrastructure and expertise to support and deliver on such ambitious projects in an academic environment. The Unit allows such projects to be realised at a small fraction of the normal commercial costs of such endeavours.



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Continuing public and animal health and economic challenges posed by prions

The arrival of vCJD and the confirmation that it was caused by the prion strain type responsible for epidemic BSE, led to a major public and animal health crisis. In the UK, there was widespread population exposure to BSE prions and concerns that a significant human epidemic would follow. The total UK cattle epidemic was estimated at around 2 million infected animals[5]. Thankfully, numbers of confirmed clinical cases of vCJD (around 200) have been modest, given that the majority of the UK population born before 1996 will have been exposed. Annual numbers of cases have been falling for some years. While this is encouraging, there are strong grounds for caution.

Human prion infections are associated with extremely prolonged and variable incubation periods which can exceed 55 years[6] and major genetic effects on incubation period at multiple loci have been identified in mouse and now in humans[7-13]. It would be surprising were vCJD to have peaked and disappear so quickly given our experience with both iatrogenic CJD caused by treatment with contaminated pituitary hormones and kuru, each with a mean of around 12 years. For vCJD to indeed have peaked in clinical onsets in 1999, this would suggest a mean incubation period of BSE in humans of less than 10 years (given the peak exposure of the UK population to BSE prions occurred around 1990). Transmissions of prions between species are invariably associated with prolongation of incubation periods when compared to within-species passage. It is possible therefore that cases to date have been in individuals with short incubation time alleles at several loci, in addition to being *PRNP* codon 129 MM, and that others may follow.

Further, a retrospective study of archived surgical lymphoreticular specimens estimated a much higher population infection prevalence in the UK population of 237 per million (95% confidence interval 49-692 per million) [14] than the number of clinical cases would so far suggest. A much larger, prospective, national-scale anonymous screen of discarded tonsillectomy tissue is being performed by the Health Protection Agency, and autopsy screening is recommended by SEAC to get better estimates of infection prevalence. The national tonsil study, although negative to date, has not been powerful enough to significantly narrow these estimates[15]. In addition, the sensitivity of such tests at different points in the prolonged incubation period and in different *PRNP* codon 129 genotypes is unknown. A further issue is that subclinical carrier states of prion infection are now well recognised in animals[16-22], including following low dose oral exposure[23,24], and the Unit plans to investigate whether human subclinical states exist. Such carriers, who cannot currently be indentified, will represent a risk to others via blood or tissue donation or contamination of surgical instruments.

Four patients have now been identified since 2004 with vCJD prion infection from a small cohort of surviving individuals (currently around 23) known to have received blood transfusion from asymptomatic donors who subsequently developed vCJD[25-27]. All were exposed to only a single unit of vCJD-implicated red cells. It appears therefore that vCJD may be transmitted relatively easily by blood transfusion and the risk to the

remaining surviving recipients is very high. Over 6000 individuals in the UK known to have received implicated plasma products from these same donors remain at risk and have been notified of their exposure. Recently, one of these was found to have vCJD prion infection at autopsy[28].

A number of risk reduction measures have been introduced, including leucodepletion of all UK blood, importation of all plasma (from the USA) for production of plasma products and importation of blood for transfusion to BSE-unexposed children. Use of prion-reduction filters for all blood is being considered. As there is no screening test for blood to date, it is unknown how many in the population have been exposed to contaminated blood products from healthy individuals carrying vCJD prion infection. The development of blood tests to detect vCJD prion infection is likely in the next few years and this may give better estimates of infection prevalence. Since first generation tests are likely to have a significant false positive rate, this will also raise considerable ethical and practical problems as the Department of Health has indicated that donors testing positive would be notified and no treatment is yet available.

Prions resist conventional sterilisation techniques and adhere rapidly and avidly to metal or plastic surfaces from which they can efficiently transmit infection in experimental models[29]. Classical CJD has been transmitted iatrogenically by surgical instruments and transmission of vCJD by this route, although not yet confirmed, is likely to occur. Over 100 individuals have already been notified by HPA of their risk status after exposure to surgical or medical instruments previously used on a prion-infected patient. The tissue distribution of infectivity is much more widespread in vCJD than in classical CJD, with marked colonisation of lymphoreticular tissues[30] and blood is now known to be infectious. Until new sterilisation methods are widely introduced into the NHS, and/or blood screening tests to identify infected patients are developed, there will be continued distress and significant costs from quarantine and destruction of instruments including fibreoptic endoscopes used on a patient subsequently recognised to have a prion infection or disease. Of course there may also be considerable preventable transmission of a lethal infection. Major concerns of iatrogenic transmission by dentistry are being considered at present and the Chief Dental Officer has circulated new guidance recently on reuse and sterilisation of dental instruments.

Regardless of the prevalence of vCJD infection, the other prion diseases, although rare, present on ongoing challenge. Sporadic CJD occurs in all countries with a random case distribution and an annual incidence of 1-2 per million (around 6-12 thousand cases annually worldwide). Acquired prion diseases include iatrogenic CJD arising from accidental exposure to human prions through medical or surgical procedures. Inherited prion disease is a major cause of inherited early onset dementia, associated with over 30 coding mutations in the prion protein gene (*PRNP*)[31], and healthy at-risk family members also pose an potential iatrogenic risk to others.

The origin of the BSE strain causing epizootic disease remains unclear and the emergence of other lethal human pathogenic prion strains from animal prion diseases clearly remains a possibility. Scrapie is a worldwide endemic disease of sheep and goats and a number of different strains have been isolated from natural cases. Modern humans are clearly highly resistant to dietary infection with sheep scrapie although our genetic studies of human populations suggests that major prion disease epidemics may have occurred in human evolution[32]. Recently "atypical scrapie"[33], perhaps selected by the breeding programmes that aimed to eradicate sheep scrapie, has been recognised and its transmission characteristics in models of human susceptibility are being examined currently at the Unit and elsewhere. The emergence of other new or newly recognised strains is to be anticipated, and two novel bovine prion strains have recently been identified; transmission studies suggest a clear potential for human pathogenicity[34,35]. Prion strains are known to adapt and mutate on passage in new species, and within species as a result of PrP polymorphisms and other genetic factors (see[4] for review). For some time it has been EU-wide policy to move to eradication of animal TSE's, in large part because of these uncertainties.

The BSE epidemic has been a disaster for UK and EU agriculture. GRO-B GRO-B GRO-B Despite the low numbers of vCJD clinical cases to date, the economic costs continue, in part because of major uncertainties about risk. Over GRO-B has been invested in improving UK hospital sterilisation practices and the costs associated with measures to protect the blood supply discussed above runs into GRO-B £M annually for the NHS. Internationally, even the detection of a single infected bovine can lead to economic costs of $billions, for example most recently in Canada and Japan.

The *scientific* case for continuing a long-term strategic investment in prion research can be made in view of the intrinsic interest and importance of the fundamental biology and its growing relevance to other far commoner neurodegenerative diseases. However, the public health and economic case to follow through UK investment in prion research to effective diagnostics and therapeutics also seems clear. Prion research remains a strategic priority for both MRC and the Department of Health.

Key objectives for next quinquennium

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- To understand the basis of differential cellular susceptibility to prion infection and develop vCJD prion-permissive cell lines suitable for their rapid bioassay
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Here I briefly outline our principal research themes to illustrate how we propose to achieve the strategic targets described above. Our mission is ambitious, challenging, and in part high risk - as it should be. We plan to achieve this by the following approaches:

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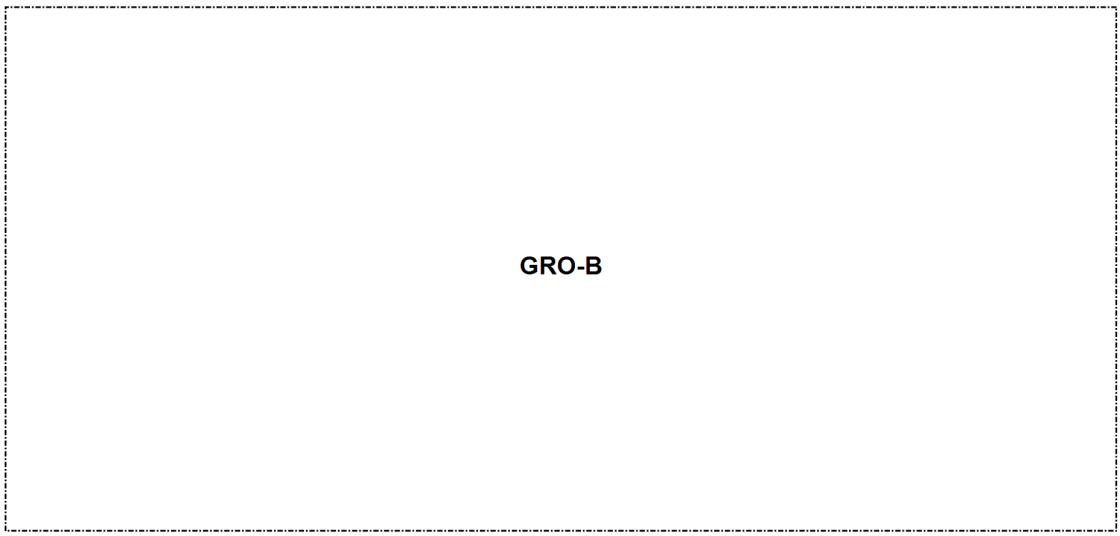
Understanding prion strains and transmission barriers
How prion strain diversity is encoded by an apparently protein-only agent remains a challenging questions in biology and the precise molecular determinants remain unclear. A major long-term effort in the Unit to purify PrP^{Sc} from multiple well defined animal and human strains to near homogeneity has now reached the point where structural studies are possible and a key aim is to define the molecular basis of prion strain diversity. In addition to the intrinsic biological interest in addressing this question, such characterisation will have immediate practical consequences in studying human prion disease where classification of human strains has been hindered by the difficulty and

variability in the biochemical methods used to distinguish PrP^{Sc} types. Such molecular classification would facilitate aetiological and epidemiological studies of so-called "sporadic CJD" and evaluation of relationship between human and animal strains. Major biochemical characterisation studies in the Unit (Dr Jonathan Wadsworth, Programme 6) are closely integrated with transmission studies in cellular (Dr Peter Kloehn, Programme 5) and wild type and transgenic mouse models (Dr Emmanuel Asante, Programme 3) to biologically characterise strains. The potential of newly recognised animal prion strains to pose risks to public health is under evaluation by examining their ability to transmit infection to transgenic mice expressing human PrP in comparison to our extensive transmission experience of all forms of human and animal prion diseases.



Prion cellular models and assays
The Unit's development of an automated cell-based prion bioassay (ASCA) has transformed much of our work in that we can perform up to 600 bioassays weekly (Programme 8). We will further optimize and robotize this method to allow even higher throughput and consistency of assays and to meet increasing Unit-wide demand. Importantly we have developed cells capable of propagating Sc237 hamster prions and vCJD human prions and plan ASCAs for these during the next quinquennium. Our PrP-silenced neuroblastoma cell lines, that can be reconstituted to propagate RML prions by re-introduction of mouse PrP will allow a reverse genetics approach to determine which parts of PrP^{C} are critical for efficient propagation. Although PrP^{C} is an absolute requirement for the propagation of prions, relative cellular susceptibility does not relate to PrP^{C} expression level and clearly factors other than PrP^{C} are necessary. Dr Peter Kloehn (Programme 5) has isolated cell lines that are either highly susceptible or resistant to prion infection but show no difference in PrP^{C} levels and Professor Jat and Dr Kloehn will use genome-wide screens to identify these susceptibility factors.

Animal models of prion disease and preparation for pre-clinical studies
The Unit has made a major long-term investment in the development of a range of transgenic mouse models to tackle a series of key questions in prion disease. Our long term work modelling human susceptibility to BSE and vCJD strains, and characterising novel strains emerging on passage of BSE in different *PRNP* codon 129 genotypes continues, as does work to characterise and isolate the full range of naturally occurring human prion strain types in serial passage studies. Programme 3 will generate many mouse models to support work across the Unit, including prion modifier and resistance genes, role of PrP glycosylation in strain propagation, modelling frontotemporal dementia and production of improved human models. Long term modelling of inherited human prion diseases continues, in particular to address the controversial issue as to whether *de novo* infectivity can be generated in mice. Importantly, studies will be completed in several models to prepare for preclinical assessment of candidate therapeutic agents against peripheral and CNS propagation of RML and CJD prions.

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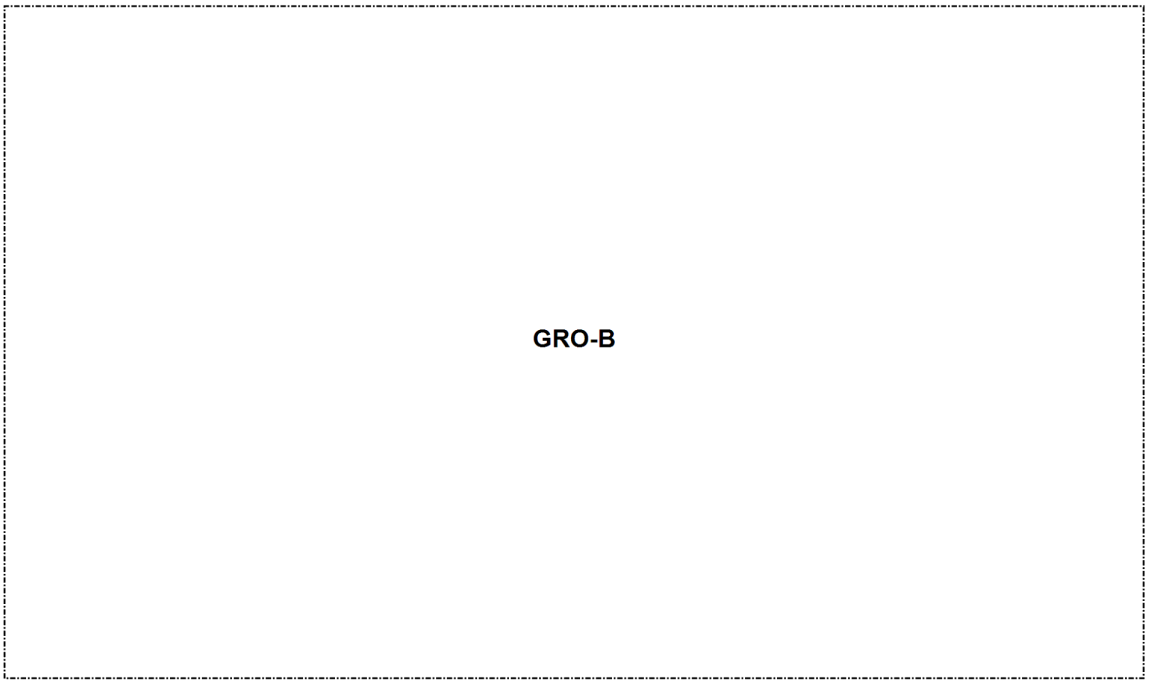
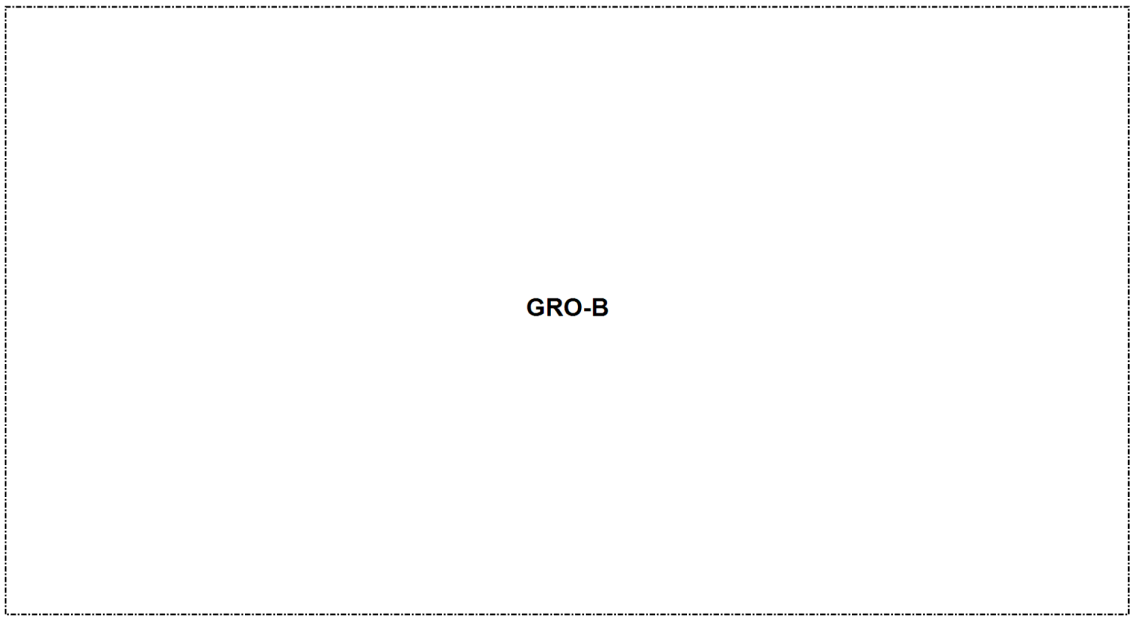
Molecular diagnostics

A new programme was established in the Unit in response to the arrival of blood transfusion-associated secondary vCJD and the consequent threat to the UK blood and blood product supply (Dr Graham Jackson, Programme 9). A blood test to detect the infected state would allow screening of donated blood and patients undergoing surgery, and reduce both the risk of secondary transmission and the major (several hundred £M annually) current costs to the NHS arising from these uncertainties. The realistic potential for effective therapeutics also makes the development of means for diagnosis at the earliest clinical stages, before extensive irreversible CNS damage has ensued, imperative. Significant advances have been made at the Unit recently with parallel work on prion capture from whole blood (using novel monoclonal antibodies developed at the Unit which can now immunoprecipitate native PrP^{Sc}), *in vitro* amplification of PrP^{Sc} (using a range of published and novel methods developed at the Unit) and exquisitely sensitive in-house ELISA. Combinations of these methods, on which MRC have recently filed patent protection, are now allowing prion detection in blood from mouse models during the asymptomatic incubation period. Much further basic as well as development work will be required, following this proof of principle, to achieve effective detection from vCJD prion infected blood. These studies are crucially supported by Programme 11 enabling collection of samples for blood test development and validation, including

collection of serial samples from patients known to have received vCJD-implicated blood transfusions where the incubation period is precisely defined.



Our long term kuru field work will complete its study of determinants of the kuru epidemic. Our field base in the remote Eastern Highlands of Papua New Guinea is perhaps the farthest flung outpost of the MRC. However, this highly successful project is of wide ranging relevance, as we are studying not only the major epidemic of human prion disease, but one that has now essentially completed its course with direct relevance to understanding vCJD. A particular aim in the forthcoming quinquennium is to study a cohort of elderly survivors known to have been exposed to kuru over 50 years ago, in particular with respect to whether subclinical carrier states of prion infection occur and how its pathogenesis differs from conventional infection.



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Monitoring of progress

Programme plans, and all significant changes to them during the quinquennium, are agreed in writing between Programme leaders and the Director which includes outline timelines for completion of projects, where this is appropriate. Research updates are given by all staff at a weekly Unit-wide laboratory meeting on a rotating timetable. More rigid project planning is necessary in some cross-programme activities and particularly so in our translational work with external and commercial partners. The Director reviews progress in regular (flexible, but generally monthly) meetings with Programme leaders who provide brief monthly to quarterly reports. Some large-scale cross-cutting activities are managed by formal virtual groups with regular meetings of participating Programme leaders usually chaired by the Director and *ad hoc* attendance of other staff and collaborators as required.

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Unit setting, collaborations and major interactions

Alignment of the Unit with MRC plans
The Unit has a key role in MRC's research investment in neurodegeneration and is also firmly engaged with the MRC translational and public health agenda as was acknowledged by the MRC *Strategic Review of Neurodegeneration*[1].

The Unit will continue to build its collaborations nationally and internationally. We are keen to support capacity building and training in UK neurodegeneration research and to assist others to utilise the fundamental molecular mechanisms studied by the Unit which are clearly of wide relevance[4,53,54]. This was, again, firmly endorsed by the *Strategic review* (page 5): "Whilst the MRC should strive to increase its investment in other areas of neurodegenerative disease, the knowledge gleaned from prion research should inform research on other neurodegenerative diseases since several of these involved protein misfolding."

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Host University and other research establishments
The MRC Unit is a key part of UCL Biomedicine strategy and of fundamental importance to the ION which now has one of the largest programmes on neurodegenerative disease research in Europe, and which is fully competitive with leading European and US centres. The Unit is the core of the ION Department of Neurodegenerative Disease and together they occupy two floors of Queen Square House (and will from mid-2009 occupy a third being jointly refurbished by MRC and UCL).

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Participation in EU research networks
The Unit Director has led major (10 centre) EU concerted action programmes continuously for around 10 years prior to the consolidation of EU prion research funding into the *Neuroprion Network of Excellence* of which we are a participating centre and the Director is a member of the Executive Committee. We were also a partner in the EU TSELAB collaboration (*Human transmissible spongiform encephalopathies: The European diagnostic laboratory*) led by Professor Herbert Budka in Vienna.

The Unit leads the international (UK London and Cambridge, Sweden, Denmark) FReJA consortium which studies frontotemporal dementia.

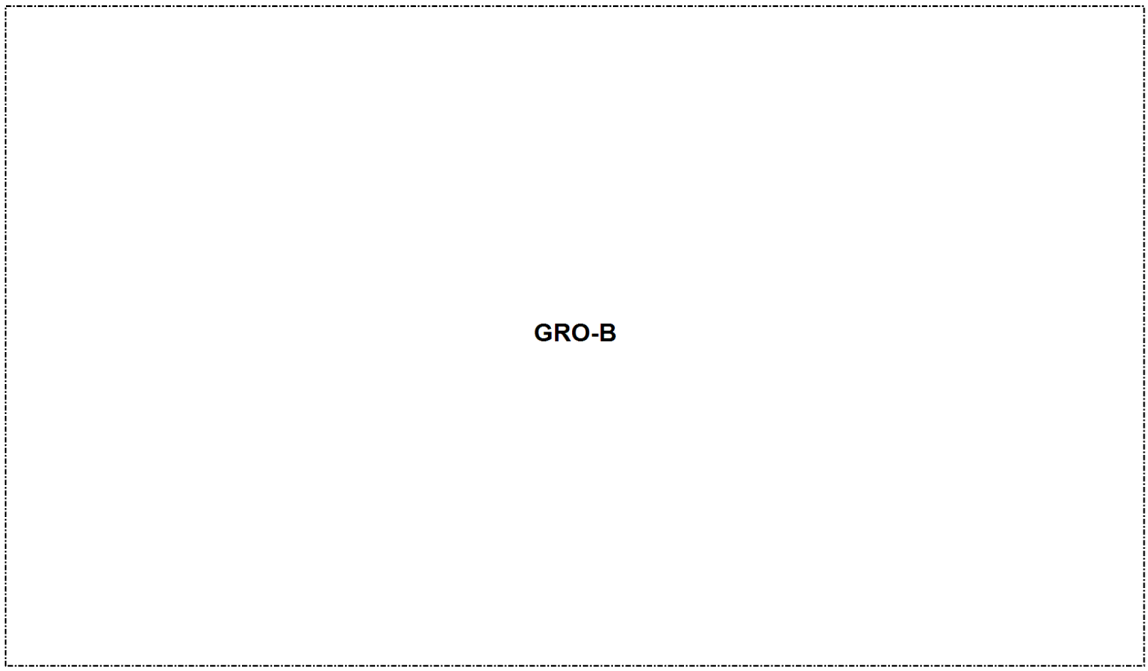
National Institute for Health Research (NIHR)
The Unit is fully engaging in NIHR initiatives and the Unit Director was recently appointed as one of the inaugural NIHR Senior Investigators of the NIHR Faculty. The NIHR funded UK coordinating centre in clinical neurodegeneration research (DENDRON) is led from the Department (Professor Rossor) and our recently funded (Department of Health) National Prion Monitoring Cohort is being incorporated into this important network. UCLH Trust hosts an NIHR Comprehensive Biomedical Research Centre. This has a major neurodegeneration theme group linking translational neurodegeneration research across UCL Hospitals, with the MRC Unit Director as the current theme leader.

NHS

The Unit is adjacent to the National Hospital for Neurology and Neurosurgery, part of UCLH NHS Foundation Trust, at which all clinical staff of the MRC Unit hold honorary appointments. Unit consultant staff run the Department of Health funded NHS National Prion Clinic (Clinical Lead: Dr Simon Mead), an NHS tertiary referral service at the National Hospital. Under a UK-wide agreement instituted by the Chief Medical Officer, all suspect cases of prion disease are referred by neurologists to both the National Prion Clinic and the National CJD Surveillance Unit in Edinburgh (NCJDSU) with which we jointly coordinate all patient-related activity to support their surveillance function and epidemiological research.

Government Departments

We have extensive interaction with Government Departments at all levels (Department of Health and DEFRA) and Unit staff sit on many advisory committees at local, national and international levels including NIHCE and WHO. The Director has often been called for direct meetings with the Chief Scientific Advisor, Chief Medical Officer (CMO) and Cabinet Ministers. The Unit performs collaborative work with DEFRA via the Veterinary Laboratories Agency and we have research collaborations with the Health Protection Agency (National Prion Monitoring Cohort) with whom we work closely on management of at-risk groups accidentally exposed to prions via NHS treatment. The Director is a member of SEAC.



Translational successes

Given the major public health issues raised by BSE, it was always envisaged that the Unit would have a key strategic role in addition to its core objectives in both basic and clinical research. We have been asked to take on a number of additional projects to address public health questions and aid policy makers. Some are already completed and have a direct impact on patient care and public health policy:

- Developed an ultra-sensitive immunoassay for PrP^{Sc} in human tissue and determined its tissue distribution in vCJD[30] (to inform the Department of Health's risk assessment with respect to iatrogenic transmission of vCJD via contaminated surgical instruments)

- Developed and validated tonsil biopsy for antemortem (and early) diagnosis of variant CJD[55], now part of national and WHO diagnostic criteria.

- Performed anonymous screen of 2000 tonsils for evidence of prion infection for Department of Health[56]. These methods are now being used in testing the National Anonymous Tonsil Archive by the Health Protection Agency.

- Developed novel enzymatic methods to decontaminate prions and study their properties when adherent to metal surfaces[29,57,58], and novel bioassays with extended dynamic range to validate decontamination protocols[58]; we have developed a practical decontamination product with GRO-B GRO-B now available for use in NHS for surgical and dental instrument decontamination (reduces surface bound infectivity by a million-fold following a non-corrosive simple 10 minute pre-soak procedure)

- Developed a highly sensitive rapid tissue screening test for PrP^{Sc} now licensed to and marketed by GRO-B for animal testing to protect the food supply.

- Successfully completed the UK's first clinical trial in prion disease, PRION-1, formally evaluating the drug quinacrine and establishing a protocol and infrastructure for subsequent trials[59].

Principal scientific achievements during review period and key future aims

Programme 1: Human molecular genetics and bioinformatics

GRO-B

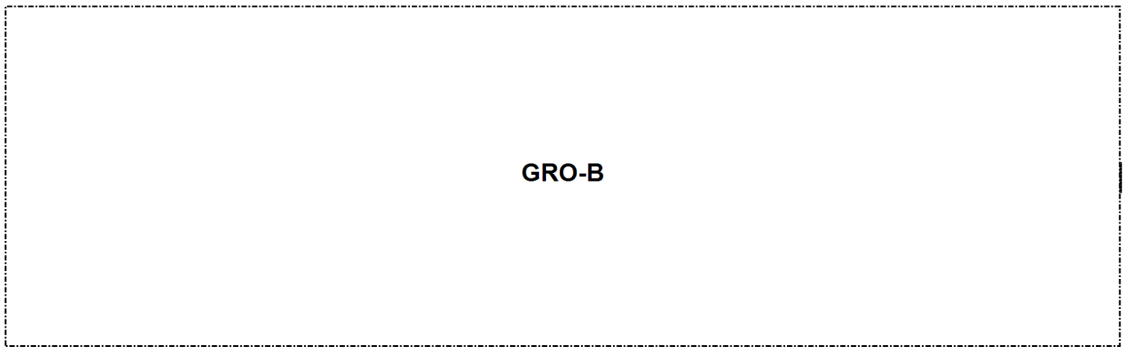
GRO-B In the UK, we were one of the first research groups to embark on genome-wide association; these studies have identified several candidate common variants in vCJD to take forward to functional analyses. Integration of mouse and human genetic studies (Programme 2) led to the characterisation of *Hectd2* as an incubation time factor in both species. We have developed a data integration framework to incorporate several Unit transcriptome experiments. We continue to build a large sample cohort in neurodegeneration and contribute to UK collaborative efforts to identify genes in related dementias. In the next quinquennium we will conduct international collaborative genome-wide association and meta-analysis in sporadic CJD. We will monitor re-sequencing technologies with the advent of genome re-sequencing in mind. GRO-B

GRO-B

Programme 2: Prion genetic modifiers in the mouse and Annex 1: CELLGENE virtual group

During the last review period our goal was to identify quantitative trait genes for prion disease incubation time in mice. Previous studies identified multiple loci across the genome, however, these were too large for candidate gene analysis. Using a heterogeneous stock (HS) of mice we successfully reduced the regions of interest to 1-2Mb on mouse chromosomes 8, 9, 11, 15 and 19. Detailed sequencing, expression analysis and genotyping identified individual candidate genes on *Mmu*11 (*Sp6*), 15

(*Cpne8*) and 19 (*Hectd2*) and significantly reduced the gene list for *Mmu*8. Further, a joint project with Programme 1, showed an association between *HECTD2* haplotypes and susceptibility to both vCJD and kuru and a genotype-dependent differential expression of *Hectd2* mRNA and a significant up-regulation in mice at the terminal stage of disease. We broadened our search for candidate genes by combining incubation time data from inbred mouse strains with whole genome mRNA profiling. In addition, we developed two new transgenic models where our existing human PrP (V129 and M129) transgenic mice were backcrossed onto an SJL background with the aim of providing a model with a shorter incubation time for human prions. We have also extended our work characterising two prion strains derived from BSE (MRC1 and MRC2) and showed that MRC1 is indistinguishable from the RML mouse-adapted scrapie prion strain. A key aim for the coming quinquennium is to continue our search for candidate genes by carrying out genome-wide screens of our existing crosses and examining new crosses as they become available.



Programme 3: Transgenic modelling of human prion diseases, intermammalian transmission barriers and assessment of candidate therapeutics

The *PRNP* codon 129 polymorphism has been of seminal importance in understanding human susceptibility to acquired prion infection and has been comprehensively modelled in transgenic mice. Prior to the review period, we established that vCJD is the human counterpart of BSE and we were able to reproduce the characteristic neuropathology and molecular strain type of vCJD in transgenic mice expressing 129M human PrP. We have since provided an explanation using transgenic models as to why vCJD has been restricted to the *PRNP* 129MM genotype: human PrP V129 appears unable to adopt the characteristic PrP^{Sc} conformation of vCJD and indeed may have a dominant negative effect. We further showed that mice modelling the *PRNP* 129MV genotype can develop four distinct disease phenotypes after challenge with BSE prions or vCJD prions, with a dissociation of the molecular and neuropathological phenotype of vCJD, suggesting that human heterozygotes infected with BSE prions may present with a phenotype distinct from vCJD. These findings are consistent with the Unit's *conformational selection model* of prion strains and transmission barriers. We have also performed extensive modelling of inherited prion disease. Previous studies have modelled human pathogenic mutations on mouse PrP, where they may have different structural consequences, and it remains controversial whether infectious prions have been generated in these models. In contrast, we have studied mutant human PrP and have produced spontaneous prion disease and infectious prions in a transgenic model. Several other models are yielding insights into the effect of PrP point mutations on PrP^{Sc} assembly and strain propagation. Key future aims are to establish the full range of prion strains causing human disease using newly-derived congenic lines in large-scale transmissions of well characterised human phenotypes to try to resolve the diversity of naturally occurring human prion strains and their relationship with animal prion diseases. We will attempt to biologically clone these for biochemical study with programme 6. Mice expressing human PrP with defective glycosylation will be used to explore the role of N-glycosylation in prion strain diversity and propagation. This programme will also characterise and develop new animal models of susceptibility with novel naturally occurring and experimental *PRNP* alleles and prion modifier genes identified by Unit-wide genomic and transcriptome analysis. Mouse models of frontotemporal dementia are being characterised with

Programme 10. As much effort in the Unit is now focusing on the development of therapeutics, the development and use of standardised models for evaluation of candidate therapeutics will form an increasing part of this programme which also provides the Unit's Transgenic Core facility and supports animal research throughout the Unit

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Programme 6: Molecular and phenotypic analysis of prion strains
How prion strain diversity is encoded by an apparently protein-only agent remains one of the most interesting and challenging questions in biology, with significant evolutionary implications. Despite much worldwide research effort the precise composition and the molecular determinants of strain remain unclear. During this quinquennium Programme 6 has made highly significant progress. Using the RML prion strain in conjunction with our rapid automated cell culture bioassay (ASCA), novel protocols have been developed for isolation of highly purified infectious prions. ASCA has revolutionized our ability to systematically explore methods for prion purification and conduct meaningful structure/function studies that would be otherwise impractical. Concomitantly, significant progress has been made in further defining the diversity and molecular basis of human prion strains resulting in publications across all of the major aetiological categories of human prion disease. A major achievement during the review period has been to provide a molecular basis for how human PrP polymorphic residue 129 acts as the key genetic susceptibility factor for sporadic and acquired prion disease. Transgenic studies indicate that this polymorphism directly limits human prion strain transmission by dictating the thermodynamic permissibility of distinct human PrP^{Sc} conformers and associated patterns of neuropathology. Further achievements have been to characterise the central and peripheral pathogenesis of secondary vCJD caused by blood transfusion, definition of the strain properties of kuru prions and provision of new insight into the molecular mechanisms underlying the phenotypic diversity seen in inherited prion disease. The emergence of newly recognised, potentially zoonotic, prion strains is likely to continue during the next quinquennium and the internationally recognized expertise of programme 6 provides a key resource to safely handle and comprehensively characterise existing and emerging human and animal prion pathogens with appropriate biosecurity and experience. The central aim of Programme 6 will be to exploit our novel methods of prion purification to accurately define the molecular composition and ultra-structure of infectious prion strains working with Programme 7, and to apply these methods to fully define the diversity of human prion strains.

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Programme 9: Molecular diagnostic strategies in prion disease

This Programme was formed in 2007; previous work by Dr Jackson was performed under the former Programme 6 where key achievements included producing unique monoclonal antibodies capable of reacting with and selectively enriching disease-associated PrP. We have made significant progress in using these antibodies to devise methods for capturing low levels of PrP^{Sc} spiked into large volumes of whole blood. GRO-B

GRO-B

GRO-B

Programme 11: Clinical research studies in the UK and PNG

A: UK Clinical research

The Unit's clinical research is vital to our translational agenda and is based at the NHS National Prion Clinic (NPC). Under a national referral protocol agreed early in the current quinquennium, the NPC, together with the National CJD Surveillance Unit, is notified of all patients with suspected prion disease. Close liaison with the NCJDSU is maintained to coordinate respective roles. This agreement has greatly facilitated our clinical research as well as allowing all patients that wish access to our specialised NHS clinical service at the National Hospital, led by Unit staff. We
have completed the first UK controlled trial in prion disease, the largest conducted internationally to date, establishing the infrastructure to conduct trials in these rare and rapidly fatal neurodegenerative diseases. While we found no evidence that quinacrine prolonged survival in prion disease, much was learned from detailed assessment of the measures and protocol used to refine future trial design. GRO-B

GRO-B

GRO-B We will have a particular focus on identifying the earliest stages of disease in iatrogenic and genetic at-risk individuals and will develop a prion disease staging scale. Serial blood samples are taken for our diagnostics research (Programme 9) and also provided to the CJD Resource Centre at NIBSC. Clinical samples contribute to a substantial proportion of the Unit's research,

notably the genome-wide association studies and discovery of novel *PRNP* mutations and genotype-phenotype studies (Programme 1).

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B: Kuru field studies in Papua New Guinea

Our field studies on kuru, the principal example of epidemic human prion disease, have provided much information of direct relevance to understanding vCJD in the UK. They have also been key to major advances over the current quinquennium in understanding genetic susceptibility to acquired prion disease. We have identified and studied all cases of kuru since 1996, provided new insights into cultural practices across the remote kuru-affected region of the Eastern Highlands Province that affect kuru epidemiology and characterised the remarkable range of incubation periods, up to 55 years so far, possible in human prion infection. The spiritual significance and cosmological beliefs surrounding the mortuary practice of transumption, the consumption of the bodies of the dead by living relatives, have been documented for the first time. We performed an autopsy on a recent case, the first for nearly 30 years, allowing comprehensive examination of peripheral pathogenesis. Allied with transmission studies, we determined the prion strains causing kuru and were able to conclude that the distinctive tissue distribution of infectivity in vCJD related to prion strain type, not the oral route of exposure. Our genetic studies unexpectedly provided wider insights into human evolution, with evidence that kuru-like epidemics may have occurred before in our early ancestors and led to discovery of a new genetic resistance factor that may provide complete resistance to kuru. For future plans, we propose a continuation of epidemiological surveillance to follow the epidemic to its end, to complete a comprehensive kuru database (now fully maintained by this programme) and use this to perform comprehensive epidemiological analyses in conjunction with emerging information on mortuary feast practices that affected transmission. Our key field task is to investigate whether subclinical carrier states of kuru exist and if so how its pathogenesis differs from conventional infections. Elderly survivors of the kuru epidemic, now half a century after cessation of prion exposure, provide a unique opportunity to address this issue. The issue of human carrier states is of considerable public health importance, and scientific interest, and kuru offers a unique opportunity to address this. Detailed characterisation of the tissue distribution of infectivity in kuru will continue with Programme 6 and studies on genetic resistance factors to acquired prion disease with Programmes 1 and 2. Elderly survivors are the main focus of our work: they are highly enriched for resistance genes, having been exposed to the strongest episode of selection yet documented in humans, and are the only members of the community left with direct experience of the traditional cultural practices essential to a full understanding of the kuru epidemic. Given the age of this cohort, this is the very last opportunity to conduct such studies and obtain information of major cultural, scientific and biomedical importance.

CV - *completed for each programme leader and programme leader track scientist.*

Name	Professor John Collinge
Current Post incl. start date	Honorary Director, MRC Prion Unit: 1998-current Established Chair; Professor of Neurology and Head, Department of Neurodegenerative Disease, UCL Institute of Neurology: 2001-current Honorary Consultant Neurologist and Clinical Director, National Prion Clinic, National Hospital for Neurology and Neurosurgery: 2001-current
Employer	University College London
Programme	Director and Programme 8 – Prion Kinetics, Toxicity and Synthesis

Previous Posts (including studentships and fellowships)

Institution	Position Held	Start Date	End Date
Imperial College London	Professor of Molecular Neurogenetics and Honorary Consultant Neurologist	1994	2001
	Wellcome Principal Research Fellow in the Clinical Sciences	1996	2000
	Wellcome Senior Research Fellow in the Clinical Sciences	1992	1996

Educational Qualifications

Degree Type	Degree Class (if appropriate)	Year	University	Subject
BSc(Hons)	First	1981	Bristol	Pharmacology
MB,ChB		1984	Bristol	Medicine
MRCP		1988	Royal College of Physicians	Medicine
MD		1992	Bristol	Medicine
FRCP		1998	Royal College of Physicians	Medicine
FRCPath		1999	Royal College of Pathologists	Neuropathology
DSc *Honoris causa*		2008	Bristol	Medicine

Clinical Qualifications

Are You Clinically Qualified?	Yes
Are You Clinically Active?	Yes
Are You Active In Clinical Research?	Yes

Personal Awards/Achievements/Membership of prestigious academic research based bodies (e.g. Fellowships of the Royal Society, Academy of Medical Sciences)

Linacre Medal, Royal College of Physicians 1992
Graham Bull Prize, Royal College of Physicians 1993
Alfred Meyer Medal, British Neuropathological Society 1997
Founder fellow, Academy of Medical Sciences 1998
Howard Taylor Ricketts Medal, University of Chicago 2001
Commander, Order of the British Empire, HM The Queen 2004
Jean Hunter Prize, Royal College of Physicians 2004
Fellow of the Royal Society 2005
Doctor of Science *Honoris causa*, University of Bristol 2008
Inaugural Senior Investigator of the Faculty, National Institute for Health Research 2008
Nineteen named lectureships

Publications (List all publications arising from current QQR period and if relevant up to 10 other key publications)

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CV - *completed for each programme leader and programme leader track scientist.*

Name	Dr Graham S Jackson
Current Post incl. start date	Programme Leader (March 2007)
Employer	MRC
Programmes	Programme 9 - Molecular Diagnostics, March 2007-present. Programme 6 - Structure of normal and mutant prion proteins – March 2003 to February 2007

Previous Posts (including studentships and fellowships)

Institution	Position Held	Start Date	End Date
MRC Prion Unit	Programme Leader Track	December 2001	February 2007
MRC Prion Unit	Postdoctoral Researcher	April 1999	November 2001
Department of Neurogenetics, Imperial College, London.	Postdoctoral Researcher	April 1996	March 1999
Department of Chemistry, University of Bristol.	Postdoctoral Researcher	January 1994	March 1996

Educational Qualifications

Degree Type	Degree Class	Year	University	Subject
PhD		1994	Bristol	Biochemistry
BSc (Hons)	Upper Second	1990	Leicester	Biological Science

Clinical Qualifications

Are You Clinically Qualified?	No
Are You Clinically Active?	No
Are You Active In Clinical Research?	No

Personal Awards/Achievements/Membership of prestigious academic research based bodies (e.g. Fellowships of the Royal Society, Academy of Medical Sciences)

Fellow of the Royal Society of Medicine

Honorary lecturer, Department of Neurodegenerative Disease, UCL Institute of Neurology (2003 to Present)

Member of the Health Protection Agency (HPA) Expert Advisory Group on the Laboratory Testing Strategy for Large Scale Abnormal Prion Prevalence Studies. (2006 to Present)

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Programme 1

HUMAN MOLECULAR GENETICS AND BIOINFORMATICS

1A: HUMAN MOLECULAR GENETICS OF PRION AND RELATED DEMENTIAS

Dr Simon Mead

GRO-B

GRO-B

PROGRESS REPORT

1. Mutation detection in PRNP and modifiers of inherited prion disease (IPD) phenotype

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c) Use of IPD pedigrees to estimate phenotype heritability

We have developed direct evidence for heritability of prion disease phenotype in the last quinquennium by considering whether the quantitative traits of age of disease onset/death are heritable. Over 30 mutations in *PRNP* are known with a remarkable range of age of clinical onset (adolescence to old age). We investigated the correlation of age at onset and death between parents and offspring in three large UK IPD kindreds[28]. Our analysis suggests that overall heritability of a composite phenotype is 0.55 (95% CI 0.35-0.75). These data suggest a significant heritable component to phenotypic variability in IPD. We have also documented the first example of familial concurrence of sCJD[19]. Recently, we have collaborated with Spanish prion disease physicians who have diagnosed a mother and son with vCJD, the first familial concurrence of this disease. Collectively, these findings support attempts to identify human prion disease modifier genes.

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3. Association studies

a) Candidate Gene studies

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Hectd2 is the most promising candidate from a region of linkage to mouse prion disease incubation time. We found evidence of human genetic association at the locus in vCJD (P=0.0049), sCJD (P=0.012) and kuru (0.0009, haplotype association test), and an association between genotype and level of blood expression (see Programme 2 for details) [91]. Collectively these data support a role for *HECTD2* in mammalian prion disease[91].

GRO-B

b) Genome-wide association study

In 2005-2007 we were one of the first UK research teams to start GWA[47-51]. Initially, we compared vCJD with UK controls and tested for replication of our findings in approximately a thousand samples from multiple categories of human prion disease. Unsurprisingly, we found the *PRNP* locus was strongly associated with risk across multiple markers and all categories of prion disease (best single SNP association in vCJD $P=2.5x10^{-17}$; best haplotypic association in vCJD $P=1x10^{-24}$). The top ranked SNP was in strong linkage disequilibrium with *PRNP* codon 129. Although the major contribution to disease risk was conferred by *PRNP* polymorphic codon 129, we have long speculated that genetic variation near to *PRNP* might confer additional susceptibility through modification of PrP expression[52]. In 2001 we reported the association of rs1029273 (SNP-1368), upstream of *PRNP* exon 1, in sporadic but not vCJD[53], a finding that has been replicated in a large study based in Germany[54], although not in some smaller studies[55,56]. In our genome-wide association studies a downstream SNP (rs6116492) conferred increased risk of vCJD, beyond that conferred by codon 129 (P=0.001, OR 2.63 95% CI 1.43-4.82).

Aside from *PRNP*, one technically validated SNP association achieved nominal genome-wide significance, according to the statistical criterion used by the WTCCC, upstream of the retinoic acid receptor beta, *RARB* ($P=1.9x10^{-7}$). A similar association was found in a small sample of iatrogenic CJD (P=0.030), but not sCJD or kuru. Upstream of *STMN2* we found evidence of an association in acquired prion disease, including vCJD ($P=5.6x10^{-5}$), kuru incubation time (P= 0.017) and resistance to kuru ($P=2.5x10^{-4}$). The risk genotype upstream of *STMN2* was not associated with risk of sCJD, but did confer a significantly earlier age of onset in sCJD; a similar effect was noted in vCJD but this was not independently statistically significant. The first prion disease GWAS thus identified two biological pathways of interest for functional studies (figure 1a.2). In cell culture, retinoic acid has been shown to regulate prion protein expression[57]; SCG10 the protein product of *STMN2* is involved in regulation of microtubules and neurite outgrowth[58].

GRO-B

5. Kuru genetics

Kuru was a devastating epidemic prion disease affecting a highly geographically restricted area of the Papua New Guinea highlands which, at its peak, predominantly affected adult women and children of both sexes (Programme 11 for detail of the fieldwork we have established for over 12 years)[60,61]. Its incidence has steadily declined since the cessation of its route of transmission, endocannibalism[62]. The importance of kuru in understanding human prion disease epidemics can not be overstated because of its huge scale, oral route of transmission, parallels with vCJD and that we have witnessed a *completed* epidemic. The clinical and genetic data we have acquired from fieldwork over 50 years is completely without rival in this historic disease of contemporary relevance.

It was the practice amongst the Fore for kinship groups to consume deceased relatives at mortuary feasts, resulting in human-human prion transmission. A peak annual mortality of over 2% was recorded in some Fore villages[63]. Around the start of the last quinquennium we reported provocative genetic evidence that balancing selection had occurred intensely and recently in the kuru region and but also older and globally[9]. We showed that marked selection pressure results in extreme Hardy-Weinberg disequilibrium at codon 129 in albeit a small sample (n=30) of elderly women survivors of mortuary feasts[9]. Genetic susceptibility has thus had a profound effect on the epidemiology of kuru.

In the last quinquennium we have progressed these studies further, analysing over 3000 individuals from a wide range of Eastern Highland populations, including an almost complete sampling of elderly participants of cannibalistic mortuary feasts (n=632), 152 of whom subsequently died of kuru. Genotyping of these samples unequivocally confirms that heterozygosity at polymorphic codon 129 of the prion protein gene (*PRNP*) confers powerful resistance to kuru. Kuru-exposed survivors of the epidemic in PNG *and* kuru patients with extraordinarily long incubation times, extending over 50 years in some cases, are predominantly heterozygotes[9,14,64-66]. This effect has resulted in an increasing cline in 129V frequency of modern populations centring on the kuru exposed region.

Aside from this profound and important effect we have recently discovered an entirely novel *PRNP* variant at codon 127 found exclusively in the kuru region, and present in half of the otherwise susceptible *PRNP* codon 129 methionine homozygous women from the region of highest exposure. Whilst common in the area of highest kuru incidence, this allele is absent from kuru patients and unexposed population groups, both globally and indeed, the codon does not vary in mammals. Genealogical analysis reveals a significantly lower incidence of kuru in 127V pedigrees when compared with geographically matched control families. We have shown that the 127V polymorphism is a new acquired prion disease resistance factor - a response to, rather than a trigger of, the kuru epidemic. Variants at codons 127 and 129 of *PRNP* thus demonstrate the population genetic response to a prion disease epidemic and record perhaps one of the strongest and best documented episodes of recent human selection[67].

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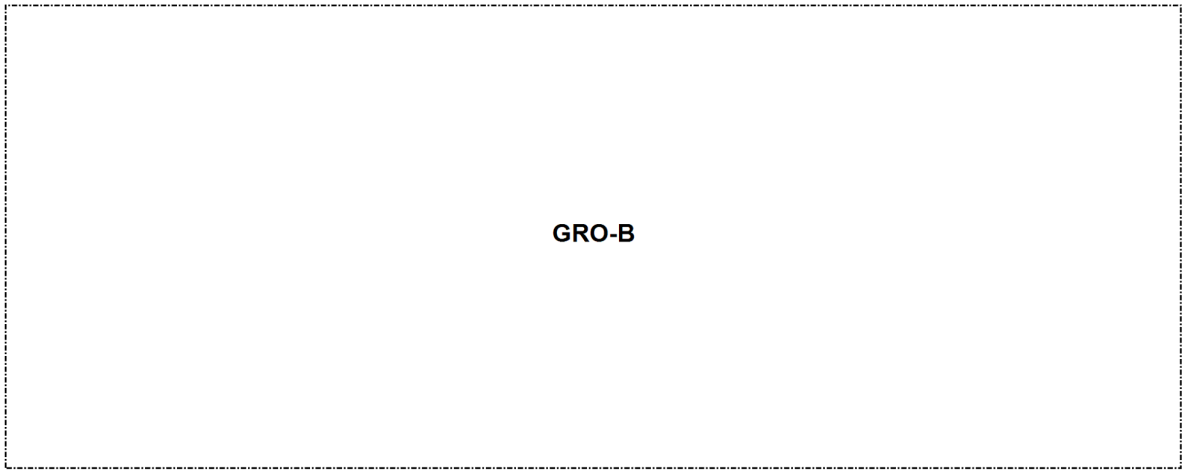
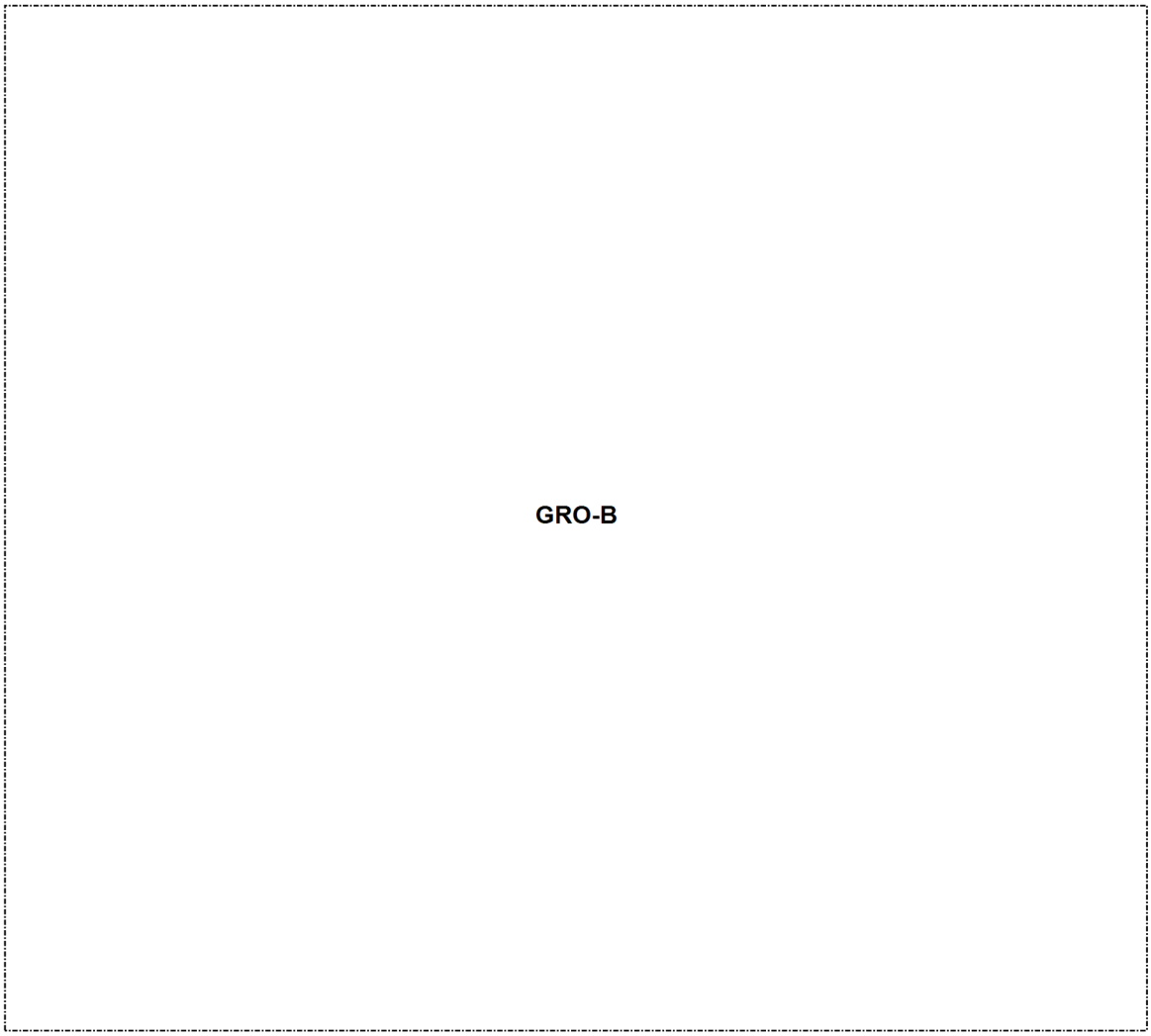


Kaski D, full author list not decided. Variant CJD in a PRNP codon 129 heterozygous individual (in preparation)



GRO-B

Smithson S, Leonard S, James MA, King C, Newbury-Ecob RA, Wroe SJ, Collinge J, Mead S Inherited Creutzfeldt-Jakob disease: phenotypes, genotypes and public health implications. *J Med Genet* (2007) 44 S19-S19

GRO-B

External collaborations

Professors Robert Will, Richard Knight, Hester Ward, James Ironside, National CJD Surveillance Unit, Edinburgh.
Important longstanding National Agreement to share all UK resources of CSF and DNA and well-established joint patient referral system.

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Dr Mike Hubank, Institute of Child Health(ICH), London.
Dr Hubank manages the ICH gene array facility, which we used for our vCJD GWA and are currently using for ongoing GWA.

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Professional Activities

Dr Simon Mead
Member of several national committees including the Department of Health CJD Incidents Panel and the National CJD Resource Centre Oversight Committee.

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FUTURE PROPOSALS

Abstract
We know that human and animal prion diseases are under strong genetic control. An understanding of the molecular and cellular processes central to prion disease may have public health importance and wider relevance to diseases of protein misfolding. Aside from the prion protein gene we have only recently started to identify human susceptibility genes and this process is ongoing. The MRC Prion Unit is an international leader in prion genetics. Our aims in the next quinquennium are to capitalise on this success by coordinating large international genetic studies towards a complete understanding of human prion disease susceptibility. Central to our plans are unbiased genome-wide association studies using a diverse cohort of several thousand patient samples. The discovery phase of a planned study comprises >1300 sCJD samples from the UK and Germany compared with large samples of publicly available population control genotype data, together with cohorts of vCJD, iatrogenic CJD, inherited prion disease (using age of clinical onset as a quantitative trait), kuru, elderly women resistant to kuru, and appropriate Papua New Guinea population control samples. The rationale for the study design is that it incorporates both the statistical power afforded by large sample collections of sCJD with the public health importance of the acquired prion diseases. We have already established a precedent in prion disease (with *SPRN*) that rare probably functional variants may confer a high risk of vCJD. In expectation of technological developments enabling whole genome re-sequencing, we plan three experimental phases, triggered by cost effectiveness and technological thresholds: candidate gene sequencing, whole coding or transcript genome sequencing and sequencing of the entire genome. Extended genotype-phenotype studies across our collection, together with new patients followed in detail by the National Prion Monitoring Cohort (see Programme 11) will add to the portfolio of evidence for a gene. Hypotheses generated by these studies, principally a list of prion disease "genes of interest" will move to functional genetic analyses coordinated across multiple Unit Programmes (see Annexe 1b and CELLGENE, Programme 2). Studies will include: (i) association of the high risk genotype with altered expression of the gene of interest and/or in blood brain tissue, these studies being conducted both genome-wide and validated for each important candidate (ii) association of patient tissues or infection in a prion disease model system with altered expression of a candidate or genome-wide, enabling a ranking of candidate against all other genes (iii) modification of prion infection in a model system or animal by perturbing expression of the candidate gene or an established pathway, including the generation of new transgenic or knock-out animals with Programme 3 (iv) biophysical studies to investigate interactions between the prion protein and candidate proteins with Programme 7. Our work will be integrated with other Unit and publicly available genome-wide studies.

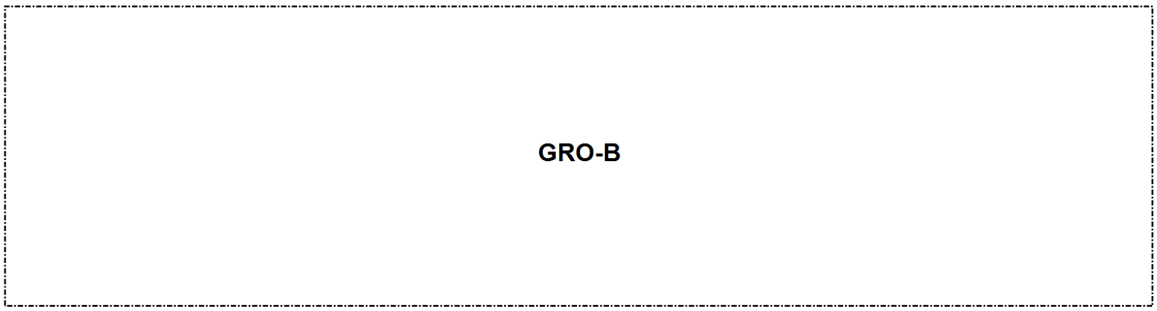
AIMS

1. Detection of common genetic variants that confer susceptibility to human prion disease using genome wide association
2. Detection of rare genetic variants that confer susceptibility to human prion disease by sequencing studies
3. Develop understanding of the prion-modifying mechanism of "genes of interest" derived from above studies with a portfolio of functional genetic analyses
4. Moving towards a complete global description of inherited prion disease both in terms of mutational spectrum and genotype-phenotype studies
5. Capitalise on our major patient sample resource for other studies in neurodegeneration by collaboration

GRO-B

In man, we have developed direct evidence for heritability in the last quinquenium by considering the familial concurrence of IPD to test whether the quantitative trait of age of disease onset is heritable. Our analysis suggests that overall heritability of a composite phenotype is 0.55 (95% CI 0.35-0.75)[28]. These data suggest a significant heritable component to phenotypic variability in IPD. A very strong and common prion disease genetic risk factor is already well known: *PRNP* is polymorphic in Europeans at codon 129, between methionine (~60% allele frequency) and valine[7]. All vCJD patients are methionine homozygous, representing the strongest association of a common genotype with any disease[77-80]. Homozygous genotypes at codon 129 (and codon 219) also strongly associate with sCJD[7,81], with iatrogenic CJD[8] (acquired by dura mater transplantation or cadaver-derived growth hormone therapy) and early age of onset or other phenotypes of inherited

prion disease (IPD)[25-28,82] and kuru, an epidemic prion disease of the Fore linguistic group of Papua New Guinea[12,64,66]. Codon 129 heterozygosity strongly associates with long-term survival after exposure to kuru prions[9,12], with long incubation time in kuru patients[14], and late clinical onset of IPD. An understanding of susceptibility conferred by *PRNP* codon 129 has had a major impact on prion biology, but can only explain a minority of the population risk and phenotypic heterogeneity of human prion diseases[25,28]. Collectively, these findings support attempts to identify human prion disease modifier genes. We aim to discover new variants with moderate or strong effects across all human prion disease, exemplified by the effects of codon 129.



The success of a GWA is dependent on a number of factors. These include:
(i) The frequency of the risk allele. If the risk genotype is rare, then corresponding statistical power is low and huge numbers of samples will be required.
(ii) Sample size. The larger the sample size, the power correspondingly increases. In our studies we consider this parameter as critical and have sought to maximise the samples available to us over several years, see Progress Report.
(iii)The degree to which the functional SNP is predicted by the "tagging" SNPs genotyped by the array, usually measured by r^2. The Illumina 660 array we have chosen gives >90% coverage of Hapmap Phase I and II SNPs with an r^2 >0.8.
(iv)The risk conferred by the genotype. Herein lies the major uncertainty in prion disease as there are no straightforward ways to estimate effect sizes at undiscovered loci. The WTCCC has decided the sample sizes of around 2000 cases and 2000 controls are required to justify analysis of complex traits by GWAS, however, there are several examples (*CFH* in age-related macular degeneration[85], *APOE* in Alzheimer's disease[86] and, of course, *PRNP* codon 129 in vCJD[12]) of valid, strong and important genetic effects in neurological diseases that have been detected with small numbers of samples. Whether the strong effects are the *only* loci worth identifying will be discussed further[87]. Large sample sizes will result in very robust evidence for strong genetic effects, and the role of these important SNPs in prion disease subgroups, also the potential to identify more modest genetic effects. Even though genetic effects may be moderate, the importance of the implicated pathway in prion disease is best determined by functional genetics rather than association study.
(v) The array used providing adequate coverage of the type and location of polymorphism that confers risk of disease. For example, genotyping platforms vary considerably in their ability to predict the presence of copy number variation. To date, the general importance of structural variation in common diseases has not been established.
(vi) Accurate phenotyping. The distinctive phenotype of prion disease with well validated international diagnostic criteria and the high proportion of patients that have diagnosis established at autopsy[12] are favourable for our studies. In this respect our large collection contrasts with other neurodegenerative diseases such as Alzheimer's disease, which are arguable more complex, heterogenous, and with less reliable clinical diagnosis and lower autopsy rates.
(vii) Avoidance of pitfalls associated with GWAS such as poorly matched population controls, or inclusion of samples with cryptic ethnicity. Standard methods for the detection of these problems are available[88].

This work will be performed with Dr Holger Hummerich (computational, database and bioinformatic expertise, see Programme 1B) and Professor J Whittaker (statistical genetic expertise).

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GRO-B

3. Resequencing studies – detecting rare functional susceptibility variants

GRO-B

Massively parallel re-sequencing

The advent of massively parallel sequencing will undoubtedly impact on this study design in the next quinquennium. We are currently monitoring the development of four products, GRO-B GRO-B GRO-B The technology is improving rapidly, we expect high quality sequencing of the coding genome for around GRO-B sample by 2011, and the entire genome for GRO-B /sample by 2013, resulting in a typical total study consumable cost of GRO-B in either circumstance. We also expect that the leading technologies will be acquired by several commercial entities and/or large publicly funded organizations such as the GRO-B and that we would establish service contracts for our work.

It is our intention to obtain these sequence data for our vCJD patient collection and several hundred sCJD patients if satisfied about cost-effectiveness and quality. It is our expectation that publicly available UK control DNA would be made available prior to embarking on our studies. The statistical framework for analysis of these studies not yet absolutely clear. Most straightforward would be a model which considers rare alleles as SNPs and tests

association by standard case-control methods. An additional complexity, with some advantage, would be a model which considers the likelihood that a rare variant was functional (e.g. a frameshift in/del or premature stop codon in a transcript would be highly likely to be functional) and allows for testing of the hypothesis that any of several likely-pathogenic variants in a gene are over-represented in cases versus controls. Professor John Whittaker will continue to provide advice as this issue develops over the next few years.

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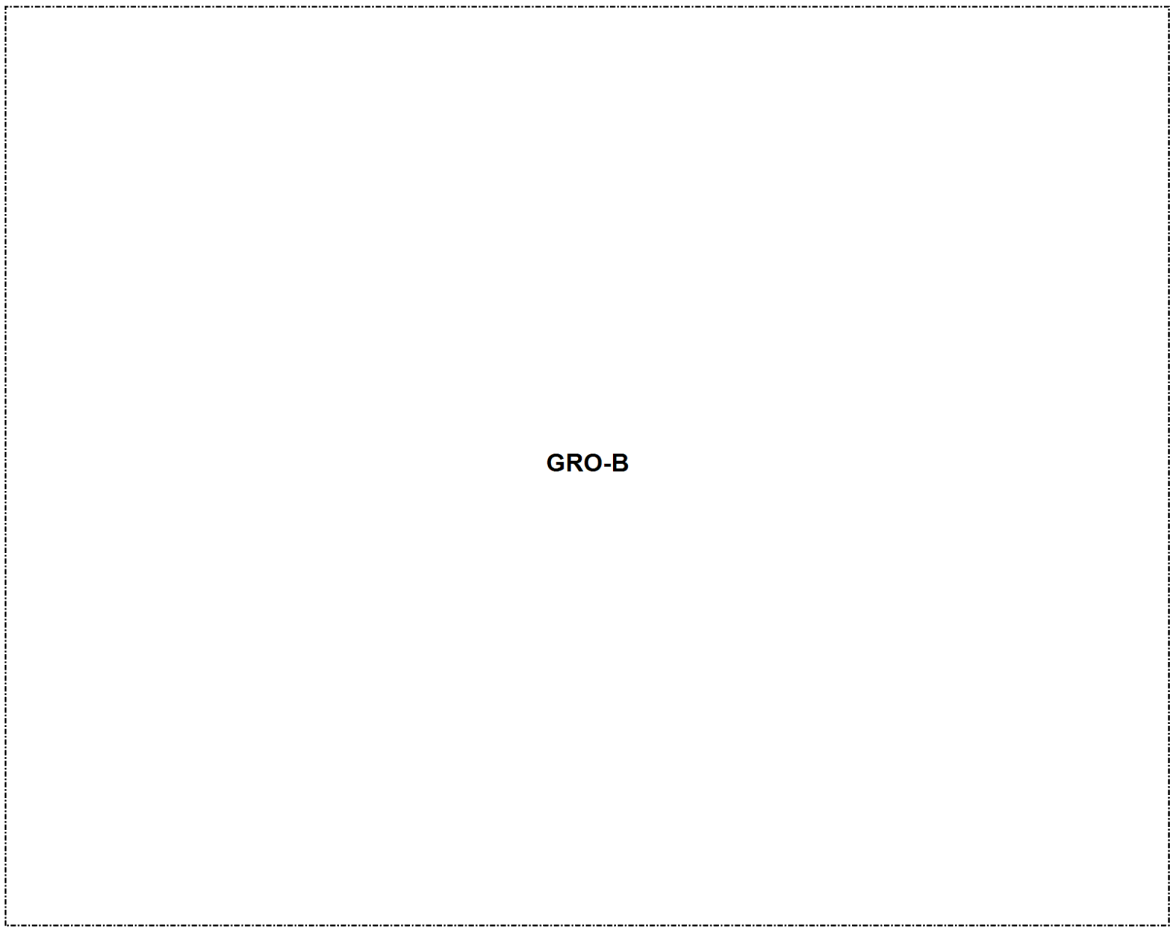
RNA samples and expression study design
Programme 1 has archived a unique resource in human prion disease of around 1600 blood samples from patients including PAXgene tubes (used to preserve RNA from degradation), and/or fractionated EDTA/citrated blood. These samples may be associated with detailed clinical phenotype data derived from National Prion Clinic hospital or domiciliary visits as part of the NPMC. The RNA preserved sample collection is derived from around 450 patients and UK controls (some of the patients have been sampled on many occasions through the course of their illness). Additionally we have brain tissue from autopsy available in around 300 patients. We plan to acquire a collection of ~100 PAXgene preserved blood samples from healthy UK elderly individuals and ~100 elderly non-prion disease dementia patients (primarily Alzheimer's disease) with the assistance of the Dementia Research Centre. Initially we intend to look at the blood resource as this has been taken directly into RNA preserving chemicals and couriered to the Unit's laboratory so we are confident about sample quality. For brain samples, we plan exploratory studies of RNA quality (using bioanalysis to generate an RNA Integrity Number (RIN), a measure of RNA quality) and pilot expression studies. The study comparison is four-fold,

- human prion disease patients (stratified by vCJD, sCJD, IPD) blood transcriptome versus genotypes at risk loci
- human prion disease patients (stratified as above) blood transcriptome versus healthy aged matched controls
- human prion disease patients (stratified as above) blood transcriptome versus non-prion dementias (primarily AD)
- correlation of expression with disease severity measured by established clinical rating scales (see Programme 11)

Data will be normalized across the array and compared using a two-class unpaired test with a Benjamini-Hochberg (False discovery rate) p-value correction. Further analyses will include generating networks and pathways (see Bioinformatics, Programme 1b).

An important potential spin-off of these expression studies is the identification of candidate genes/pathways of value in diagnostics. There is a pressing need to develop a blood test to

help protect the blood supply from donors infected with vCJD (see Programme 9). It would also be of considerable value to develop a rapid and widely accessible blood test to enable early diagnosis at a time when therapy is more likely to be useful (see Programme 11). The vulnerability of RNA to degradation means that it is not ideal for a practical blood test, however, these studies may target gene products worthy of development a more stable protein assay. Such candidates would be further studied by Programme 9.



ETHICS AND RESEARCH GOVERNANCE

All of the work performed within this Programme has ethical and health and safety approval and is conducted under the highest standards of research governance. Storage and genetic analysis of human blood/tissue samples is performed with consent from relatives, with approval from the Local Research Ethics Committee of the Institute of Neurology/National Hospital for Neurology and Neurosurgery, and complies with the code of practice specified in the Human Tissue Authority licence held by UCL Institute of Neurology and the Unit's local research governance procedures which are subjected to regular audit.

RESOURCES

The infrastructure and key collaborations required for conducting the future work of the Programme are already established. We request level funding to maintain existing resources including expected replacement of equipment. No additional new posts are required. The consumables for the first phase of genotyping array studies are in place (Wellcome Trust grant).

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Staff

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Programme 1

HUMAN MOLECULAR GENETICS AND BIOINFORMATICS

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PUBLICATIONS, AWARDS, COLLABORATIONS AND PROFESSIONAL ACTIVITIES

Publications since last quinquennial report

Peer reviewed articles

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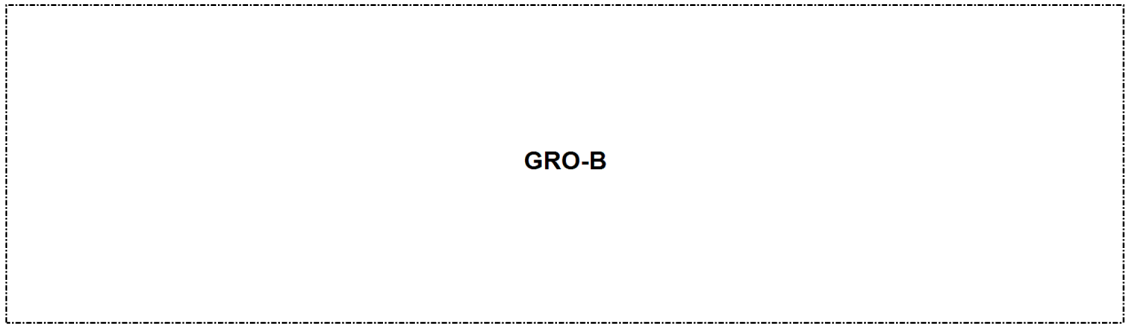
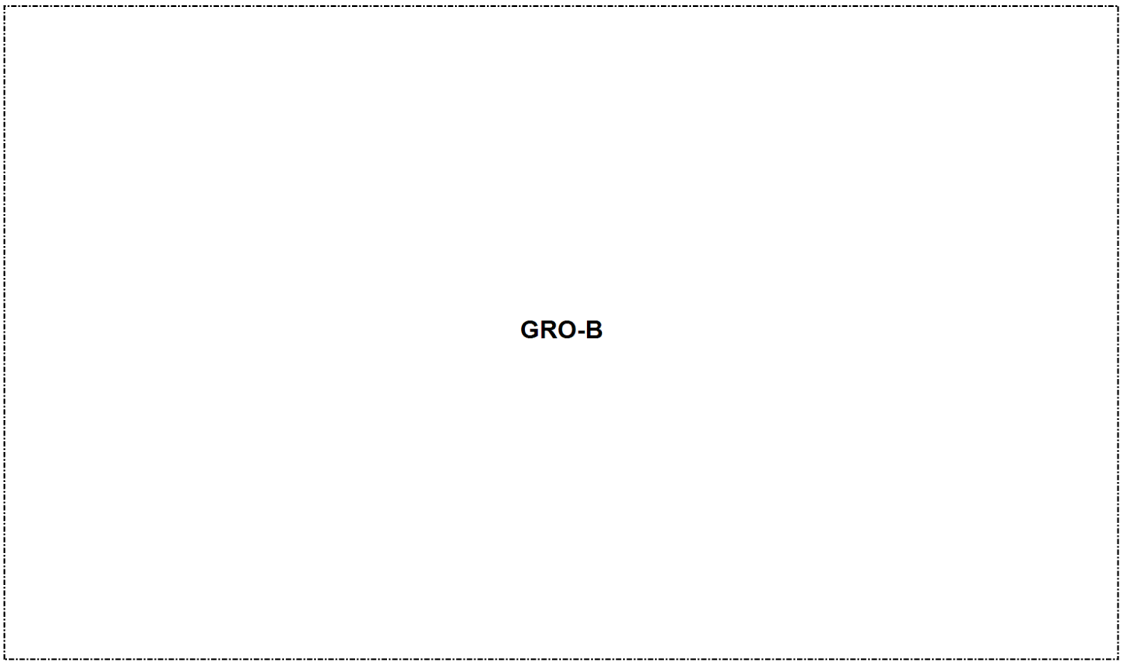


Mead S, Poulter M, Uphill J, Beck J, Whitfield J, Webb TE, Campbell T, Adamson G, Deriziotis P, Tabrizi SJ, Hummerich H, Verzilli C, Alpers MP, Whittaker JC, Collinge J. Genetic risk factors for variant Creutzfeldt-Jakob disease: a genome-wide association study. *Lancet Neurol.* 2009*;* 8: 57-66



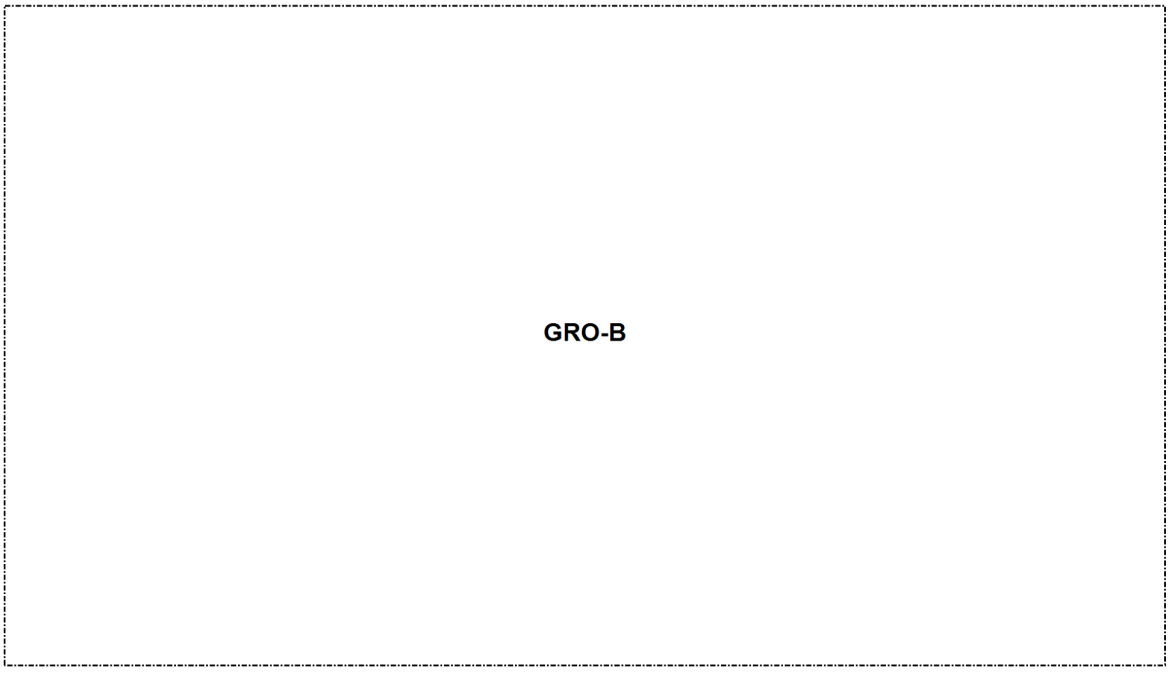
Programme 2

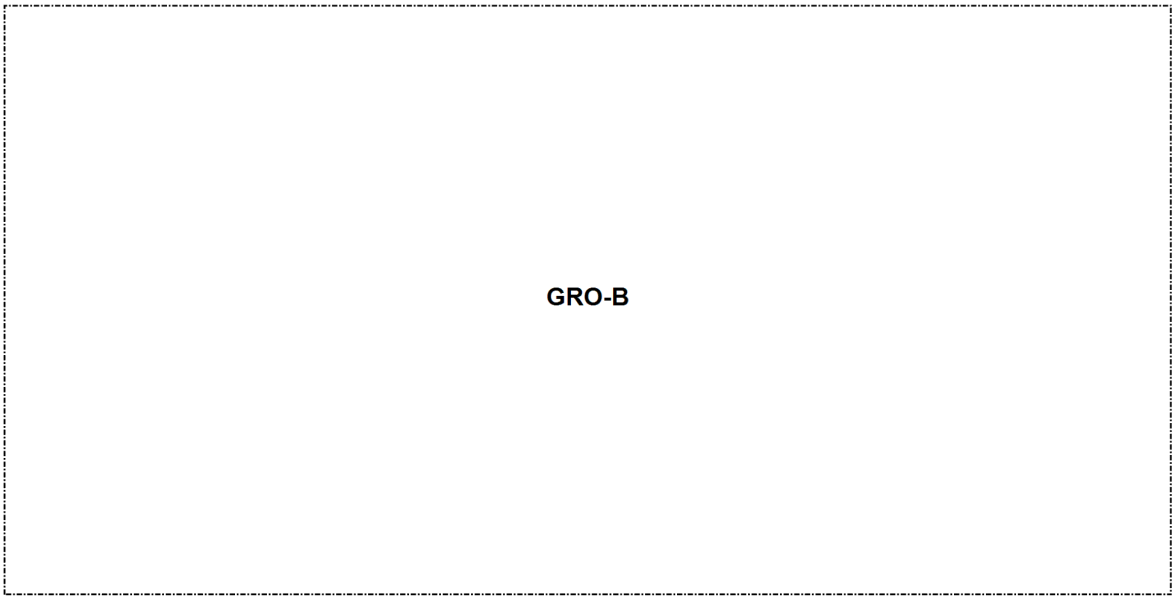
PRION GENETIC MODIFIERS IN THE MOUSE

Dr Sarah Lloyd

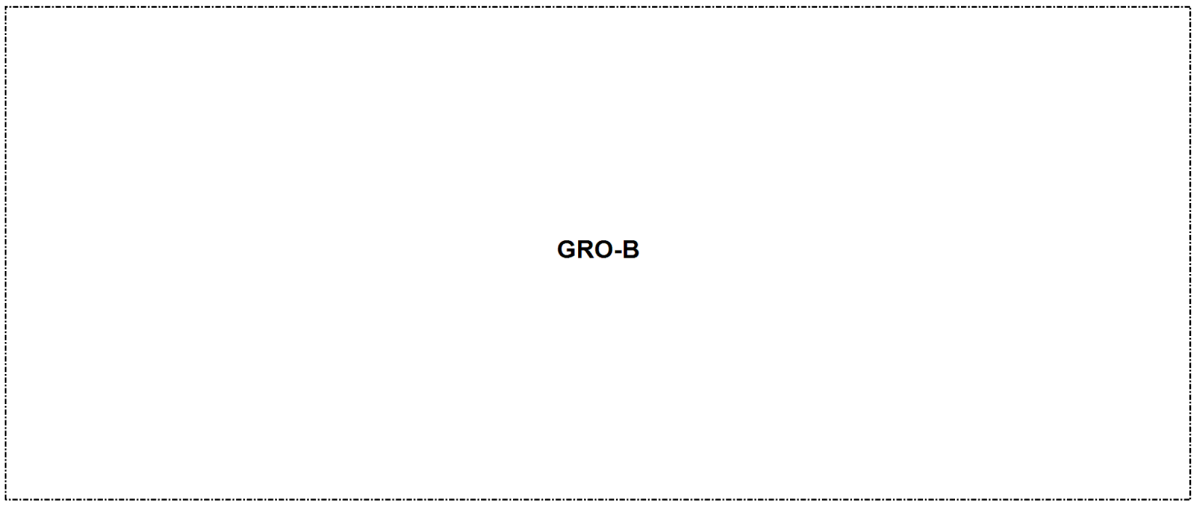
Background to programme and contribution to Unit mission

Prion diseases are naturally occurring transmissible diseases affecting many mammalian species including Creutzfeldt-Jakob disease in humans and scrapie in sheep. All are associated with remarkably long and clinically silent incubation periods, which in humans can exceed 50 years[1]. It is well established in several species that susceptibility and incubation time have a major genetic component that is partly determined by variation in the prion gene (*PRNP*)[2-4], however, there is also clear evidence that other genes play an important part in modifying this phenotype[5-9]. The Unit has made a long-term investment in understanding genetic susceptibility to prion disease by co-ordinated studies in human disease populations (Programme 1) and in laboratory mouse strains, allowing comparative genomics for confirmation of candidate genes. While accurate animal modelling of many neurodegenerative diseases has proved challenging, prion disease models are without parallel in terms of their faithful recapitulation of human pathology. Indeed, it could be argued that these are not really models at all, since laboratory mammals are naturally susceptible to these diseases. Our primary aims are to identify genes and pathways crucial to prion propagation and to explain the remarkable phenotypic diversity in prion diseases including the extremely prolonged and highly variable clinically silent incubation period which can approach, and appears to be related to, the natural lifespan of the species concerned. These studies principally aim to understand fundamental processes, and potentially identify new therapeutic targets, which may be of generic importance in neurodegeneration and the ageing brain. Additionally however, they will directly contribute to integrated work in the Unit to improve patient diagnosis and genetic counselling and are expected to inform epidemiological modelling of the consequences of population-wide exposure to BSE prions where the number of silently infected individuals from primary or secondary (iatrogenic) exposure remains unclear. The aim is to identify and characterise the principal genetic modifiers by a co-ordinated combination of methods in the Unit; these are inherently long-term studies.

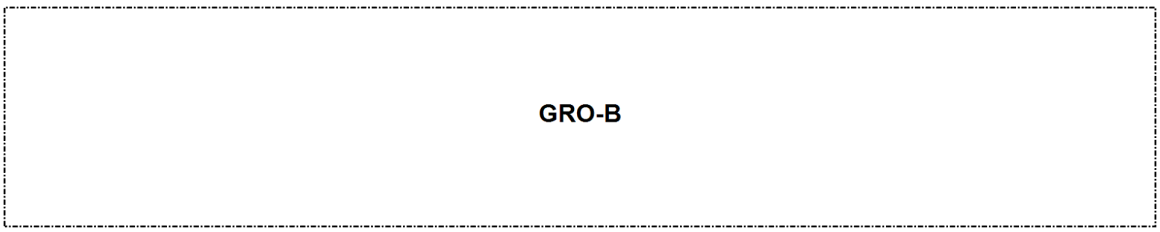


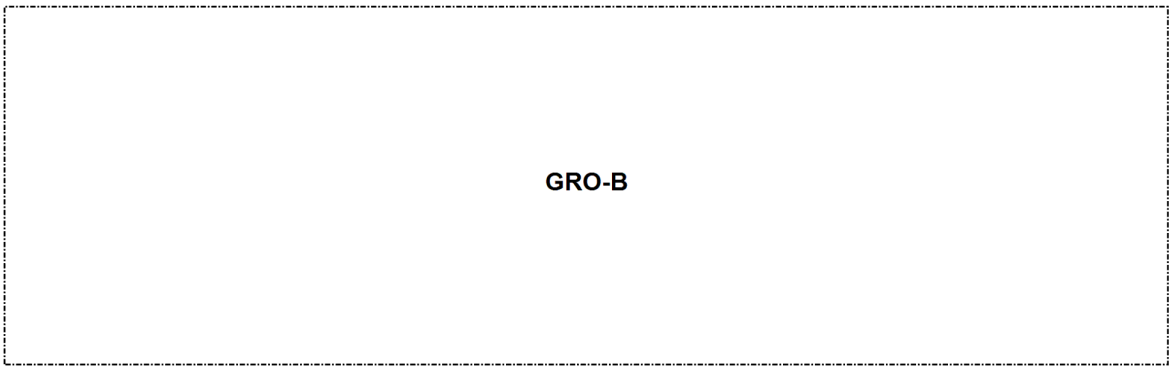


Chromosome 19

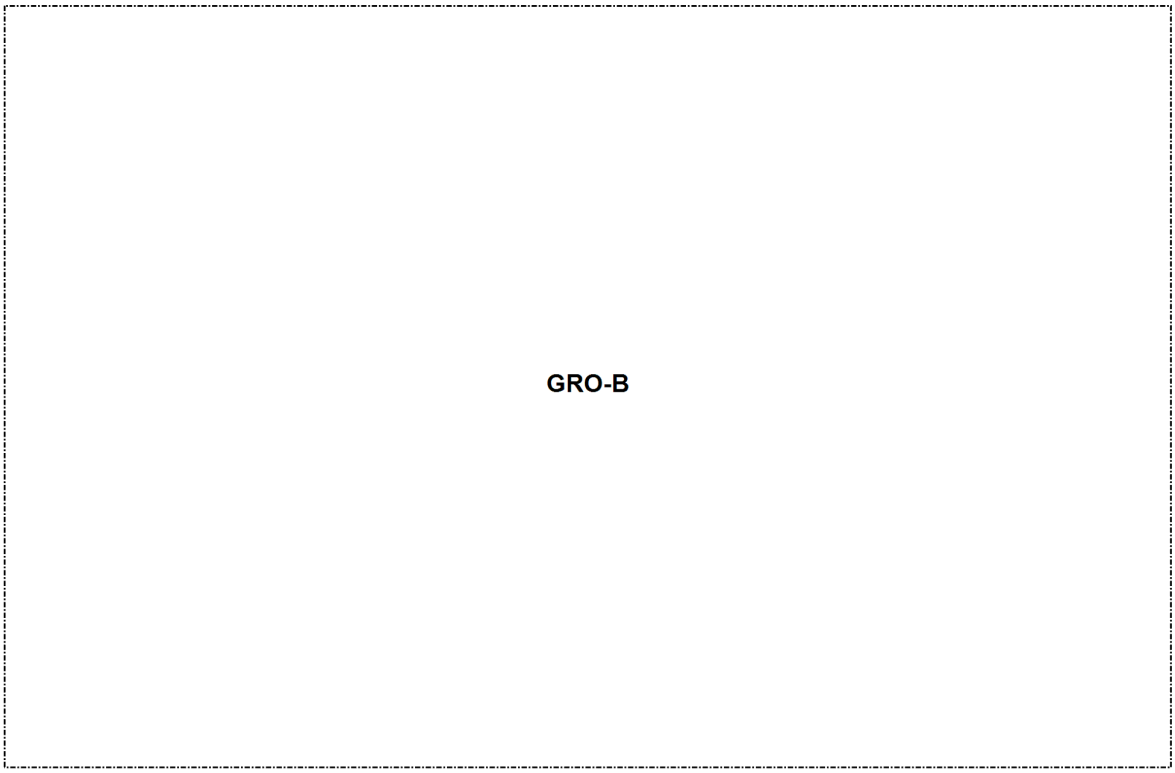


Human association study
In a joint study with Programme 1, we analysed *HECTD2* in an association study of human prion disease. We tested whether genetic variation at *HECTD2* was associated with a phenotype of variant and sporadic CJD. We also genotyped patients who died from the epidemic prion disease kuru, transmitted by endocannibalism in Papua New Guinea (PNG), and compared these data with elderly women known to have had multiple exposures to kuru at mortuary feasts prior to the cessation of endocannibalism in the late 1950's, but who are long-term survivors (see programme 11b).





Cpne8 is a member of the copine family of proteins which are Ca^{2+} dependent phospholipid binding proteins thought to be involved in membrane trafficking which may have a relevance for PrP processing[25]. In conclusion, we believe that *Cpne8* is the most promising candidate gene for the *Mmu15* QTL and its detection in a BSE and RML prion inoculated cross suggest that its influence may be independent of prion strain (Lloyd *et al*, in preparation).



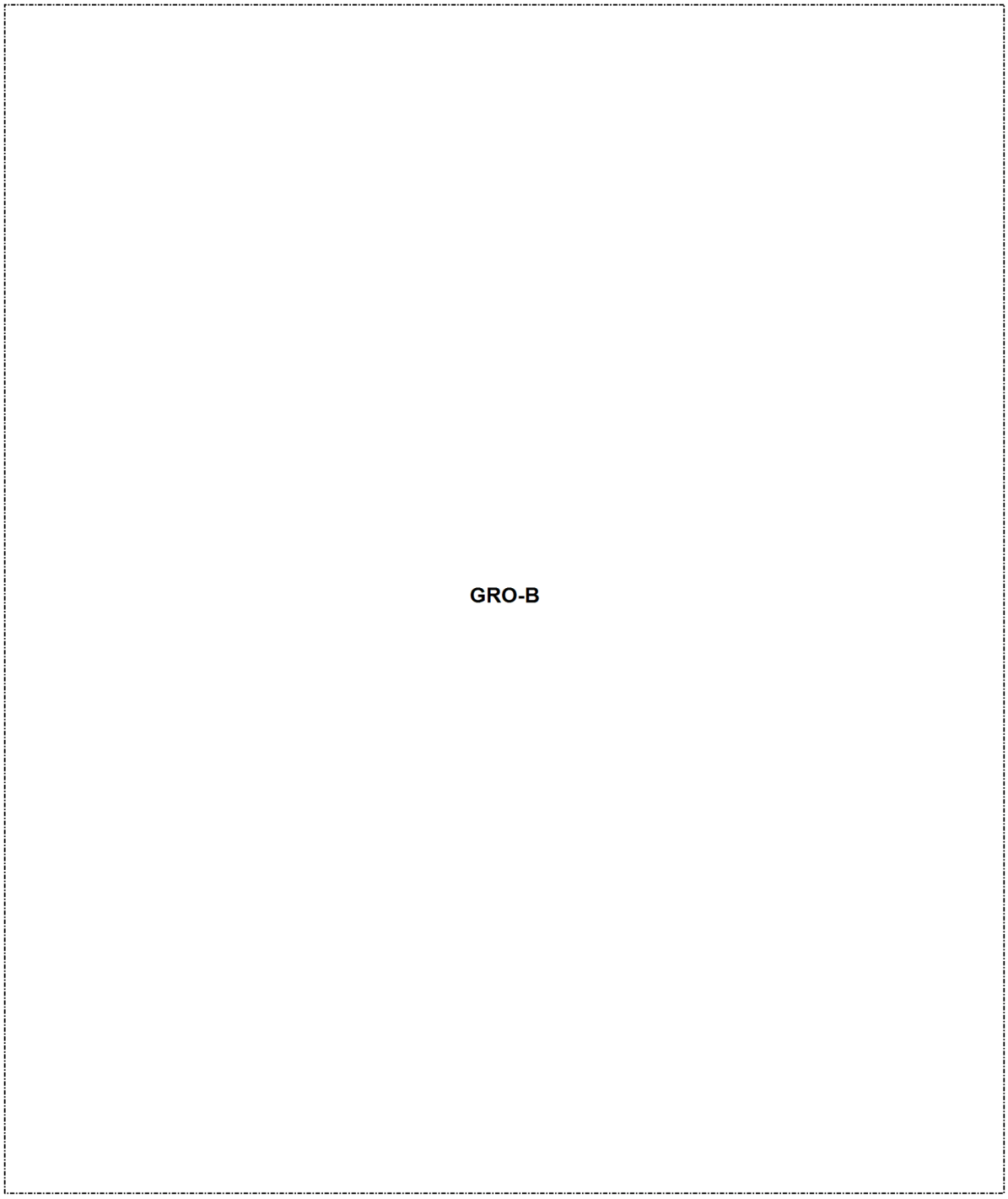
MRC1 and MRC2 prion strains

In the last quinquennium, we demonstrated that on primary passage to inbred mice, BSE could produce two different prion strains as defined by incubation time, PrP^{Sc} type on western blot and neuropathology[36,38]. We provisionally designated these MRC 1 and 2 pending further characterisation. MRC2 was found in most mouse strains challenged with BSE prions, including C57BL/6, and exhibited the classical features of the BSE strain including a di-dominant glycoform ratio on western blot. MRC1 was seen only in SJL and RIIIS mice and did not resemble the BSE strain by any of these parameters. In this quinquennium (joint project with Programme 6) we have continued to passage these strains in both C57BL/6 and SJL to further characterise the strains towards eventual biological cloning.

BSE has now been passaged five times in SJL mice (MRC1) and its strain characteristics remain stable. We have also shown that MRC1 prions can infect PK1 cells with a similar log infectivity titre as determined for RML prions using our Scrapie Cell Assay (SCA) (see programme 8). PK1 cells are highly strain specific, recognising only RML and 22L prion strains[39,40]. From these data, we conclude that on passage in SJL mice our BSE inoculum has undergone strain selection or "mutation"[41] to produce a new strain that is indistinguishable from RML (Cronier *et al*, in preparation).

MRC2 is derived from BSE passaged twice in C57BL/6 and we have now passaged this, a further three times in SJL mice. MRC2 is predominantly stable and unlike MRC1, is not infectious to PK1 cells. Between third and fourth passage we observed a strain split where 4/5 MRC2 inocula produced an MRC2 phenotype upon passage but 1/5 produced an MRC1-like phenotype. The source MRC2 inocula was examined by SCA and no infectivity was detectable suggesting that the new strain was converted spontaneously from MRC2 in the SJL mice or that MRC1 was able to propagate very efficiently in SJL mice from a minor subpopulation amongst an ensemble of molecular species[41].

Very little is understood about the nature of strains and how strain switching or selection may occur[41]. The strain selection between MRC1 and MRC2 appears to be specific for the genetic background of the mice therefore it may be possible to identify the genes responsible for this phenomenon. We had previously shown that no MRC1-like strain was observed in a C57Bl/6 x SJL F1 cross therefore we generated a C57BL/6 x SJL backcross (n=500) and inoculated them with BSE prions. Samples were taken for both genotyping and strain typing and will be analysed during the next quinquennium.



GRO-B

Wadsworth JD, Asante EA, Desbruslais M, Linehan JM, Joiner S, Gowland I, Welch J, Stone L, Lloyd SE, Hill AF, Brandner S, Collinge J. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science* 2004; **306:** 1692-1693

Lloyd SE, Linehan JM, Desbruslais M, Joiner S, Buckell J, Brandner S, Wadsworth JDF, Collinge J. Characteriziation of two distinct prion strains derived from bovine spongiform encephalopathy transmissions to inbred mice. *J Gen Virol* 2004; 85: 2471-2478

GRO-B

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Published abstracts

Lloyd SE, Maytham EG, Pota H, Grizenkova J, Molou E, Hummerich H, Mead S, Collinge J. HECTD2, an E3 ubiquitin ligase, is associated with prion disease incubation time in mice and susceptibility to vCJD and Kuru. *Prion2008*; P06.19.

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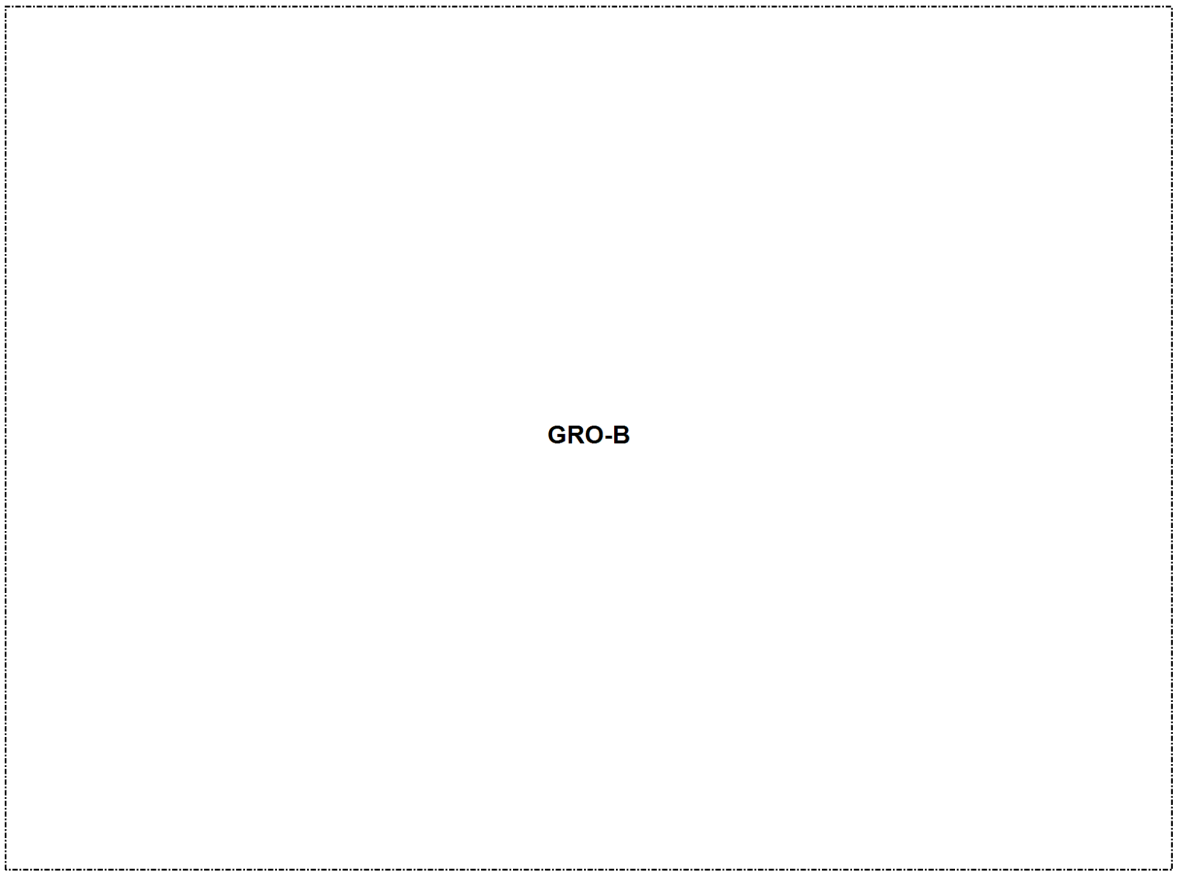
AIMS AND EXPERIMENTAL PLANS

Identification of candidate genes

Incubation time
Heterogenous stock mice:
Our studies to date have identified several potential quantitative trait genes and much work remains to establish their role in prion disease incubation time. To date, we have used our HS mice for fine mapping only, relying on previous two way crosses or other studies to provide the broader map locations. This strategy has proved very successful and in the next quinquennium we will complete these studies, focusing on *Mmu9*.

Although very successful, this approach does not take full advantage of the cross as it cannot identify novel loci. To do this a genome-wide screen is necessary. To capture the diversity of the HS, genotyping is required at a higher density than in standard two way



We previously set up a C57BL/6 x SJL backcross (n=500) inoculated with BSE prions. We will now characterise the molecular strain phenotype present in the brains. In the first instance an ELISA will be used for higher throughput screening and if necessary western blots will be used for confirmation. If we are able to detect the MRC1 strain we will carry out a genome-wide screen using SNP arrays for linkage analysis as described for experiments above. If successful, further crosses such as the generation of congenics will be required for fine mapping and gene identification.

All the genome-wide experiments described above generate large amounts of data requiring specialised analysis software and data handling tools therefore all projects will be performed with Unit bioinformatician Holger Hummerich (Programme 1b) who also co-ordinates Unit-wide data integration between programmes. In addition, data from these experiments will be integrated with other Unit wide experiments to maximise the value obtained and to identify the common genes and pathways.

Identification of candidate genes
As described above, candidate genes identified from any source will be assessed by sequence analysis and mRNA expression. Genes derived from the mouse studies will be compared to data obtained from existing and new human genome-wide association studies (GWAS) (Programme 1). Genes that also show an association in these studies will be considered a high priority for future studies. However, human GWAS may not identify all disease associated genes for example where association is attributable to multiple rare variants. In these cases re-sequencing may be required. Where there is strong genetic evidence from the mouse studies to implicate a gene in prion disease the human gene will be re-sequenced. This will be joint project with Programme 1 and no funding is requested here.

GRO-B

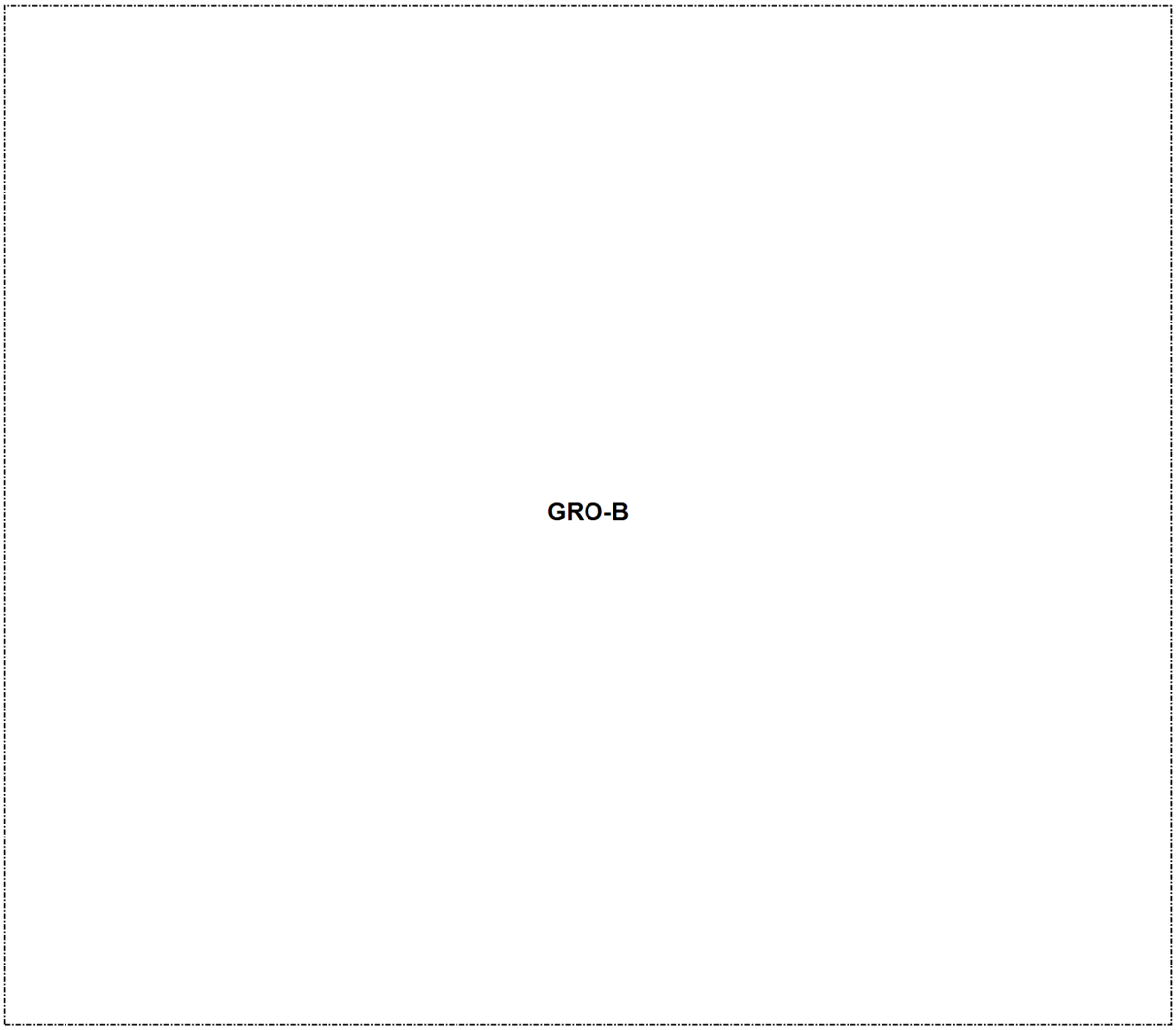
The viability and gross phenotypes of our new mouse models will be described by long term observation and if appropriate basic behavioural testing using the SHIRPA protocols[55]. Mice will be challenged with a variety of prions strains including mouse-adapted scrapie (e.g. RML, Me7) and mouse-adapted BSE (e.g. MRC2) to determine whether the genetic manipulation has influenced incubation time and to see whether the effect is prion strain specific. We will also characterise the clinical syndrome and pathology to determine the spectrum of phenotype modification. If these models significantly reduce the incubation time we can potentially improve our current bioassay models by crossing our various new lines to produce more complex models. There is also the possibility of crossing these lines to our existing human PrP transgenics to test the effect on human prion strains. Within the Unit, Programme 3 has developed models of spontaneous prion disease based on human mutations known to be associated with inherited prion disease. The most well characterised model is a transgenic mouse overexpressing human PrP carrying the A117V mutation (Tg30)[56]. These mice develop late onset clinical signs of prion disease together with PrP plaques which is transmissible on further passage. If our new transgenic models significantly reduce the incubation time in an acquired model of prion disease it would be possible to generate a cross with Tg30 to look for phenotype modification both in terms of disease onset and pathology.

GRO-B

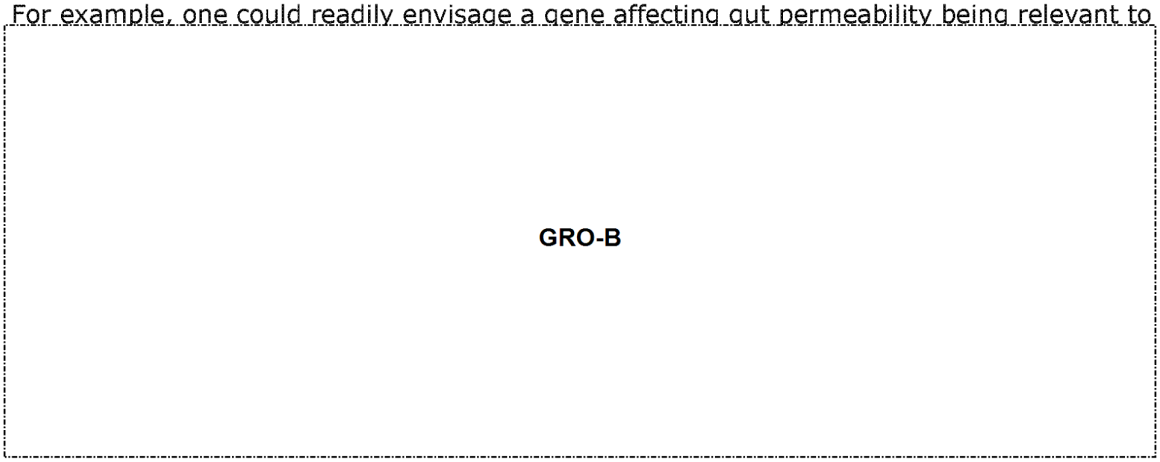
ANNEXE 1

CELLGENE: a cross-programme virtual group to evaluate and prioritise candidate prion-modifier genes

GRO-B



Our candidate gene list is being compiled from a diverse range of experiments including linkage to prion disease incubation time in mice and an association with susceptibility to human prion disease. These are complex phenotypes that are likely to be the result of multiple genes, pathways and cellular mechanisms and may also reflect prion strain and route of infection specific events. It is therefore probable that some genes, which may have a genuine role in prion disease, will not produce a detectable effect in our *in vitro* assay. For example, one could readily envisage a gene affecting gut permeability being relevant to

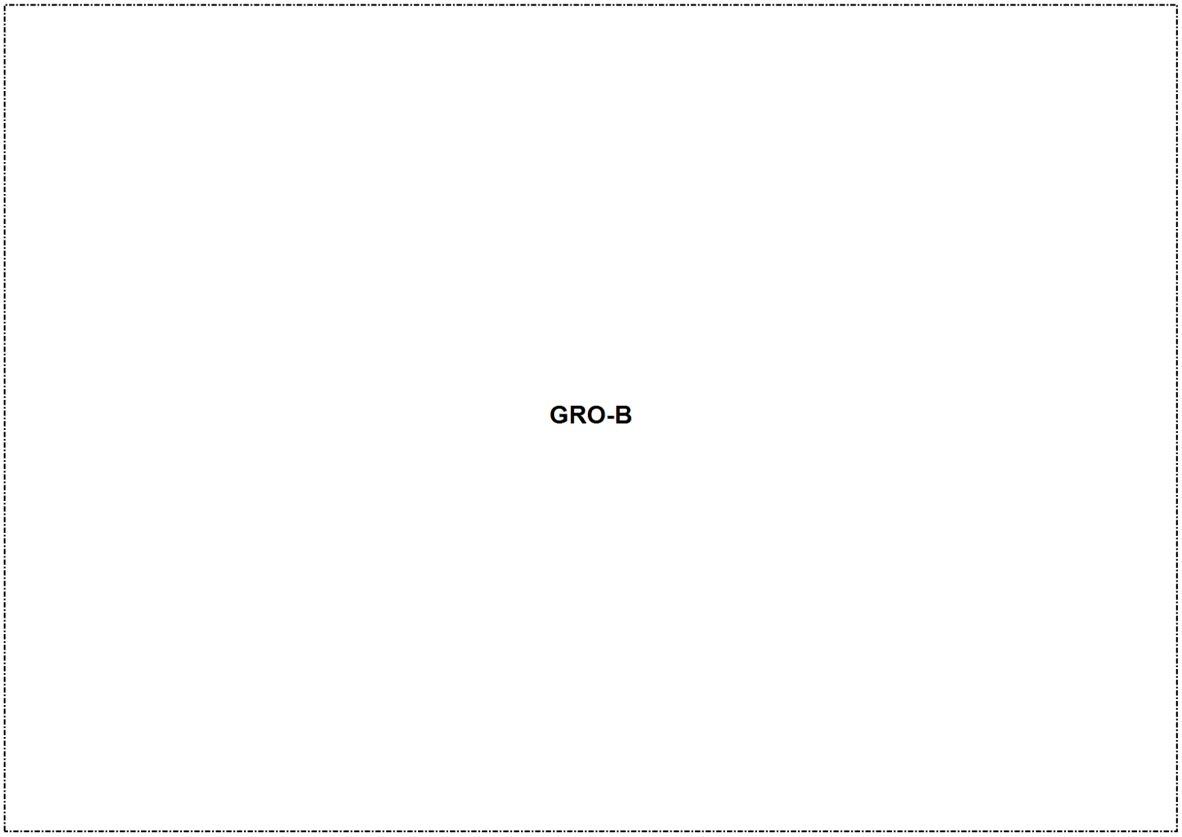


Programme 3

TRANSGENIC MODELLING OF HUMAN PRION DISEASES, INTERMAMMALIAN TRANSMISSION BARRIERS AND ASSESSMENT OF CANDIDATE THERAPEUTICS

Dr Emmanuel A Asante and Professor John Collinge

Background to programme and contribution to Unit mission:
The prion diseases are a closely related group of neurodegenerative conditions which affect both humans and animals. They are both experimentally, and in some cases naturally, transmissible within and between mammalian species. Cross-species transmission is generally much less efficient than within-species transmissions, being limited by a so-called species or transmission barrier. The arrival of vCJD, and the subsequent demonstration that it was caused by the same prion strain responsible for the epidemic of BSE in cattle[1-4], dramatically highlighted the need to understand the basis of such barriers and to model human susceptibility to this, and other, potentially zoonotic animal prion diseases. While the number of clinical cases of vCJD has been thankfully modest, the number of silently infected individuals is unknown[5,6]. However, studies of archived surgical tissues suggests a considerably higher population infection prevalence than the clinical case load would suggest[5]; prion incubation periods can exceed 50 years in humans[7]. Our animal model studies have also suggested that subclinical carrier states of BSE prion infection and other disease phenotypes may occur[4,8,9]. The *PRNP* codon 129 polymorphism has been of seminal importance in understanding human susceptibility to acquired prion infection and has been comprehensively modelled in transgenic mice. These studies led us to propose a "conformational selection" model of species barriers, with the recognition that these barriers and prion strain diversity are intimately related and indeed may be considered opposite sides of the same coin[10-12].



GRO-B

Ongoing study of intermammalian transmission barriers and role of *PRNP* codon 129 in mediating susceptibility to, and phenotype of, infection with BSE prions

Modelling bovine to human species barrier

We have conducted a long-term series of transmission experiments in mice homozygous for PrP null alleles (*Prnp*$^{o/o}$) and transgenic for human PrP 129M, 129V or 129MV, studying their susceptibility to a wide range of types of classical (sporadic or iatrogenic) CJD, vCJD, kuru, and many inherited prion diseases[1,3,4,8,9,16]. These transmissions have established relationships between *PRNP* codon 129 genotype in the human case and transgenic mouse. Detailed comparative studies with cattle BSE transmissions were performed and indeed played a key role in establishing the link between BSE and vCJD and exploring the molecular basis of prion strain diversity and transmission barriers. Further, we developed an animal model which faithfully exhibited the molecular and neuropathological hallmarks of human vCJD, with abundant florid PrP plaques, and propagation of type 4 PrP^{Sc} indistinguishable from PrP^{Sc} in the vCJD inocula[4,8,9].

To date, all clinical cases of vCJD worldwide (approximately 200) have been *PRNP* codon 129 methionine homozygous (MM)[13] (although recently a probable MV clinical case has been identified at the National Prion Clinic (Manuscript in preparation)). Notably, it also became apparent that transmission of BSE to humanised transgenic mice expressing human PrP M129 may result in subclinical infections and divergent molecular and neuropathological phenotypes, one recapitulating the features of vCJD and the other of the commonest molecular subtype of sporadic CJD[4]. The latter finding led us to suggest that BSE infection of humans of the MM genotype might develop a condition indistinguishable from sporadic CJD, in addition to vCJD[4].

While transgenic mice homozygous for human PrP V129[17] lack a species barrier to classical CJD prions regardless of their residue 129 status[1,3,8,16,17], transmission of vCJD and BSE prions was inefficient. Notably, whereas PrP^{Sc} was undetectable in any of the BSE inoculated mice that developed clinical disease, a different type of PrP^{Sc} (designated type 5 PrP^{Sc}) was propagated in 129VV mice challenged with vCJD prions[3] and this was accompanied by histopathology quite distinct from that of vCJD patient brains. In the current quinquennium, in joint studies with Programme 6, we established that V129 prevented the propagation of the unique type 4 molecular strain type of vCJD and of the characteristic vCJD neuropathological phenotype, which appears dependent on the expression of human PrP M129. It appears that human PrP V129 may not be able to adopt the prion conformation of vCJD and has a dominant negative effect. This can explain why vCJD has been restricted to the MM phenotype in humans[8]. These studies argued that BSE prion infection of VV humans may result in a molecular and clinicopathological phenotype distinct from vCJD.

We went on to model the susceptibility of 129MV heterozygotes, the commonest human *PRNP* genotype, to BSE and vCJD prions in 129MV transgenic mice. We simulated the heterozygous genotype by crossing two transgenic lines homozygous for *PRNP* codon 129M and 129V. We challenged these heterozygous 129MV mice with BSE and vCJD prions and observed a remarkable dissociation between propagation of type 4 PrP^{Sc} and abundant

florid plaques[9]. Type 4 PrP^{Sc} was propagated in the 129MV genotype after inoculation with vCJD prions, but florid plaques were very rare. Depending upon the source of the inoculum these 129MV mice can develop four distinct disease phenotypes after challenge with BSE prions or vCJD (human-passaged BSE) prions[9] and this study argued that human *PRNP* 129 heterozygotes will be more susceptible to infection with vCJD prions than to cattle BSE prions, and may present with a neuropathological phenotype that may not be easily recognised as vCJD. This interpretation was supported by subsequent complementary studies by others in human PrP expressing mice generated by gene-targeting (and with endogenous levels, rather than overexpression, of PrP)[18].

BSE can result in two strains in 129MM transgenic mice
As stated above, we reported in the last quinquennium that primary transmission of cattle BSE to Tg(HuPrP129M$^{+/+}$ *Prnp*$^{o/o}$)-35 (129MM Tg35) mice resulted in the propagation of type 4 PrP^{Sc} accompanied by abundant florid plaques, the hallmark of vCJD disease phenotype. However, the same BSE inocula also produced a concurrent alternate phenotype characterised by the propagation of type 2-like PrP^{Sc} with corresponding synaptic type PrP plaque deposits usually associated with classical CJD. We have since conducted serial passages to compare this apparently new strain derived from BSE to sporadic CJD with the same type 2 PrP^{Sc} type (London PrP^{Sc} type classification[19]). These transmission experiments ran for several years and have now been completed. Comprehensive biochemical and immunohistochemical analyses are well advanced and the data are being prepared for publication. Briefly, passages from BSE-inoculated transgenic mice with type 2-like human PrP^{Sc} and from sporadic CJD patients with type 2 PrP^{Sc} showed similar consistent short incubation periods and immunohistochemical staining patterns when inoculated into Tg(HuPrP$^{129V+/+}$ *Prnp*$^{o/o}$)-152 (129VV Tg152) and 129MM Tg35 mice. In comparison, passage from BSE-inoculated mice which had human type 4-like PrP^{Sc} displayed inefficient transmission in 129VV Tg152 mice which propagated type 5 PrP^{Sc} in this genotype as previously reported[3]. The same inocula also produced type 4 PrP^{Sc} accompanied by 100% total infection rate and abundant florid plaques in 129MM Tg35 mice. Taken together, these data suggest that BSE transmissions to transgenic mice expressing human PrP 129M can indeed result in generation of a strain closely similar to that seen in the commonest molecular subtype of classical sporadic CJD (Asante et al, in preparation).

Use of transgenic mice for bioassay of human prions
One of the distinguishing features of vCJD is that prion infection is readily detectable in lymphoreticular tissues[20-23]. Further, it is now clear that vCJD is transmissible by blood transfusion and possibly blood products[24-26]. Given the unknown population prevalence of asymptomatic vCJD infection, there is public health concern that secondary transmission of vCJD prions will occur through a wide range of medical and surgical procedures[27]. We have therefore performed a number of transmission studies of vCJD tissues to extend our earlier studies on PrP^{Sc} distribution in vCJD tissues[20,21,28].

In a study with Dr Wadsworth (Programme 6), we inoculated brain or rectal tissue from a previously characterised patient with vCJD to our transgenic mice expressing only human PrP, 129MM Tg35 and Tg(HuPrP$^{129M+/+}$ *Prnp*$^{o/o}$)-45 (129MM Tg45) mice. We observed efficient transmission of prion infection to transgenic mice inoculated with vCJD rectal tissue containing PrP^{Sc} at a concentration $10^{4.7}$-fold lower than that in vCJD brain[29]. These data confirm the potential risks for secondary transmission of vCJD prions via gastrointestinal procedures such as rectal biopsy[30]. Further, the good correlation between PrP^{Sc} levels and prion titre in vCJD tissues supports the use of protease-resistant PrP^{Sc} as a quantitative marker of prion infectivity in vCJD tissues[29,31]. We currently have ongoing experiments in which a range of vCJD tissues including spleen and tonsil are being titrated and plan to extend these studies to dental tissues where there have been particular concerns by the Department of Health recently. In the longer term we hope to replace the use of mice for

human prion bioassay, as we have successfully achieved for RML mouse prions with the development of rapid cell-based assay systems (see below and Programme 8)

Evaluation of potential therapeutic agents
We have been characterising transgenic models and developing procedures to prepare for extensive therapeutic testing in the next quinquennium (see future proposals). Pilot studies have been performed using the well established Tg20/RML model which have developed the infrastructure and expertise to undertake preclinical drug trials in our facility.

Development of "second generation" human transmission models: towards shorter incubation periods and barrier-free assay of vCJD prions
While our existing models have proved very successful, shorter incubation periods in human transmission models would be highly advantageous. Further, while we achieve a 100% infection rate in our transgenic mice expressing human PrP 129M when challenged with vCJD prions, this is predominantly a subclinical infection, limiting its use for therapeutic studies on clinical progression. We do not feel that further increases in human PrP expression levels alone will add much to existing models and also risks production of spontaneous pathology (see Programme 8). It is clear that the transmission barrier to vCJD prions in mice as assessed by onset of clinical prion disease is not abolished by expression of homologous human PrP M129 (although all inoculated mice are *infected*)[4]. While use of transgenic mice expressing bovine PrP or chimaeric PrP's have been proposed for vCJD assay, these models are also not species-barrier-free[32]. Many of our studies are aimed at studying prion strains and it is important to compare biochemical features of PrP^{Sc}, which would be complicated by expression of chimaeric PrP or other foreign PrP. Use of such models for therapeutics (see below) also necessitates replication of authentic human PrP^{Sc} and prion strains. Our focus has been on exploiting the clear evidence for the importance of other non-PrP genes that have a major effect on incubation period. Major advances have been made in the Unit in characterising prion modifier genes, with a long-term effort to identify genes in mice controlling incubation period (see Programme 2). Investigation of these genes in animal models is underway (see future proposals and Programme 2). However, in the course of these QTL studies of incubation period in defined inbred mouse lines, we identified a line (SJL) with incubation periods on primary passage of vCJD of as little as 139 days[4]. We have therefore exploited this by backcrossing the SJL genetic background genes into Tg(HuPrP$^{129M+/+}$ *Prnp*$^{o/o}$)-45 (129MM Tg45) and 129VV Tg152 mice. This project was led by Dr Lloyd (Programme 2) and involved 10 generations of conventional backcrossing. We now have SJL-congenic Tg45 and Tg152 versions that will be evaluated with a panel of prion isolates. This project is further discussed under "Future Proposals".

In addition, in large scale transmission studies completed during the last quinquennium, it was apparent that the variability in genetic background of our human transgenic models (which had contributions from FVB, C57Bl6 and 129Sv as a result of crossing with the Zurich *Prnp*$^{o/o}$ line[33]) was impairing our ability to discriminate different human prion strains and we have also reported that background genes affect strain selection or mutation. We therefore backcrossed the PrP knockout line onto an inbred FVB background (our line of choice for generating new transgenics) and new transgenic lines are produced by microinjection of fully inbred FVB *Prnp*$^{o/o}$ zygotes.

We therefore put three of our principal transgenic lines 129MM Tg35, 129MM Tg45 and 129VV Tg152, on which we have a huge body of long-term transmission data, through the 'speed congenics' programme commercially available at Charles River (UK). This project lasted about 2 years and during this period 84 FVB-specific PCR microsatellite makers covering 19 chromosomes at approximately 20cM intervals were used to screen 4 generations each of 129MM Tg35, 129MM Tg45 and 129VV Tg152 lines, all backcrossed to FVB wild type at each generation. At every generation, tail biopsies were shipped back to

the Prion Unit for genotyping so that only human PrP positive mice would be used for the next round of backcrossing to the FVB/N mouse strain.

We can report that we now have congenic versions of the principal lines listed above and, working closely with Dr Wadsworth (Programme 6), these have now being challenged with a panel of 50 classical CJD, vCJD and cattle BSE prion isolates for further characterisation of prion strains on homogeneous genetic background. These inocula have been carefully selected to represent a comprehensive molecular and phenotypic spectrum of human prion disease for analysis of molecular and biological prion strain types by biochemical (Dr Wadsworth) and immunohistochemical analyses (Professor Brandner). These ongoing studies will be completed in the next quinquennium and resources for completion are being requested under this programme.

Transgenic modelling of inherited prion disease

GRO-B

GRO-B

Absence of spontaneous disease and comparative prion susceptibility of transgenic mice expressing mutant human prion proteins

Transgenic mice expressing human PrP with P102L (1.5-3 fold compared to normal human brain pool) or E200K (2-3 fold) mutations and methionine (M) at polymorphic residue 129 did not develop spontaneous disease even at advanced age. However, these mice were readily susceptible to prion infection from patients with the homotypic pathogenic mutation. However, while vCJD prions transmitted infection efficiently to both lines of mice, markedly different susceptibilities to classical (sporadic and iatrogenic) CJD prions was observed. Prions from E200K and classical CJD M129 homozygous patients transmitted disease with equivalent efficiencies and short incubation periods in human PrP 200K, 129M transgenic mice. However mismatch at residue 129 between inoculum and host dramatically increased the incubation period. In human PrP 102L, 129M transgenic mice, short disease incubation periods were only observed with transmissions of prions from P102L patients, whereas classical CJD prions showed prolonged and variable incubation periods irrespective of codon 129 genotype. Analysis of disease-related PrP (PrP^{Sc}) showed marked alteration in the PrP^{Sc} glycoform ratio propagated after transmission of classical CJD prions, consistent with the PrP point mutations directly influencing PrP^{Sc} assembly. These data indicate that P102L or E200K mutations of human PrP have differing effects on prion propagation that depend upon prion strain type and can be significantly influenced by mismatch at polymorphic residue 129[48].

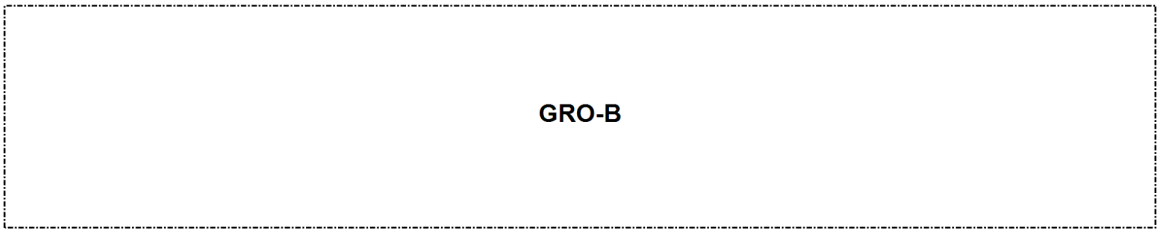
GRO-B

GRO-B

GRO-B

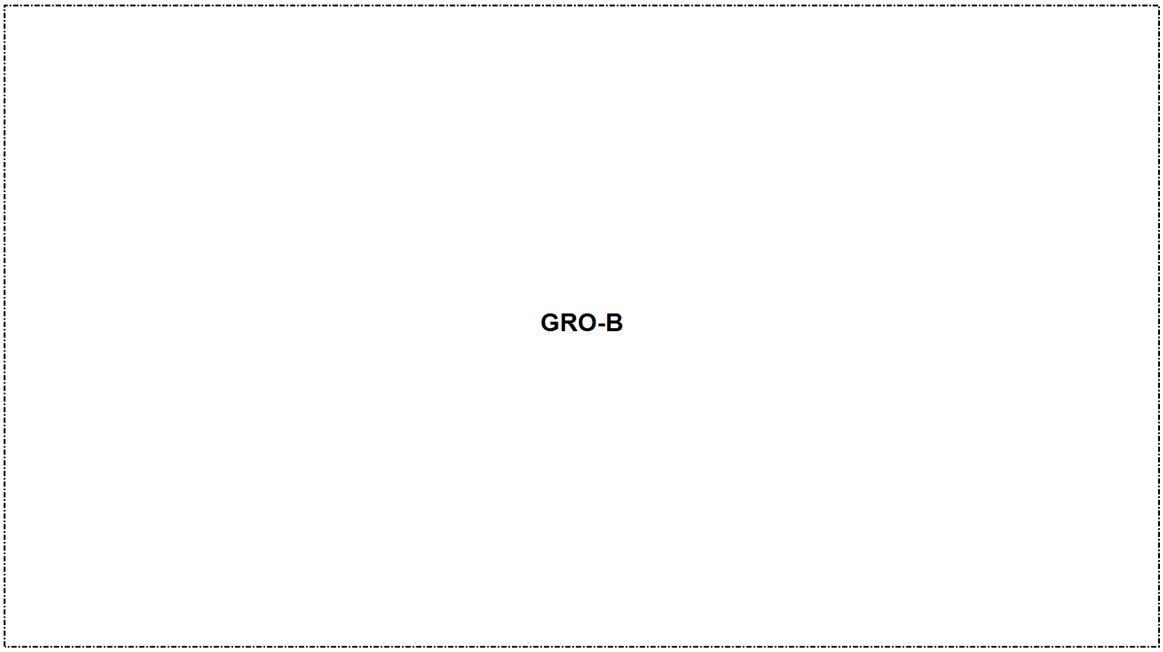
Investigating transmissibility of spontaneous disease due to other mutations
We have also observed spontaneous clinical disease and PrP plaques in the brains of transgenic mice expressing D178N-CJD (129VV) and D178N-FFI (129MM), and in an experimental mutation (M213A) and attempts to determine if this is transmissible to transgenic or wild type animals is underway. We have also challenged the D178N transgenic mice with classical CJD prions and brain homogenates from both IPD D178N CJD and FFI cases in order to assess the susceptibility of these mice. These inevitably long term

primary transmissions and serial passages will be completed and samples analysed in the next quinquennium.



Glycosylation deficient human prion transmission models

One of the most controversial aspects of the protein-only hypothesis is how the existence of multiple prion strains can be explained. Distinct human prion strains are associated with highly discrete ratios of the principal PrP glycoforms, which are maintained on serial passage[11,12]. However it remains unclear whether glycosylation is involved in encoding strain phenotype or is simply an epiphenomenon. We are investigating the role of glycosylation in strain determination by generating PrP^{C} glycosylation-deficient mice in which either one or both N-linked glycosylation sites have been disrupted.



We have generated, characterised and maintained two homozygous lines for each of the 6 permutations of single and double mutations on either M129 or V129 allele.
We have already challenged all but 2 lines with a carefully selected panel of human CJD isolates specially chosen to allow a study of their particular strain properties. While 2 lines remain to be inoculated because of poor breeding performance, some of the earlier groups have already succumbed to clinical disease and detailed biochemical studies of PrP^{Sc}, neuropathology and sub-passaging will be performed to investigate these transmissions.

GRO-B

Studies of vCJD pathogenesis in transgenic mice

In collaborative studies with Professor John Jefferys in Birmingham aimed at understanding the pathophysiology specific to vCJD infection, *in vitro* electrophysiological studies were performed in a mouse model in which human-derived vCJD prions were transmitted to transgenic mice expressing only human PrP. Electrophysiological changes were studied *in vitro* using a hippocampal slice preparation from infected animals.

Paired-pulse stimulation of the Schaffer collaterals evoked hypersynchronous bursting in the hippocampus of vCJD-inoculated mice, but comparable bursts were never observed in control or *Prnp* knockout mice, or in transgenic mice inoculated with classical CJD prions[58]. Furthermore, NMDA receptor-mediated excitation was increased in vCJD-inoculated mice. Using pharmacological experiments and computer simulations, we demonstrated that the increase in NMDA receptor-mediated excitation is necessary and sufficient to explain the distinctive bursting pattern in vCJD infected mice. These unique pathophysiological changes appear to result from a prion strain-specific gain-of-function and may explain some of the distinguishing clinical features of vCJD[58].

GRO-B

GRO-B

PUBLICATIONS, AWARDS, COLLABORATIONS AND PROFESSIONAL ACTIVITIES

Peer Reviewed Articles

GRO-B

Hill AF, Joiner S, Wadsworth JD, Sidle KC, Bell JE, Budka H, Ironside JW, and Collinge J. Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain* 2003 Jun; 126:1333-46

GRO-B

Wadsworth JD, Asante EA, Desbruslais M, Linehan JM, Joiner S, Gowland I, Welch J, Stone L, Lloyd SE, Hill AF, Brandner S, Collinge J. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science* 2004 Dec 3; 306:1793-6

GRO-B

Asante EA, Linehan JM, Gowland I, Joiner S, Fox K, Cooper S, Osiguwa O, Gorry M, Welch J, Houghton R, Desbruslais M, Brandner S, Wadsworth JD, Collinge J. Dissociation of pathological and molecular phenotype of variant Creutzfeldt-Jakob disease in transgenic human prion protein 129 heterozygous mice. *Proc Natl Acad Sci U S A* 2006; 103:10759-64

GRO-B

Wadsworth JD, Joiner S, Fox K, Linehan JM, Desbruslais M, Brandner S, Asante EA, Collinge J. Prion infectivity in variant Creutzfeldt-Jakob disease rectum. *Gut* 2007; 56:90-4

Wadsworth JD, Joiner S, Linehan JM, Desbruslais M, Fox K, Cooper S, Cronier S, Asante EA, Mead S, Brandner S, Hill AF, Collinge J. Kuru prions and sporadic Creutzfeldt-Jakob disease prions have equivalent transmission properties in transgenic and wild-type mice. *Proc Natl Acad Sci U S A* 2008; 105:3885-90

GRO-B

Ratté S, Prescott SA, Collinge J, Jefferys JG. Hippocampal bursts caused by changes in NMDA receptor-dependent excitation in a mouse model of variant CJD. *Neurobiol Dis* 2008; 32:96-104

GRO-B

GRO-B

Book Chapters

Hill A, Asante EA, Collinge J. Bioassays for prions. In: *Prions and Mad Cow Disease*, edited by Nunnally KB, Krull IS. 2003: 151-79. Marcel Dekker Inc, New York

Abstracts

GRO-B

Asante EA, Gowland I, Grimshaw A, Linehan JM, Smidak M, Houghton R, Osiguwa O, Tomlinson A, Joiner S, Brandner S, Wadsworth JDF and Collinge J.
PRNP point mutations dictate the assembly state of disease related prion protein. Joint Funder's Transmissible Spongiform Encephalopathy Workshop, University of Warwick 1-4 September 2008.

Asante EA, Gowland I, Linehan JM, Joiner S, Osiguwa O, Smidak M, Houghton R, Tomlinson A, Brandner S, Wadsworth JDF and Collinge J. Comparative transmission properties of P102L and E200K CJD prions in transgenic mice. Presented at Prion2007, Edinburgh 26-28 Sep. 2007

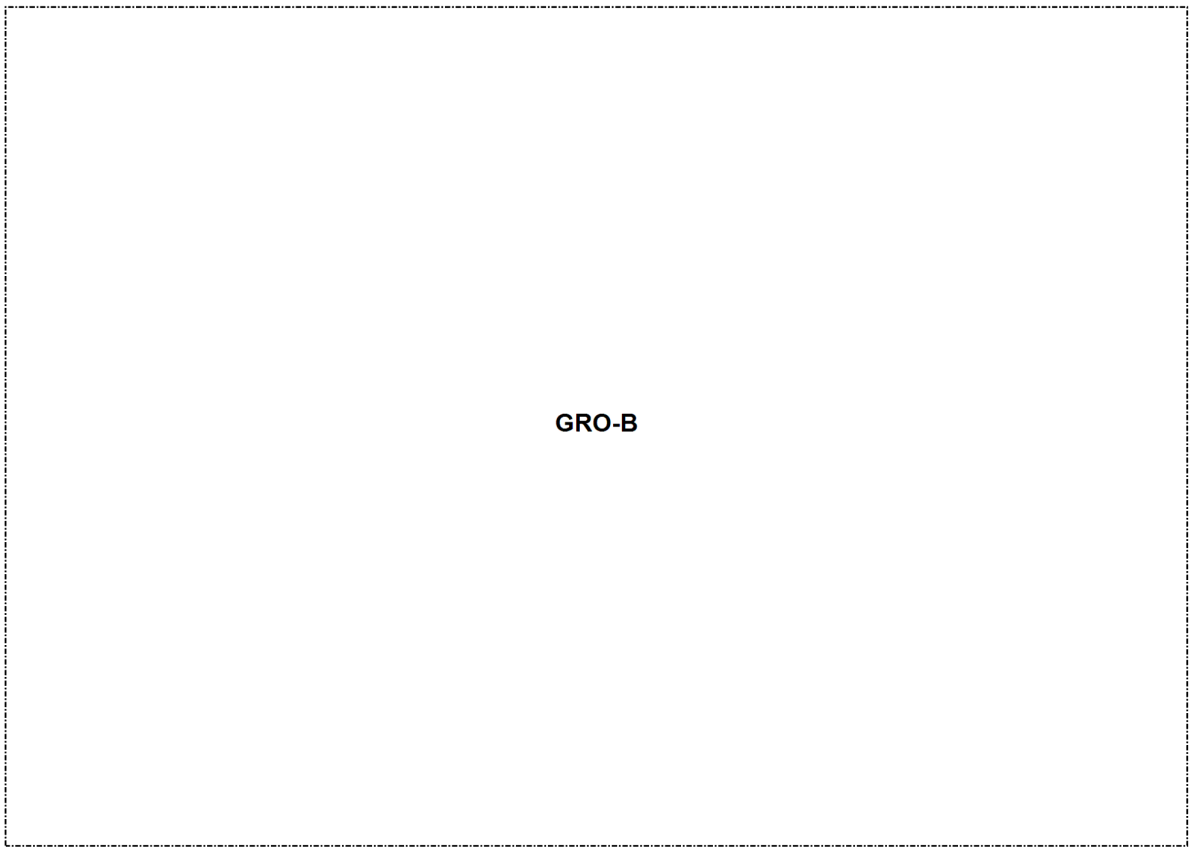
GRO-B

PROJECTS

Establishing the full range of prion strains causing human disease and to attempt to biologically clone these for biochemical study (with programme 6)

While important milestones have been achieved with our first generation 129MM, 129VV and the derivative 129MV human PrP transgenic mice[3,8,9,16,59] it has proved difficult to dissect the range of human prion strains in these mice and we have observed strain selection or mutation effects due to mixed genetic background. Understanding the genetic basis of these effects on strain propagation is a major aim of the Unit in the next quinquennium and we have exploited these findings to map genes responsible for these effects, including strain switching (see Programme 2).

As described in the report section, we have now established second generation human transgenics on two different inbred genetic backgrounds (FVB/N and SJL). The former was the major background of our previous transgenics and is now the uniform background of our new transgenic models and so will allow comparative studies with our huge body of existing transmission data; we anticipate that the SJL background will result in a significant shortening of incubation periods and provide better models to study primary and secondary vCJD.



SJL-congenic versions of Tg45 and Tg152 (see Programme 2) will be challenged with vCJD and BSE prions to determine if an expected major shortening of incubation period and increase of clinical attack rate occurs. If so, these new models will be used for bioassay of vCJD prions and preclinical studies of candidate therapeutics.

Investigation of effect of disruption of human PrP^C glycosylation on propagation of human and other prion strains

GRO-B

GRO-B Clearly, further studies are necessary to clarify the role of PrP glycosylation and in any case we wish to study human prion strains, not least with respect to the marked glycosylation differences between classical CJD and vCJD. We have challenged our newly available human PrP glycosylation deficient mice that have been generated on congenic FVB-PrP/null background, as detailed under past progress. The ongoing experiments are being conducted with Programme 6 and will be completed during the next quinquennium.

GRO-B

GRO-B

To investigate if transmissible prion infectivity has been spontaneously generated in D178N-129M (FFI), D178N-129V (CJD) and other PrP mutant models.
We have observed spontaneous clinical disease accompanied by positive PrP immunohistochemistry, and in some cases PrP plaques in the brains of transgenic mice expressing D178N 129M (FFI), D178N 129V (CJD) and M213A. However we have not been able to demonstrate the presence of typical protease resistant PrP^{Sc} at this stage. We will perform serial passage to investigate if transmissible prions are present. The M213A mutation is not one of the known disease causing mutations, but one that has been predicted to have a de-stabilising effect on PrP^{C} (Programme 7). Indeed these mice develop spontaneous clinical disease and PrP plaque deposition. Primary transmission of D178N and classical CJD prions to the D178N transgenic mice is ongoing and will be completed in the next 2 years.

GRO-B

GRO-B

Generation of new transgenic lines to model novel human PrP polymorphisms and candidate prion modifier genes (in conjunction with programmes 1, 2 and 11B)

GRO-B

GRO-B

Development and use of standardised models for evaluation of candidate therapeutics

GRO-B

GRO-B

Candidate therapeutics will also be evaluated in transgenic mouse models of human prion diseases including models of vCJD in which the human vCJD prion strain propagates and neuropathological features of vCJD (with abundant "florid" amyloid plaques), indistinguishable from that seen in vCJD-infected human brain, are faithfully recapitulated.

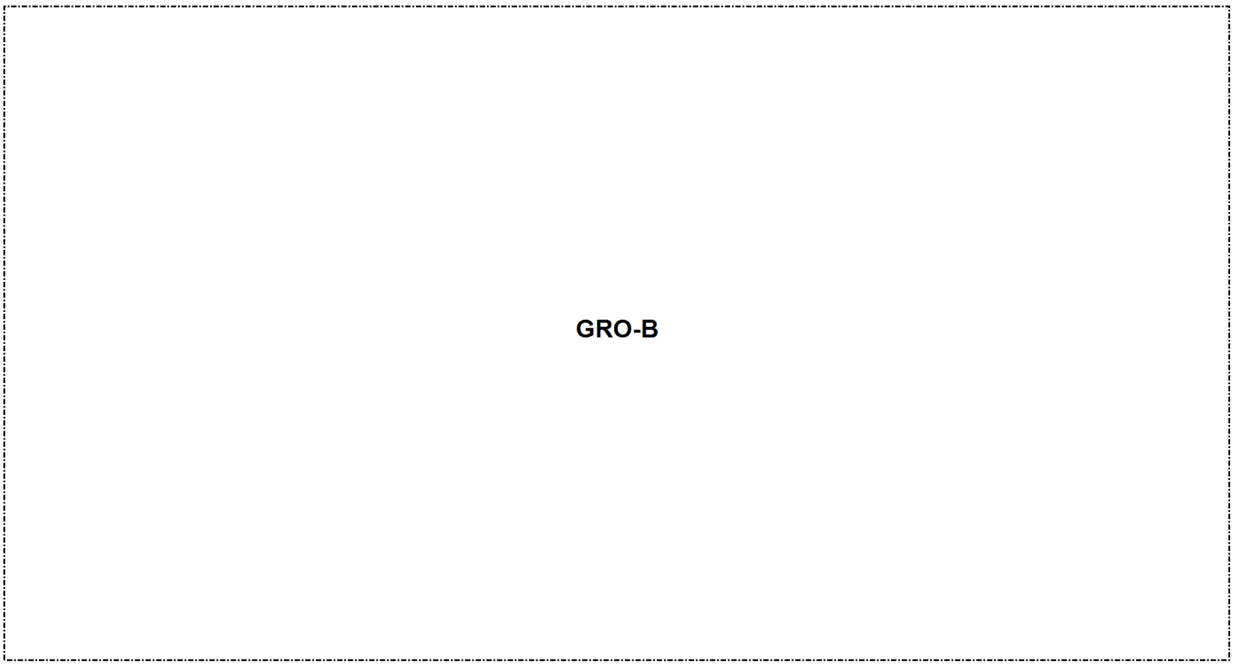
GRO-B

GRO-B

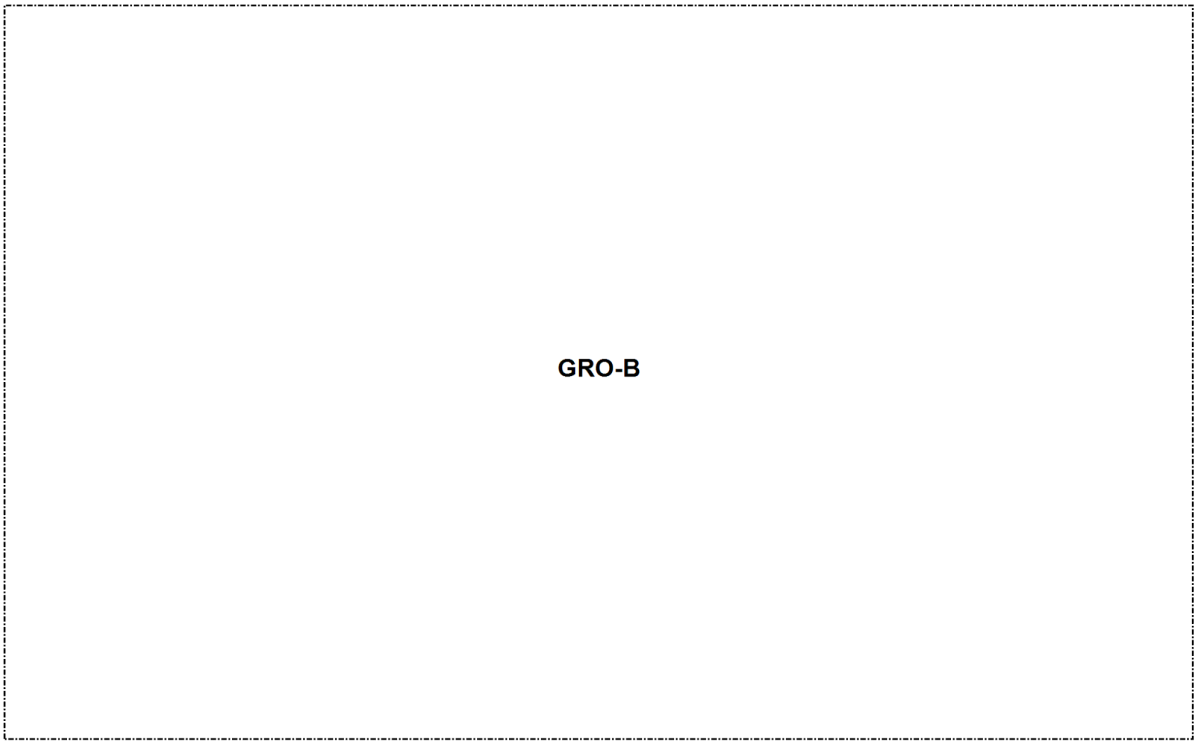
Cellular susceptibility factors for prion replication

Coding polymorphisms within *Prnp* are known to affect disease incubation times and susceptibility in human, mice, and sheep[34,35]. The most prominent example, codon 129 polymorphisms in humans, has major disease modifying effects[35] and homozygosity for methionine at codon 129 confers susceptibility to variant CJD (vCJD)[36,37]. However, significant differences in incubation times for scrapie in mice with the same *Prnp* genotype and quantitative trait genetic linkage studies indicate a major role of prion protein-independent genetic factors[38-42].

GRO-B



In Programme 8, Professor Jat is attempting to derive N2a subclones which propagate vCJD prions or Sc237 hamster prions to develop cell based bioassay for these strains in the Unit. This is being achieved by selection (see Programme 8) but advances in this Programme may allow targeted manipulation of susceptibility.



Programme 6

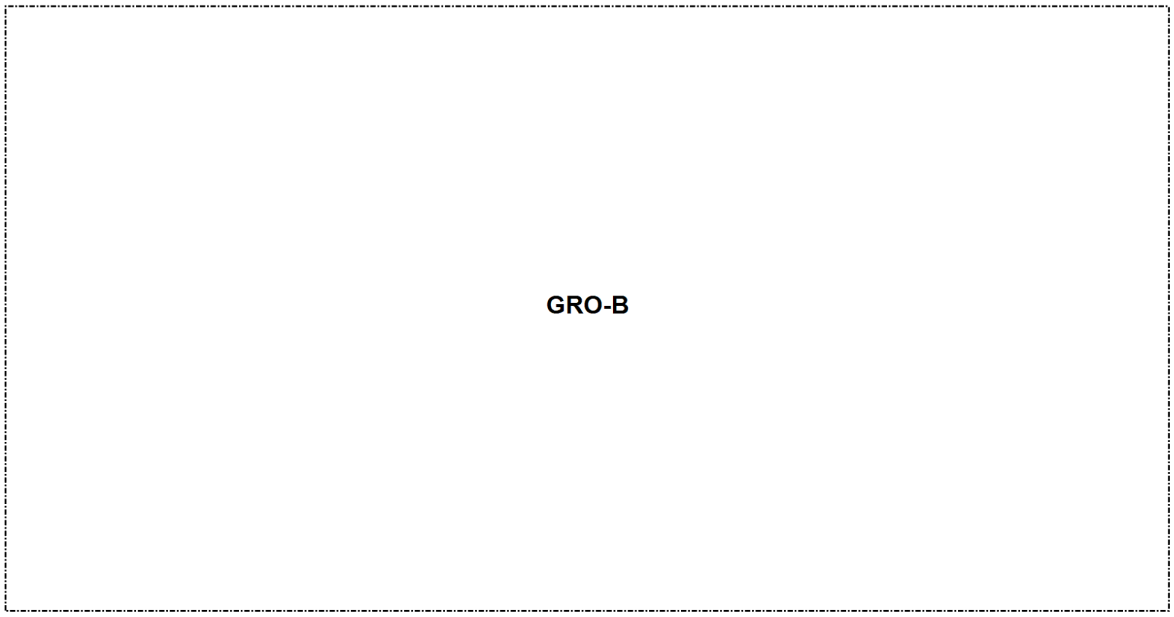
MOLECULAR AND PHENOTYPIC ANALYSIS OF HUMAN PRION STRAINS

Dr Jonathan Wadsworth and Professor John Collinge

Background to programme and contribution to Unit mission

Mammalian prions exist as multiple strains which produce characteristic phenotypes in defined hosts[1-6]. How this strain diversity is encoded by an apparently protein-only agent remains one of the most interesting and challenging questions in biology, with significant evolutionary implications[5,7]. The principles of protein conformation-based inheritance established from studying mammalian prions[1,8,9] are now also strongly supported by elegant studies with analogous systems in yeast models[10-12], indicating the very wide potential relevance of understanding the molecular basis of prion strains. Despite much worldwide research effort their precise composition and the molecular determinants of strain remain unknown[1,3-6,8,9]. Furthermore, it is clear that the barriers controlling transmission of prions between species are fundamentally related to strains, and indeed may be considered opposite sides of the same coin[1,5,13]. Such *conformational selection*, which we proposed in 1999[13], has also now been formally demonstrated in yeast[10-12]. Work in the Unit during the last quinquennium has led us to propose a *general model* of prion strains and the possibility that such phenomena may have a much wider relevance in other diseases involving aggregation or polymerisation of misfolded host proteins: including all the common neurodegenerative diseases[5].

This programme is therefore not only of fundamental importance to the basic scientific mission of the Unit - *to study the fundamental aspects of prion biology and their wider relevance* - but also to its public health and translational mission - *tackling the specific issues posed by BSE/vCJD in the UK.* The strain programme in the Unit has pioneered studies of vCJD prions and answered key questions to inform public health risk assessment, diagnosis and prevalence screening. The group works closely with the Unit clinical staff and provides molecular diagnosis and strain typing of prion disease from tissue biopsies nationally and internationally. This internationally recognised centre of expertise provides a key resource to safely handle and comprehensively characterise existing and emerging human and animal, potentially zoonotic, prion pathogens with appropriate biosecurity and experience.



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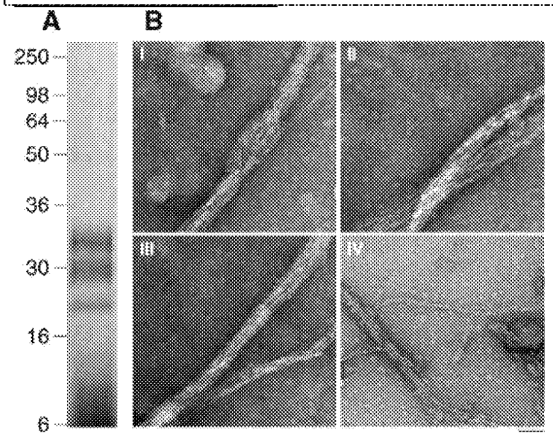


GRO-B

GRO-B

Progress with defining the diversity and molecular basis of human prion strains

GRO-B

GRO-B

Transgenic modelling of human prion disease

A major part of our work has been large-scale molecular strain typing of ongoing primary and secondary transmissions of the comprehensive phenotypic spectrum of human prion disease to transgenic mice expressing human prion protein with either methionine (M) or valine (V) at polymorphic residue 129. These studies enable the effects of genotype in inoculum and recipient transgenic mouse to be modelled and provide key information regarding the influence of PrP primary structure in influencing prion strain propagation[1,54,55]. These studies are performed in co-ordination with Dr Asante and Professor Brandner and are carried out in highly specialised animal microbiological containment facilities developed at the Unit. Dissecting the contribution of prion strain type from a growing number of other parameters known to influence human prion disease phenotype (for example, aetiology and host genome effects)[54,56-58] critically requires the development of new transgenic models that eliminate many of these variables. During this quinquennium we have worked closely with Dr Asante to initiate more than 50 primary transmissions of human prion diseases to new congenic lines of transgenic mice expressing human PrP. The congenic background of these mice will be critical for differentiating human prion strains associated with sporadic CJD from those with an animal origin and these transmissions are currently being analysed and sub-passaged for detailed molecular and neuropathological characterisation. Collectively this body of work represents the largest series of human transmissions performed internationally and are important, not only because they will help to define how many human prion strains exist (with major implications for public health epidemiology), but also because transgenic mouse brain will provide an excellent substrate for structural analysis of human prion strain variation.

Homozygosity at polymorphic residue 129 of human PrP remains the key genetic susceptibility factor for sporadic and acquired prion disease[59-64] including vCJD where it represents the strongest known genetic susceptibility polymorphism in any human disease[64-66]. A particular highlight of our research has been to provide a molecular basis for this effect. Through our transgenic studies we have shown that this polymorphism constrains both the propagation of distinct human PrP^{Sc} conformers and the occurrence of associated patterns of neuropathology[67-71]. Biophysical measurements suggest that this powerful effect of residue 129 on prion strain selection is likely to be mediated via its effect on the conformation of PrP^{Sc} or its precursors or on the kinetics of their formation, as it has no measurable effect on the folding, dynamics or stability of PrP^{C} [5,72]. These data are consistent with the conformational selection model of prion transmission barriers[5,13,69] and strongly support the protein-only hypothesis of prion infectivity by indicating that alternative conformations or assembly states of PrP provide the molecular substrate for the propagation of distinct human prion strains.

GRO-B

GRO-B

vCJD

Our studies on the molecular basis of human prion strains provided the first strong experimental evidence that vCJD was the human counterpart of BSE[25,67]. Since then we have led internationally in the characterisation of vCJD. We have demonstrated that vCJD has a pathogenesis distinct from all other forms of human prion disease with prominent involvement of lymphoreticular tissues[73-77] and have translated these findings to develop methods for tonsil biopsy[73,74,78,79] which remains the most sensitive and specific ante-mortem diagnostic procedure for vCJD and forms an established part of National and WHO diagnostic criteria. Using ultra-high sensitive methods that we developed[80], we have led on providing upper limits on PrP^{Sc} levels in vCJD peripheral tissues to inform risk assessment models to prevent iatrogenic transmission of vCJD prions[54,74]. During this quinquennium we have continued with these ongoing studies and, in particular, have focused on the risks posed by the gut for transmission of vCJD prions through endoscopy or surgery[75,81]. As part of this work we have also undertaken extensive transmissions to transgenic mice to evaluate vCJD prion titres in relevant peripheral tissues. Additionally we have also undertaken studies to investigate the potential for vertical transmission of vCJD prions by examination of reproductive tissues and have also investigated the basis of severe neurological dysfunction in a child born to a mother with vCJD (Isaacs et al, submitted). During this quinquennium four cases of secondary vCJD associated with blood transfusion have occurred and we have undertaken extensive analysis of the central and peripheral pathogenesis of both the third[82] and fourth cases (manuscript submitted). Our demonstration of tonsillar prion colonisation in these patients with secondary (iatrogenic) variant CJD indicates that tonsil biopsy will allow early diagnosis in other similarly exposed high risk patients as well as in those with primary BSE infection. We have also continued to act as a national specialist centre with the ability to respond rapidly to emerging public health issues to aid policy makers. We have completed a pilot study (at the request of and funded by the Department of Health)[83] to estimate the prevalence of vCJD infection in the UK population which has formed the basis of the Health Protection Agency's large scale National Anonymous Tonsil Archive (NATA) study.

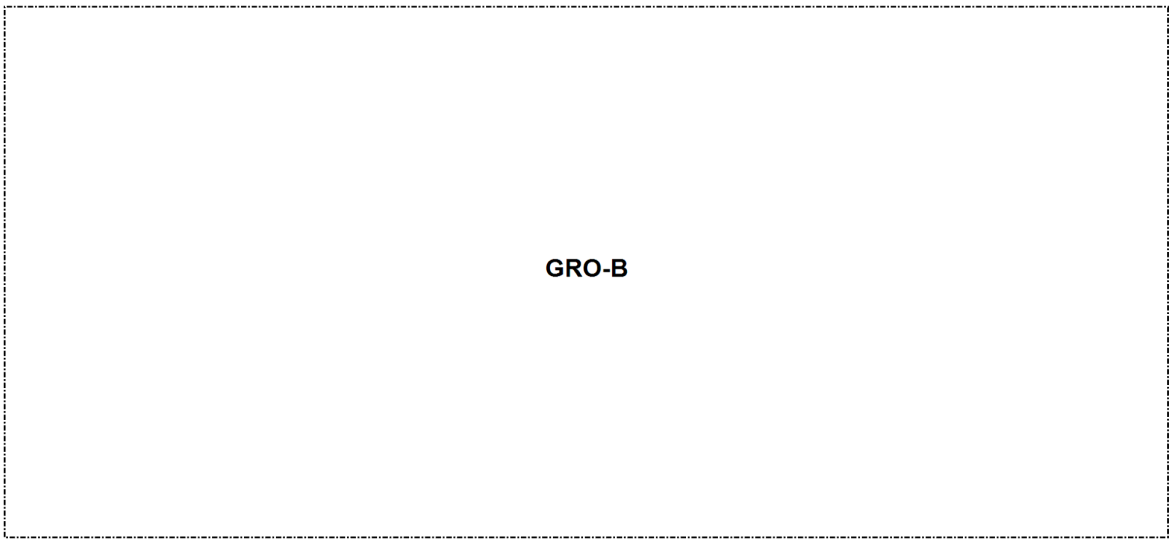
Sporadic CJD

Following on from our detailed molecular classification of sporadic CJD[84], we have continued to characterise novel and unusual forms of 'sporadic' human prion disease that have presented at the National Prion Clinic. Some of these cases have already been reported[85,86] while others will be reported in due course following analysis of transmission experiments that are currently ongoing. This research forms an essential part of our molecular surveillance that will allow early recognition of new forms of human prion disease should they occur.

Inherited prion disease

A major focus has been to investigate the molecular basis for the pronounced phenotypic diversity seen in inherited prion disease[49,76,87-90]. In agreement with existing evidence that human prion strain diversity may be generated through variance in PrP^{Sc} conformation and glycosylation[1,3,5,8,9,23-28], we have shown that cases of inherited prion disease caused by point mutations have PrP^{Sc} glycoform ratios that are distinct from those seen in classical CJD and vCJD[49,76]. These findings have been strongly supported by recent transgenic modelling studies with Dr Asante and indicate that *PRNP* point mutations directly dictate the preferred assembly state of disease related PrP isoforms[91]. A further significant finding from

these studies has been to show that the propagation of pathological isoforms of wild type PrP may make a significant contribution to phenotypic variability in P102L inherited prion disease[49]. Differences in the neuropathological targeting of distinct disease-related PrP species generated from mutant and wild-type PrP, together with differences in their abundance and potential neurotoxicity, provides a general molecular mechanism for the generation of multiple clinicopathological phenotypes in inherited prion disease[49,54].



Progress with defining prion transmission barriers relevant to public health

The molecular basis of prion strain diversity lies at the heart of understanding prion replication and transmission barriers[1,5]. Our studies with Dr Asante (Programme 3) have led internationally in characterising the bovine to human transmission barrier and this research forms a large part of the Unit mission *to tackle the specific issues posed by BSE/vCJD in the UK.* Our research was the first to demonstrate transmission of BSE prions to transgenic mice expressing human PrP and these data confirmed that vCJD was caused by human exposure to the BSE prion strain[67,68].

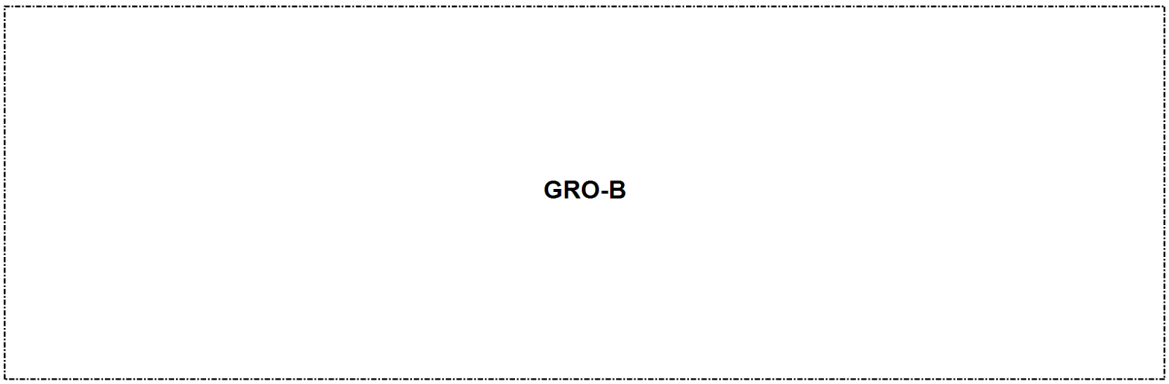
During this quinquennium our continued transgenic modelling of primary and secondary human BSE infection has established that there is no common preferred PrP^{Sc} conformation for V129 and M129 human PrP that can be generated as a result of exposure to the vCJD/BSE prion strain[69]. Because human PrP with V129 is incompatible with the PrP conformation propagated in vCJD[69], and may have a dominant negative influence on the propagation of the vCJD prion strain in codon 129 heterozygotes mice[70], this may explain why vCJD has occurred exclusively in individuals homozygous for M129. While caution must be exercised in extrapolating from animal models, even where faithful recapitulation of molecular and pathological phenotypes is possible[68-71], our data, together with more recent studies from other laboratories[95,96], argue that primary human BSE prion infection, and secondary infection through iatrogenic routes, will not be restricted to a single disease phenotype. Dependent upon the genotype of the prion source and the recipient the propagation of prion strains seen in classical CJD or other novel prion strain types are anticipated[68-71]. These data reiterate the need to continue to stratify all human prion disease patients at the molecular level to facilitate rapid recognition of novel sub-types and change in the relative frequencies of particular subtypes due to either BSE exposure patterns or iatrogenic sources of human prions.

In addition to transgenic modelling of primary and secondary human BSE infection, we have also established important collaborations to examine the potential risks to public health posed by other animal prion strains. Of particular concern at present are the BSE

prion strain passaged in sheep, atypical forms of sheep scrapie and chronic wasting disease of deer and elk. We have established collaborations with Dr Jim Hope, Veterinary Laboratories Agency, Professor Martin Groschup, Friedrich-Loeffler-Institut and Professor Adriano Aguzzi, University Hospital Zurich who have provided us with highly characterised isolates of these prion diseases. Findings with CWD prions have now been submitted for publication (Sandberg et al, submitted) and transmissions of a comprehensive panel of sheep isolates to more than 800 transgenic mice are currently ongoing. Positive transmissions will be characterised by sub-passage followed by substantial characterisation of molecular and neuropathological prion strain phenotypes and correlation with the phenotypes of human prion strains propagated in the same congenic mice.

Translation and knowledge transfer

In addition to our core research functions we provide a tissue diagnostic service to the National Prion Clinic and receive referrals from neuropathologists worldwide. We have trained, and supervise, a technician funded by the National Prion Clinic which facilitates the close integration of basic and clinical research and has a direct impact on clinical care. Our development of tonsil biopsy is now an established part of National and WHO diagnostic criteria for ante-mortem diagnosis of vCJD and this procedure together with other diagnostic methods that we developed[80] are now used throughout the world. We continue to offer training in these techniques and act as a WHO international reference centre in this regard. We also interact closely with patient groups and the PhD studentship of Programme 6 receives a scholarship endowment from the CJD Support Network. We provide regular written research updates to the CJDSN newsletter and give research presentations at family support days. All of the work of Programme 6 is conducted in ACDP microbiological containment level 3 facilities and we are providers of specialist training and knowledge. In this regard we continue to make contributions to all other research programmes in the Unit and contribute substantially to MRC codes of safe laboratory practice for working with prion isolates. We also continue to act as a national specialist centre and have made major contributions to the Department of Health's vCJD prevalence studies and have continued to focus on informing policy makers on the pathogenesis of vCJD with respect to potential routes of iatrogenic transmission. Professor Collinge and Dr Wadsworth are members of various expert advisory committees allowing the rapid dissemination of knowledge and preliminary research findings to assist in the formulation of healthcare policy[97]. Professor Collinge is a Director, and Professor Collinge and Dr Wadsworth are shareholders and consultants of D-Gen Limited, an academic spin-out company working in the field of prion disease diagnosis, decontamination, and therapeutics. D-Gen's institutional shareholders include The Medical Research Council, Imperial College London, The Wellcome Trust and the University of Bristol. During this quinquennium D-Gen has successfully licensed two discoveries made by the Unit leading to commercialisation of products for testing cattle for BSE (Roche Diagnostics) and for prion sterilisation of surgical instruments (DuPont Corporation).



GRO-B

PUBLICATIONS, AWARDS, COLLABORATIONS AND PROFESSIONAL ACTIVITIES

Publications since last quinquennial report

Peer reviewed articles

GRO-B

Webb T, Pal S, Siddique D, Heaney D, Brandner S, Linehan JM, Wadsworth JDF, Joiner S, Beck J, Wroe SJ, Stevenson V, Mead S, Collinge J. First report of sporadic Creutzfeldt-Jakob disease occurring in two siblings. *J Neuropathol Exp Neurol* 2008; 67: 838-841.

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Mead S, Joiner S, Desbruslais M, Beck JA, O'Donoghue M, Stevens J, Rossor M, Lantos P, Wadsworth JDF, Collinge J. A young British woman with CJD, valine homozygosity at *PRNP* codon 129 and a novel PrP^{Sc} type. *Arch Neurol* 2007; 64: 1780-1784.

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GRO-B

Hill AF, Joiner S, Wadsworth JDF, Sidle KCL, Bell JE, Budka H, Ironside JW, Collinge J. Molecular classification of sporadic Creutzfeldt-Jakob disease. *Brain* 2003; 126: 1333-1346.

Book chapters, reviews and editorials

GRO-B

Wadsworth JDF, Collinge J. Molecular pathology of prion diseases. In: *Creutzfeldt-Jakob Disease: Managing the Risk of Transfusion Transmission* (Turner, M. ed) 2006; pp 1-36, AABB Press, Bethesda, Maryland, USA

GRO-B

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GRO-B

GRO-B

AIMS AND EXPERIMENTAL PLANS

Rationale

GRO-B

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The hypothesis that alternative conformations or assembly states of PrP provide the molecular substrate for clinicopathological heterogeneity seen in human prion diseases and that this relates to the existence of distinct human prion strains is supported by considerable experiment evidence[1,3,5,8,9,23-28] and also by the demonstration of protein conformation-based inheritance mechanisms of yeast prions[10-12]. Despite these advances, the precise molecular basis of mammalian prion strain diversity remains unknown. A major confounding issue in this regard has been in resolving whether relatively subtle biochemical differences in PrP^{Sc} are of biological importance and accurately reflect the propagation of distinct prion strains. This is particularly true in sporadic CJD[25,26,84,107-111] where progress has been severely hampered by a lack of transgenic modelling data to firmly distinguish the identity of distinct prion strains and their defining molecular and neuropathological phenotypes. This fundamental problem coupled with the difficulties and variability of the biochemical methods used to distinguish PrP^{Sc} types[54,84,108,109,111] has so far precluded an internationally accepted classification system for human prion strains. In this regard, the increasingly recognised co-occurrence of different PrP^{Sc} types in the same brain[26,49,84,112-119] has further confounded progress. All of these factors, together with the known ability of genetic background to influence prion strain selection[64,68,120-122], has re-emphasised the requirement to remove host variability by identifying distinct prion strains in appropriate transgenic models. In addition, it is now clear that protease-sensitive pathological isoforms of PrP may have a significant role in both animal and human prion disease[27,32-39] and therefore development of new diagnostic tests and biochemical methods that do not rely on proteinase K digestion are required for their detection and characterisation. Achievement of an accurate classification system for human prion strains by defined molecular criteria will have immediate practical consequences in studying human prion disease. This will facilitate aetiological and epidemiological studies of human disease, notably with respect to sporadic CJD where evidence for aetiological heterogeneity is mounting[123,124], and comprehensive evaluation of relationship between the prion strains causing the various forms of human disease with those causing animal prion diseases. Understanding these relationships and the risks that existing and emerging animal prion diseases pose will have direct translation to protecting public health[54,65].

Aims

1. To define the molecular composition and ultra-structure of infectious prion strains
2. To define the diversity and molecular basis of human prion strains
3. To fully characterise prion transmission barriers relevant to public health

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GRO-B

(2) Defining the diversity and molecular basis of human prion strains

GRO-B

As described in our progress report, we are working closely with Dr Asante on more than 50 primary transmissions of human prion disease to new congenic lines of transgenic mice expressing human PrP with either valine or methionine at residue 129. These human isolates represent a comprehensive molecular and phenotypic spectrum of human prion disease and the congenic background of the transgenic mice will enable differentiation of distinct human prion strains (see Programme 3). Primary screening of mouse brain from these transmissions will be conducted by PrP immuno-histochemistry overseen by Professor Brandner and we will support these studies by performing molecular PrP analysis on representative neuropathological phenotypes. Sub-passage of isolates representative of the range of molecular and neuropathological phenotypes will be performed. A major focus here will be to establish the role of *PRNP* codon 129 in defining sporadic CJD prion strains and to establish a minimum number of prion strains that are propagated in sporadic CJD. Despite notable advances[25,26,84,111], human studies have not been able to resolve this issue due to fundamental problems of host variability and a reliance on overlapping phenotypic criteria. Key molecular and neuropathological phenotypes seen in the congenic mice will be re-correlated with findings in human brain, involving molecular PrP studies within this Programme allied with further neuropathological investigations by Professor Brandner. At this point identified human prion strain isolates will be amplified through large scale sub-

passage in congenic mice in order to generate material for our detailed compositional and ultra-structure studies.

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(iii)Diagnostic research support to the National Prion Clinic

GRO-B

Our ongoing analysis of human cases, using our existing methods[80], will allow us to rapidly recognise new forms of human prion disease should they occur. Substantial characterisation of novel human cases will be performed to characterise their pathogenesis and this will including comprehensive transmission studies to conventional and transgenic mice. New forms of BSE-related human prion disease that are predicted from our transgenic modelling studies may pose new threats to public health and it is imperative that these are recognised and characterised as quickly as possible. In this regard, we will continue to place a high priority on rapid evaluation of peripheral pathogenesis states in newly emerging human prion disease cases.

Our detailed biochemical investigations of disease-related PrP allied with detailed clinical and neuropathological analysis will continue to inform on the diversity of phenotypes seen in human prion disease. As it has now clear that prion strain type, host genetic makeup, and other factors (for example route of transmission) may significantly influence prion disease phenotype[1,64,65,71] it is expected that the actual number of distinct human prion strains may be far less than the number of identified phenotypes. The combined studies of Programme 6 and Programme 3 will directly inform on how many human prion strains there are, and what the defining molecular features of PrP are for each strain. This information allied with our comprehensive transgenic modelling of human BSE infection, and ongoing transmissions of other relevant, potentially zoonotic, prion strains will inform on how many human prion strains may have an animal origin. Accurate classification of human prion disease will have major implications for epidemiological research into the causes of sporadic CJD, whose aetiology remains obscure. While spontaneous conversion of PrP^{C} to PrP^{Sc} as a rare stochastic event, or somatic mutation of the PrP gene, resulting in expression of a pathogenic PrP mutant are plausible explanations for sporadic CJD[1,49,88,131], other causes for at least some cases, include environmental exposure to human prions[123,124] or exposure to animal prions. In this regard, the number of prion strains causing sheep scrapie has yet to be established[6,132] and epidemiological data cannot exclude this as a

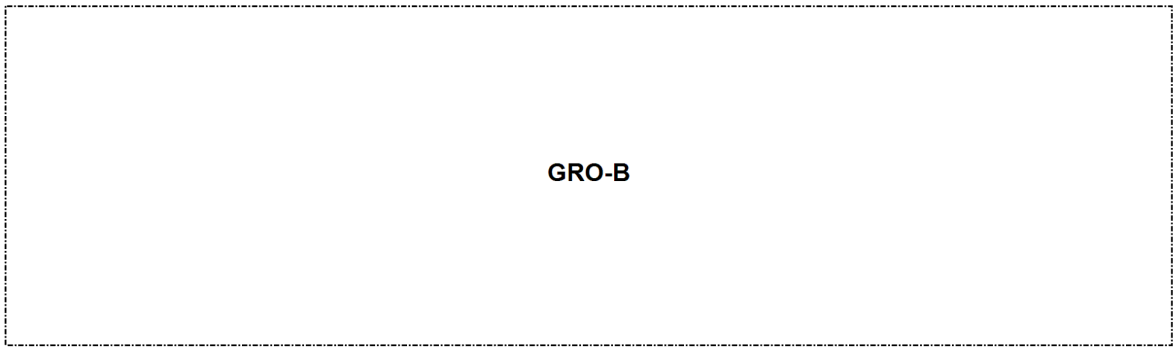
cause of a minority of cases. As our research will provide a more precise understanding of the origins of human prion disease, this will facilitate re-analysis of epidemiological data, by the National CJD Surveillance Unit and others, to reveal important risk factors that might have been obscured by analysing sporadic CJD as a single entity.

In addition to our planned research, we will also continue to contribute to the Unit's role of acting as a National Centre with the ability to react rapidly to new public health concerns. Here our membership of various expert advisory committees including those of the WHO, the Department of Health and the Health Protection Agency, means that we can directly contribute to answering questions that help formulate public heath policy. For example, at present there is renewed concern regarding dentistry as a potential route of iatrogenic transmission of vCJD prions. Accordingly we have planned to undertake analysis of relevant tissues from the oral cavity as they become available via the National Prion Monitoring Cohort Study to directly inform on this risk.

(3) Investigating prion transmission barriers relevant to public health

The Unit provides facilities with appropriate biosecurity and expertise to handle all forms of human and animal prion disease and one of the Unit's major contributions as a National Centre is to evaluate risks posed to public health by novel animal prion strains. In the next quinquennium, the major focus of our contribution to these studies will be to undertake analysis of ongoing transmissions of sheep prions to our congenic mice expressing human PrP. The panel of inocula used in these studies comprise highly characterised examples of field cases of typical and atypical sheep scrapie provided by Dr Jim Hope and Professor Martin Groschup and primary and secondary passaged experimental sheep BSE generated at the VLA. Analysis of ~800 mice will be required during the next quinquennium to fully evaluate these transmissions. Primary screening of mouse brain will be performed by both PrP immunohistochemistry and immunoblotting. Extensive sub-passage of any positive transmissions will be performed accompanied by substantial characterisation of molecular and neuropathological prion strain phenotypes and correlation with human prion strains propagated in the same congenic mice. These studies will directly inform on how many human prion strains may have an animal origin.

The emergence of other new or newly recognised potentially zoonotic prion strains is anticipated during the next quinquennium. Indeed a number of novel isolates of bovine prion disease have now already been identified which appear to be distinct prion strains[6,133-135] and the host range of atypical sheep prions[6,53,132] has not been established. Because prion strains can adapt and mutate on passage in new species[5,6,136], and also within species as a result of PrP polymorphisms and other genetic factors[56,64,68,69,137], the evaluation of their risks to public health must be directly evaluated in the most appropriate transgenic models[138]. In this regard, the collaboration that we have established with Jim Hope at the VLA and Martin Groschup at the Friedrich-Loeffler-Institut will enable us to react rapidly to new public health concerns from new emergent animal prions if identified.



Programme 7

STRUCTURAL STUDIES OF PRION PROTEINS AND THEIR LIGAND INTERACTIONS

Professor Anthony Clarke

GRO-B

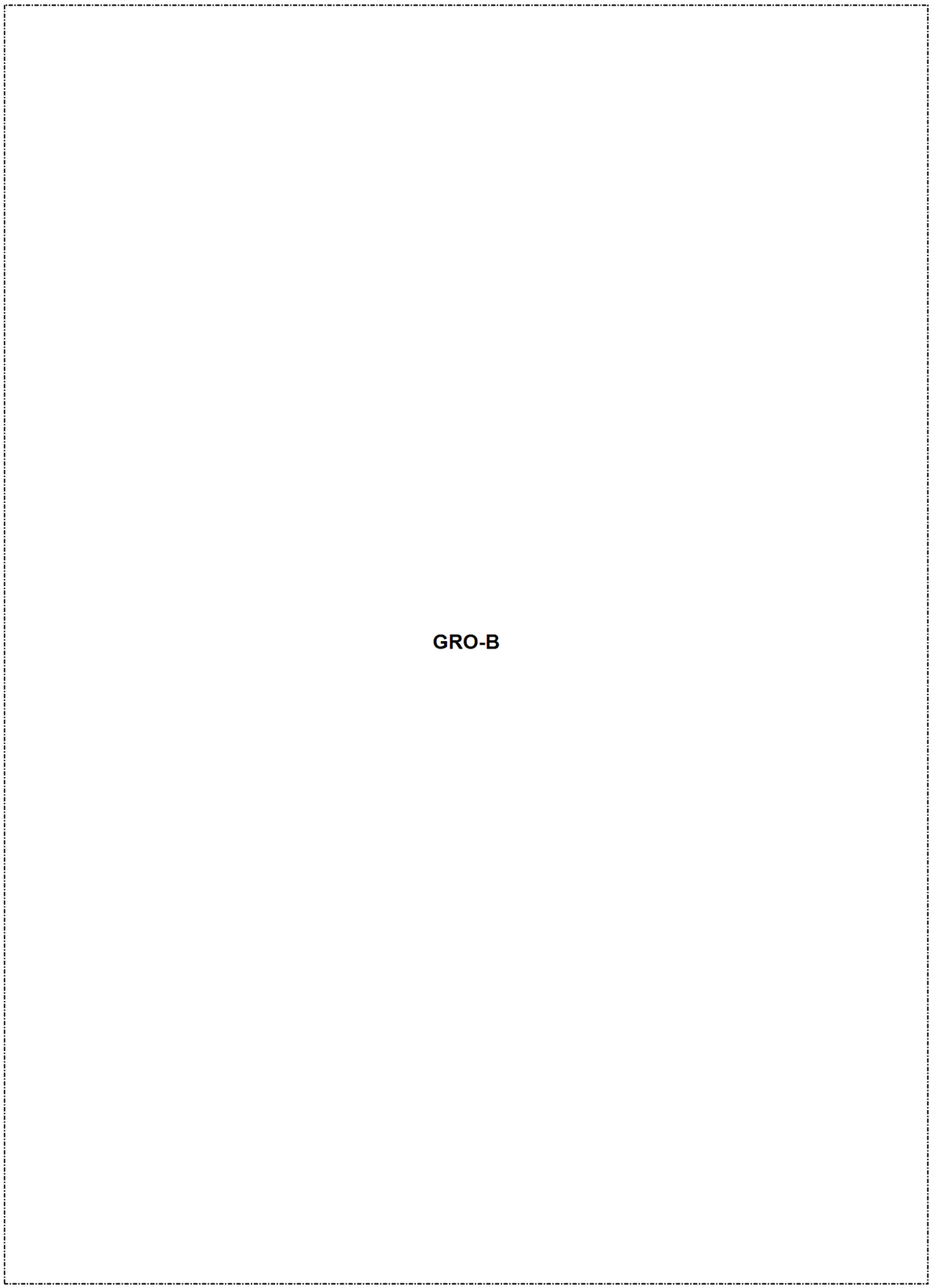
GRO-B

Translation and knowledge transfer

Enzymatic decontamination of prions

Iatrogenic transmission of classical CJD via neurosurgical instruments is well documented[79-81] and the involvement of lymphoreticular tissues in variant CJD (vCJD) [82], together with the unknown population prevalence of asymptomatic vCJD infection, has raised concerns about prion disease transmission from a wide range of surgical procedures. To address this problem, we looked for conditions that destroy infectious material adhered to surgical steel surfaces. A range of proteolytic enzymes were evaluated for their ability to digest PrP^{Sc} from vCJD brain. A combination of proteinase K and Pronase, in conjunction with SDS, was shown to degrade PrP^{Sc} material from highly concentrated vCJD-infected brain preparations to a level below detection[83]. When RML

prion-infected wires were exposed to the same enzymic treatment, intracerebral bioassay in highly susceptible hosts showed virtually no infectivity[83]. The prion-degrading reagents identified in this study are readily available, inexpensive, non-corrosive to instruments, non-hazardous to staff and compatible with current equipment and procedures used in hospital sterilization units. The technology developed within the Unit has now been licensed to the DuPont Corporation.



GRO-B

PUBLICATIONS, AWARDS, COLLABORATIONS AND PROFESSIONAL ACTIVITIES

Publications since last quinquennial report

Peer reviewed articles

Three manuscripts are currently in preparation or submitted

GRO-B

GRO-B

Hosszu LL, Jackson GS, Trevitt C, Jones S, Batchelor M, Bhelt D, Prodromidou K, Clarke A, Waltho JP, Collinge J. The residue 129 polymorphism in human prion protein does not confer susceptibility to CJD by altering the structure or global stability of PrP^{C}. *Journal of Biological Chemistry* 2004; 279: 28515-21.

Peer reviewed reviews

GRO-B

Trevitt C, Singh PN. Variant Creutzfeldt-Jakob disease: pathology, epidemiology, and public health implications. *American Journal of Clinical Nutrition* 2003; 78: 651S-6S.

GRO-B

Abstracts

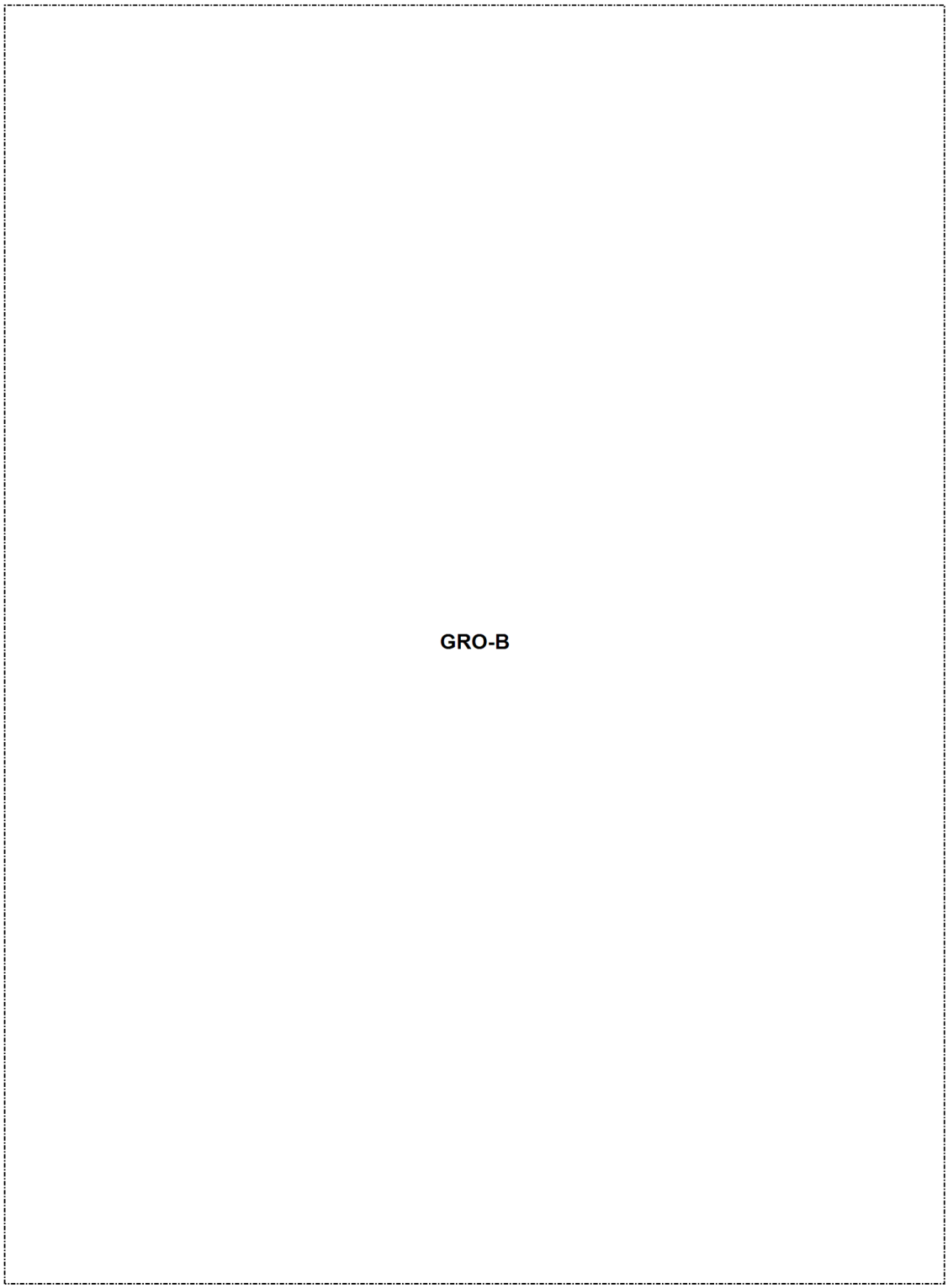
GRO-B

The residue 129 polymorphism in human prion protein does not confer susceptibility to CJD by altering the structure or global stability of PrP^{C}. Hosszu, L.L., Jackson, G.S., Trevitt, C.R., Jones, S., Batchelor, M., Bhelt, D., Prodromidou, K., Clarke, A.R., Waltho.

Programme 8

PRION KINETICS, TOXICITY AND SYNTHESIS AND ITS WIDER RELEVANCE

Professor John Collinge and Professor Anthony Clarke



PROGRESS REPORT

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GRO-B

Development of cell lines responsive to human or hamster prions

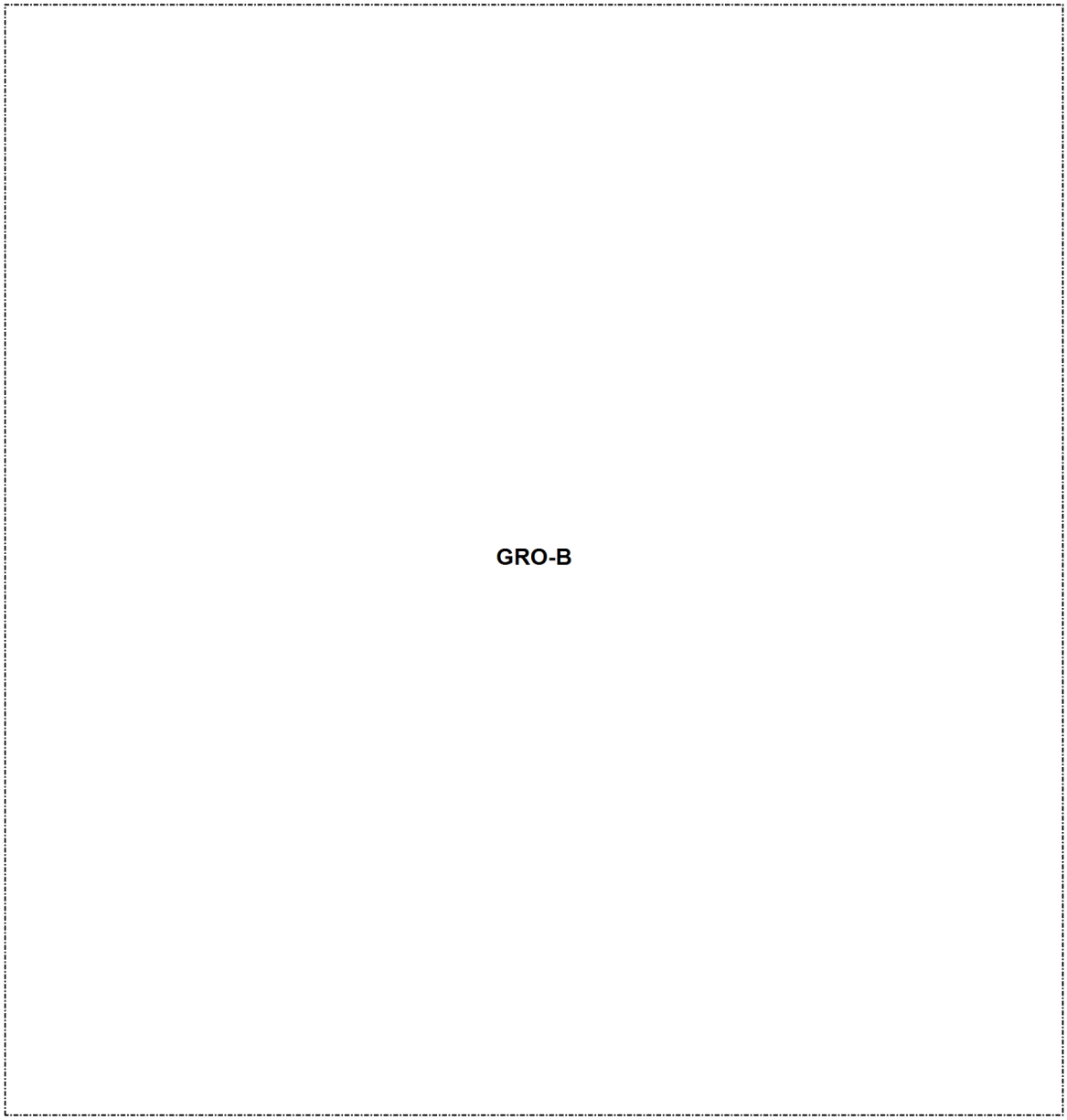
The use of *in vitro* cell culture models to study prion propagation has been limited to a few cell-lines that are susceptible either to mouse-adapted or sheep scrapie prion strains, with none yet described able to stably propagate human prions, despite world-wide efforts. Over the last five years, a wide range of strategies have been deployed by Dr Sarah Tabrizi, who joined the UCL Department staff funded by a DH Clinical Scientist Award, working with Unit scientists, to obtain a human cell model of prion disease that could be utilised both for studies of cellular pathogenesis and to provide a cell based assay for vCJD prions. The first approach utilised a high-throughput screening methodology in our microbiological containment level III facility to assess multiple mammalian cell lines for their susceptibility to infection with human vCJD-prions. This involved the exposure of each cell line to vCJD-prions, following which they were sub-cloned so that rare susceptible clones could be isolated; susceptibility to prion propagation was then assayed by monitoring PrP^{Sc} levels over time using the scrapie cell assay (SCA) or a cell blot assay. A wide range of mammalian neuroblastoma, neuroglia and epithelial cell lines were assayed in this manner, but whilst transient vCJD prion propagation was observed in two human neuroblastoma lines (SKN-SH and SHSY5Y) and a rabbit epithelial kidney line (RK13) overexpressing human PrP^{C}, none were shown to stably propagate human vCJD-prions. Attempts to prolong prion propagation in a human neuroblastoma cell line by means of retroviral infection to mimic the intercellular spread of prions via exosomes also proved unsuccessful.

Another approach has involved using a v-myc immortalised human foetal neural stem cell (NSC) line derived from human ventral mesencephalon; this is termed the ReNcell 197VM cell line. Whilst maintained in their undifferentiated state, these 197VM cells were completely resistant to vCJD-prion infection. Conversely though, once differentiated to a neuronal phenotype by the removal of certain key growth factors from the culture medium, the 197VM cells have shown some evidence of stable prion propagation following infection with a semi-purified preparation of human vCJD prions. However, it should be noted that differentiated 197VM cells do not proliferate in culture, making it difficult to rigorously apply the SCA as a means of testing for prolonged prion propagation in these cells. This cell line may prove useful to model vCJD-prion infection and study cellular pathophysiology, but is clearly not likely to be useful for cell-based bioassay.

The difficulty in obtaining a cell line suitable for human cellular pathogenesis studies, which can also propagate vCJD prions sufficiently well to form the basis of a sensitive and reproducible assay system, led us to diverge these aims particularly since the development of the latter has become an increasing priority for our collaborative therapeutics programme with GSK (see Appendix 2). Dr Tabrizi has continued to focus on the development of a human cell line for pathogenesis studies, supported by the Unit, while a different approach to developing a cell line suitable for vCJD prion assay was pursued.

The murine neuroblastoma cell line N2a has been widely used in prion research and, as described above, was used to derive the highly susceptible PK1 line in the Unit by extensive subcloning[3]. The basis of the increased susceptibility of PK1 cells is not known, but is being investigated (see Programme 5). Our aim was to make use of this cell line in an analogous fashion to the strategy used to make mouse models of human and other prion diseases by transgenically expressing PrP^{C} from different species in *Prnp*$^{0/0}$ mice[14-16]. Since PK1 cells are polyploid and contain approx 6 copies of *Prnp*, we used RNA interference to silence

expression of the endogenous mouse *Prnp* in PK1 cells and then reconstituted them with the open reading frames (ORFs) of PrP genes from other species.



Importantly, we have also reconstituted the doubly silenced N2aATCC cells with the full length ORF for human PrP (G418^{r}) containing methionine at codon 129. 1500 single cell clones were isolated and screened by SCA using vCJD prions. Five clones were identified which reproducibly show susceptibility to vCJD; N2a9-8c is the most susceptible. This strategy has therefore been successful and will be taken forward in the next quinquennium with the aim of developing a rapid accurate ASCA for vCJD and Sc237 prions in the Unit (see future plans).

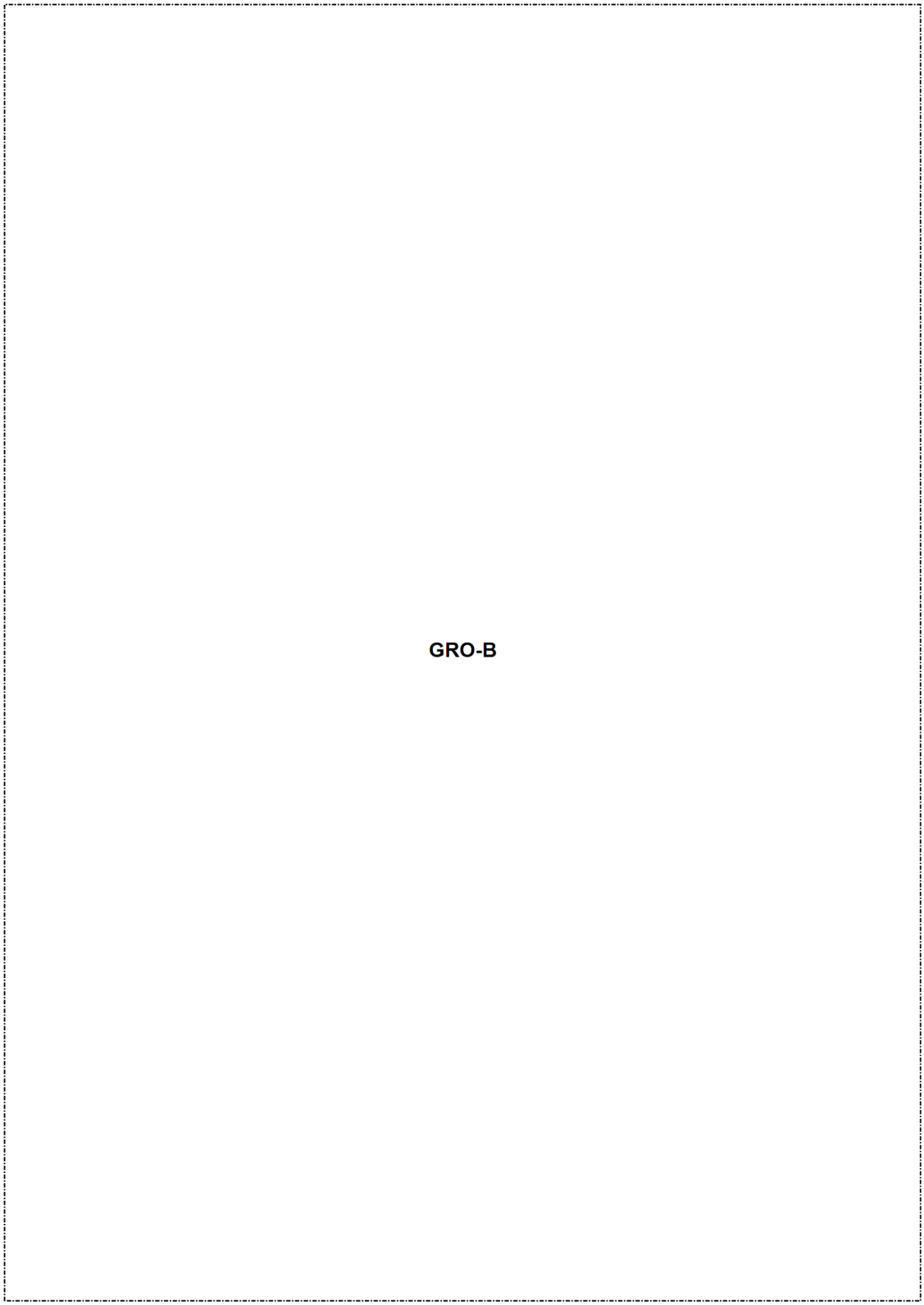
GRO-B

The study of prion binding, properties and inactivation on metal surfaces

Prions adhere avidly to metal surfaces such as steel wires and can then initiate infection in exposed cultured cells or in animals with remarkable efficiency. Work at the Unit and elsewhere demonstrated that prions bind avidly to metal surfaces and that this adsorbed material is highly infectious to mice[21,22] as well as to cultured cells[23]. Metal surface-bound prions appear to infect susceptible cells by contact and do not appear to need to leave the metal surface[23]. Serial iatrogenic transmission of classical (sporadic) CJD by a contaminated neurosurgical instrument, despite routine sterilisation, has been reported[24] and epidemiological evidence suggests a fraction of apparently sporadic CJD may be caused by unrecognised iatrogenic infection during general surgery[25,26]. The unknown, but potentially substantial[27], prevalence of clinically silent infection with vCJD prions in populations exposed to dietary BSE prions, together with the much wider tissue distribution of infectivity in vCJD demonstrated by the Unit[28-30] (see programme 6) and others, highlighted concerns of risk of infection through contact with surgical instruments. Secondary vCJD arising from blood transfusion has now been documented, indicating significant prionaemia in asymptomatic donors during the incubation period[31-33]. This wide distribution of infectivity makes common surgical and endoscopic procedures, in addition to neurosurgery and eye surgery, a potential risk factor for iatrogenic transmission of vCJD. Tissue prions withstand many forms of sterilisation techniques[34] and metal-adsorbed agent is even more resistant to both thermal and chemical treatments [35].

These concerns led the Department of Health to launch a directed programme to stimulate research into prion decontamination and under one of these awards (to Professors Collinge, Clarke and Weissmann) the Unit developed novel enzymatic methods to decontaminate prions adherent to metal surfaces[36]. This research has been

successfully translated (see *Translation and Knowledge Transfer* section below) to a practical decontamination product now available for use in surgical and dental instrument decontamination which reduces surface-bound infectivity by at least a million-fold following a simple 10 minute pre-soak procedure[37].



GRO-B

Book Chapters

GRO-B

Wadsworth J, Collinge J. Molecular pathology of prion diseases. In: Turner M, ed. Creutzfeldt-Jakob Disease: Managing the Risk of Transfusion Transmission. American Association of Blood Banks Press, 2006: 1-36.

GRO-B

GRO-B

Professional activities

Professor Collinge

Chair, UK Motor Neurone Disease Association DNA Bank Management Committee (2004-ongoing)
Ad Hoc International advisor, Deutsche Forschungsgemeinschraft (2004-ongoing)
International Advisory Board, Institute Pasteur de Lille, France (2005-ongoing)
Research Grant Board F, Royal Society (2006-ongoing)
Sectional Committee 10, Royal Society (2007-ongoing)
Member, UK Government Spongiform Encephalopathy Advisory Committee (SEAC) (2007-)
Member, SEAC, New Variant CJD Epidemiology Sub-Group (1997 – 2005)
Member, DOH/MRC Steering Group for Studies of detectable PrP^{Sc}
Member, DOH CJD Tissue Management Steering Group (2002 – 2007)
Member, DOH CJD Therapy Group (2002 – 2004)
Member, MRC New Therapies Scrutiny Group (2005 – ongoing)
Member, MRC Horizon Scanning Working Group (2006 – ongoing)
Charter member of Editorial Board, *Neurobiology of Disease*
Editorial Board, *Journal of Neurovirology*
Editorial Board, *Neurogenetics*
Guest Editor, *Proceedings of the National Academy of Sciences*
Partner and member of Executive Committee, Neuroprion, EU Network of Excellence
Lead, Neurodegeneration Theme, UCLH Comprehensive Biomedical Research Centre

GRO-B

GRO-B

Studies on the subclinical (carrier) state of prion infection

GRO-B

In addition to its obvious scientific interest, the phenomenon of subclinical infection is also of interest in public health as the discrepancy between prevalence estimates of prion infection based on anonymous screening of discarded surgical tissues[27] and the numbers of clinical cases of vCJD in the UK has become apparent. This is allied with the recognition of efficient secondary transmission of vCJD by blood transfusion[31-33], the recent report of prion infection of a (haemophiliac) recipient of blood products (factor VIII)[93], and continued concerns about the potential for iatrogenic transmission by contaminated surgical and medical instruments given the precedent[24] and epidemiological associations[25,26] with sporadic CJD and the much wider tissue distribution of infectivity in those incubating vCJD[28,29,94]. For these reasons also the Unit has maintained a strong interest in subclinical prion infection and is exploring this also in humans in the course of our kuru field studies (see Programme 11B).

GRO-B

GRO-B

(5) Development of cell lines for efficient propagation of hamster and human prions (in collaboration with Professor Parmjit Jat)

Our goal is to identify cell lines that are highly susceptible to, and can readily propagate, vCJD and hamster Sc237 prions. We have already identified clonal derivatives of N2a cells

that are susceptible to human vCJD (N2a9-8c) (see figure 8.5) and Sc237 (N2a2Y9C3) prions.

A

	post 4th		post 5th		post 6th	
Non-infected control	0	3	72	44	7	14
	3	0	51	45	5	7
	3	0	69	43	6	2
	9	2	30	37	11	2
	11	9	35	21	7	5
	16	21	48	39	14	10
	8	7	63	55	7	2
	17	20	42	50	8	6
Mean	8		47		7	
SD	7		14		4	

	post 4th		post 5th		post 6th	
vCJD infected	175	92	1065	248	29	4
	222	129	693	444	7	29
	1351	196	436	346	64	11
	283	183	543	316	5	73
	273	353	929	856	9	9
	217	164	439	553	18	11
	58	352	550	304	26	16
	216	617	562	815	19	11
Mean	305		569		21	
SD	308		241		20	

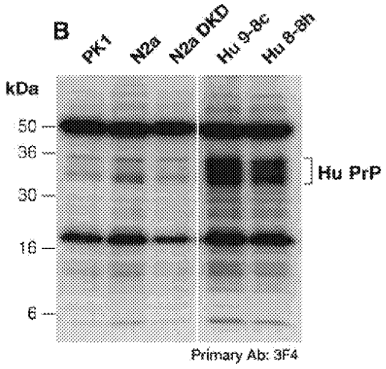


Figure 8.5: A. Cell line N2a9-8c was infected with 10^{-3}vCJD and taken through SCA. Numbers represent spots showing the number of infected cells per well in a 16 well replicate assay at post split 4, 5 and 6 compared with non-infected cells. B. Western blot analysis showing expression of HuPrP in cell lines 9-8c and 8-8h compared with N2a double-knock down and parental N2a cells.

The next step is to sequentially single cell clone them until we obtain clones that are highly susceptible. Sequential single cell cloning was the procedure employed by Peter Kloehn in the Unit to originally develop PK1 cells, the highly susceptible derivative of N2a cells[3].

If we are able to develop a highly susceptible cell line for vCJD, it will enable us to set up a human automated SCA (ASCA) for rapid and accurate titration of human vCJD prions which would be applied to many projects throughout the Unit, and immediately allow a comprehensive approach to characterising the properties of vCJD prions as has been performed with RML prions. Such an assay would have immediate applications to inform public health concerns, for example a detailed study of tissue distribution of infectivity and assessment of relative resistance to physical, chemical and enzymatic decontamination methods. It would also be applied to diagnostic testing under Programme 8 as well as facilitating strain characterisation studies of Programme 6. Importantly, a vCJD-propagating cell line will also allow us to develop a medium throughput cell blot assay, as we successfully developed for RML, for screening anti-prion drugs as part of our collaborative programme with GSK (see Programme 7 and appendix 2). Development of a highly susceptible cell line for hamster prions will permit *in vitro* experiments on hamster prions, including evaluation of subclinical infections and kinetics of prion propagation.

Ultimately this successful strategy of silencing *Prnp* in N2a cells followed by reconstitution with the PrP ORFs from other species followed by selection (or "tuning") for sensitivity to particular prion strains has the potential for being widely applicable to developing cell lines permissive to other species and strains of prions, for comparative susceptibility and *in vitro* transmission barrier studies and ultimately understanding the cellular basis of susceptibility (Programme 5) and strain selectivity (Programme 6 and this programme).

GRO-B

RESOURCES

GRO-B

GRO-B

Synthetic prions project (see above)

Prion immunotherapeutics (see appendix 1)

GRO-B

D*evelopment of cell lines with either new or wider strain susceptibility (see above)*
A hamster ASCA will largely replace the use of hamsters (since their use is almost exclusively for bioassay). A vCJD assay will generate huge demand both within Unit programmes and for diagnostic and public health application. Since human prions are ACDP category 3 pathogens, assay will need to be performed in our microbiological containment level III facility and we therefore require for biosafety, as well as logistical reasons, a second robotic system.

GRO-B

Requirements for expansion of throughput

GRO-B

A second identical robot, which will have to be located within our microbiological containment level III facility, will be essential for human prion assays. An automated system for the accurate bioassay of vCJD prions will have significant implication in the prion field. For this second automated system a new Band 5 technician will be required.

GRO-B

Programme 9

MOLECULAR DIAGNOSTIC STRATEGIES IN PRION DISEASE

Dr Graham S Jackson

Background to programme and contribution to Unit mission

Prion diseases remain a strategic priority area for both the MRC and the Department of Health. The core mission of the Prion Unit is to understand the molecular basis of prion propagation and to tackle the specific public health issues posed by BSE and vCJD in the UK. While our key focus on the basic science of prions and its wider potential relevance in pathobiology remains, we have developed an increasing translational activity. In particular the Unit has been working to develop effective means of prion sterilisation, early diagnosis, effective therapy and post-exposure prophylaxis. From the start of the current quinquennium, until February 2007, Dr Jackson has been a programme leader track scientist working with Professor Anthony Clarke on the structure of normal and mutant prion proteins (designated Programme 6 in the last quinquennial report). This work focused upon characterising the physicochemical properties of the prion protein, both in its native state and in altered, disease-associated, conformations. In discussion with the Director and Professor Clarke, it was decided to separate some of the resources of former Programme 6 (now programme 7) to form a new programme (designated 9) during this quinquennium to accelerate our work on molecular diagnostics under the leadership of Dr Jackson. The rationale was both to provide an independent programme with respect to career progression, and to better focus several ongoing projects and advances across the Unit. The arrival of blood transfusion-associated vCJD during this quinquennium highlighted the need to advance blood-based diagnostics for prion infection. Further, the advances in therapeutics research in the Unit and funding to take forward development of both conventional drugs and biopharmaceuticals necessitate parallel advances in early diagnostics. Currently, most patients are diagnosed only at an advanced clinical stage indicative of irreversible brain damage. Further, we reasoned successful molecular diagnostics are likely to require *in vitro* amplification of prions or surrogate abnormal prion proteins to attain the necessary sensitivity, and this programme also aimed to focus, integrate and expand Unit activities in this area which is of fundamental importance in understanding disease pathogenesis. This programme is closely coordinated with, and aims to translate, the molecular research in Programmes 1, 6, 7 and 8 and also works closely with our Clinical Research (Programme 11) and the National Prion Clinic.

Work in current quinquennium prior to formation of new programme

Prior to the formation of programme 9, Dr Graham S. Jackson, Dr M. Howard Tattum and Mrs Samantha Jones worked under the supervision of Prof. Anthony Clarke on Programme 6. This work is described in detail elsewhere but included the isolation of novel PrP conformers for the generation of unique monoclonal antibodies for use in the development of new diagnostic methods and immunotherapy[1-3], the defining of a role for PrP^C in the functioning of the immune system[4-7] and the development of seeded fibrillisation assays[8] that are relevant to Programme 9. In addition to elucidating the alternative modes of metal coordination by the full length mature prion protein[9,10] we have identified effective methods for the removal of prion decontamination of metal surfaces[11]. These findings have been successfully translated into a commercial product which has been licensed to the DuPont Corporation. Further work on surface binding of prions to steel and methods for their assay has led to an adaptation of the scrapie-cell-assay (Programme 8) that is capable of detecting prion infectivity with a sensitivity around 100 fold greater than conventional bioassay[12]. This has significance not only for understanding the interactions of prion infectivity with surfaces and the ability to detect surface bound prions sensitively and rapidly but also for the Unit's aim to Reduce, Replace and Refine its use of animals for experimentation.

PROGRESS REPORT (FEBRUARY 2007 TO PRESENT)

Background

The pre-clinical phase of vCJD is currently unidentifiable and presents a substantial infection risk to others via blood transfusion products, tissue and organ transplantation and other iatrogenic routes such as medical and dental procedures with contaminated instruments. The immediate solution to all of these problems would be to develop a sensitive blood-based pre-symptomatic diagnostic test for vCJD infection. Following infection a proportion of a normal host protein, PrP^{C}, is conformationally altered to disease-specific forms typified by PrP^{Sc}. Detection of aberrant forms of PrP, such as PrP^{Sc}, provides the greatest levels of disease specificity but has inherent problems of sensitivity due to the very low levels of PrP^{Sc} in the presence of very high background levels of the normal prion protein, PrP^{C}.

The detection of PrP^{Sc} in blood is considerably more challenging than other tissues with as little as 10 infectious units present in 1ml of whole blood. In order to achieve the high levels of sensitivity required a blood-based CJD diagnostic assay will need to exploit the unique ability of prions to replicate and incorporate an *in vitro* amplification step in the methodology. However, amplification alone does not provide a solution to blood based diagnosis due to the low abundance of PrP^{Sc} requiring large volumes of blood to be sampled to obtain certainty that a prion particle is present. Our research has tried to address three separate elements of sensitive detection of prion infection; capture of abnormal PrP, *in vitro* amplification and high sensitivity immunoassay.

The Unit holds around 1600 blood samples from prion disease patients as EDTA blood fractionated on arrival before storage frozen at -80°C. These samples are associated with detailed clinical phenotype data derived from the PRION-1 trial, the National Prion Clinic hospital or domiciliary visits, or as part of the ongoing National Prion Monitoring Cohort (for details refer to Programme 11; appropriate samples are also being made available to a National collection to assess putative vCJD blood tests). Some of the patients have been sampled on many occasions through the course of their illness, including patients sampled over a decade. This unique resource is unrivalled worldwide, with notable access to individuals at high-risk of vCJD who have received blood from donors that went on to develop vCJD.

Overall Project Aims

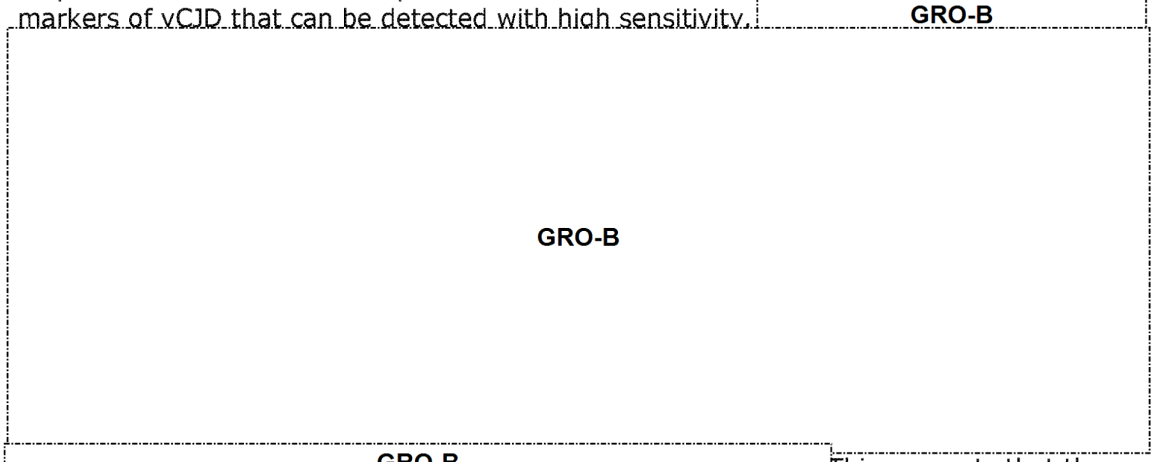
1. Proteomics screening for surrogate markers of vCJD infection.
2. Development of a sensitive immunoassay for abnormal PrP that does not require proteinase K digestion.
3. Capture and enrichment of abnormal PrP.
4. Prion replication and detection *in vitro*.

(1) Progress with proteomics screening for surrogate markers of vCJD infection

The large scale pathological damage associated with clinical disease suggests markers other than PrP^{Sc} for infection and disease progression may exist. Indeed previous studies using differential display reverse-transcriptase PCR (DDRT-PCR) have indicated that changes in gene expression occur in the spleens of experimental mice infected with the ME7 strain of prions[13]. One particular transcript was shown to be significantly depressed during the course of prion infection and was originally designated erythroid differentiation-related factor (EDRF)[13]. The role of EDRF also known as erythroid-associated factor (ERAF) has been elucidated as a molecular chaperone that binds and stabilises newly synthesised α-globulin and is now known as α-haemoglobin stabilising protein (AHSP)[14]. Although this and other erythroid specific genes display altered expression profiles in murine models of prion disease, translation of these findings to human blood samples from sporadic CJD and vCJD patients failed to differentiate

infected samples from controls[15,16] highlighting the importance of using authentic patient samples for such studies.

Although the specificity of PrP^{Sc} detection as a diagnostic marker of prion infection remains absolute, this advantage is balanced by the low levels available for detection in blood. Therefore, in parallel with developing new methods for the detection and amplification of PrP^{Sc} we have pursued the alternative strategy of identifying surrogate markers of vCJD that can be detected with high sensitivity. GRO-B



GRO-B This suggests that the measurement of the ratios of these various fragments could be used as the basis for further investigation as an alternative method for the diagnosis of vCJD and other prion diseases. Peaks observed to change by SELDI profiling could be correlated to fragments of albumin and a crude assay involving cleavage of an adrenocorticotropic hormone (ACTH) substrate demonstrated the anticipated proteolyic activity in normal plasma although the identity of the protease responsible remains to be established. The concept of using peptide substrates to monitor levels of protease activity in disease versus control samples may offer an alternative basis for the diagnosis of prion and perhaps other neurological diseases[17]. An additional benefit of these experiments has been the identification of specific changes between the HD and control group which has led to the identification of three proteins, namely Clusterin, β-actin and Apolipoprotein A IV, which may have value as biomarkers to track HD progression and responses to nascent drug treatments[18].

(2) Progress with Development of a sensitive immunoassay for abnormal PrP that does not require proteinase K digestion

Detection of disease-associated, abnormal forms of the prion protein such as PrP^{Sc} is the most specific criterion for the diagnosis of prion disease in humans and animals[19,20]. However, the high specificity associated with PrP^{Sc} detection has always been counterbalanced by a limit on the sensitivity of detection. This limit is a product not only of the absolute levels of PrP^{Sc} present in a particular diagnostic specimen but also the ratio of PrP^{C} to PrP^{Sc} in that sample. Detection of PrP^{Sc} therefore requires pre-treatment of samples before analysis, typically proteolytic digestion with proteinase K (PK) which selectively degrades PrP^{C}. However it is now accepted that prion infection is associated with deposition of protease-sensitive abnormal isoforms of PrP^{21-27}. Indeed, the majority of disease-related PrP may be destroyed by PK under conditions that are typically employed to detect prototypical PrP^{Sc}[24,27,28].

From an existing resource of over 80 monoclonal antibodies (Mabs) we determined to identify those capable of discriminating between PrP^{C} and PrP^{Sc} using an optimized ELISA format. The ability to specifically detect disease-associated material was assessed initially by comparing signals obtained using mouse brain homogenate infected with the RML strain of prions versus uninfected mouse brain homogenate without the use of PK. Preliminary pairings were subsequently tested with vCJD-infected human brain homogenate versus control brain to confirm reactivity with human prions. As whole blood is the ideal analyte and presents the biggest challenge in terms of potential

background and non-specific reactivity this was used as a diluent for vCJD brain homogenate to determine the sensitivity of an ICSM10/ICSM35B sandwich ELISA. The use of whole blood initially led to significant problems with high background signals using normal brain homogenate spiked into whole blood. To overcome this limitation a range of detergents were tested alone and in combination to ensure disruption of the cellular components of whole blood. GRO-B

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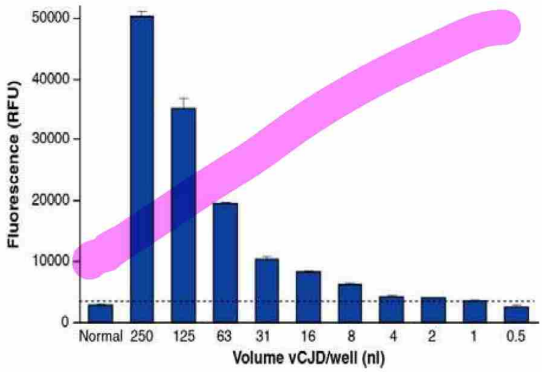

GRO-B

(3) Progress with capture and enrichment of abnormal PrP

To improve the sensitivity of immunoassay for prion infection we require the ability to capture PrP^{Sc} from larger volumes of whole blood. We have investigated two methods to achieve this; sodium phosphotungstic acid (NaPTA) precipitation and immunoprecipitation. GRO-B

GRO-B

GRO-B In this limited study the method was found to be 100% specific and 100% sensitive with six infected brain homogenates being correctly identified from a set of twenty samples analysed in a blinded manner. The discrimination between PrP^{C} and PrP^{Sc} was absolute with no detectable background of PrP^{C31}.

GRO-B

(4) Progress with in vitro prion replication and detection

Further significant increases in the sensitivity of detecting prion infection in blood can best be achieved by exploiting the ability of prions to replicate *in vitro* to achieve both amplification of abnormal PrP and a decrease in the ratio of background PrP^{C} to PrP^{Sc}.

Protein Misfolding Cyclic Amplification (PMCA) is a technique which can amplify small amounts of seeded abnormal PrP to a level detectable by conventional methods[33]. Although PMCA has been widely described in the literature including its use to detect prion infection in buffy coat fractions of rodent blood[34,35] it is a technique that was initially treated with scepticism by the prion field. This was the result of numerous technical details required for the technique to be reproduced outside of the host laboratory.

GRO-B

GRO-B

GRO-B

One key element to successful PMCA reactions is the availability of ideal substrate materials.

GRO-B

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GRO-B

All of the work in this programme is carried out within ACDP microbiological containment level III laboratories and staff and students associated with this programme receive specialist training and knowledge of how to work in such an environment and how to handle prion infectivity safely. Dr Jackson is chair of the joint Departmental and Unit safety committee charged with overseeing the safe running of these facilities and the specialist training required to work within them. This contributes to other Unit and Departmental research programmes and MRC codes of safe laboratory practice for working with prion infectivity.
Through membership and contributions to various expert advisory committees and groups Dr Jackson contributes knowledge to healthcare policy makers outside of the MRC.

GRO-B

PUBLICATIONS, AWARDS, COLLABORATIONS AND PROFESSIONAL ACTIVITIES

Peer reviewed articles

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Book chapters, reviews and editorials

Isaacs JD, Jackson GS, Altmann DM. The role of the cellular prion protein in the immune system. *Clinical and Experimental Immunology* 2006; 146: 1-8.

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Recombinant PrP based methods for the detection of PrP^{Sc} by replication *in vitro*., Tattum, M.H., Jones, S., Collinge, J. and Jackson G.S. (2008) Joint Funders TSE workshop, University of Warwick, UK.

Awards, research prizes and honours
Dr Graham Jackson
Fellowship of the Royal Society of Medicine
Honorary lecturer, Department of Neurodegenerative Disease, UCL Institute of Neurology (2003 to Present)

Competitive Fellowships
HLA polymorphisms and resistance to variant Creutzfeldt-Jakob disease, an investigation of molecular mechanisms. MRC (Clinical Research Fellowship) awarded to Dr Jeremy Isaacs, Dr G.S Jackson principal supervisor; £137,411, 2003-2006.

Major collaborations
Professor Elizabeth Fisher,
Department of Neurodegenerative Disease, Institute of Neurology, Queen Square, London, WC1N 3BG, UK.

Professor Claudio Soto,
UTMB, Galveston, Texas, TX 77555, USA.

Professor David Anstee,
International Blood Group Reference Laboratory, Southmead Road, Bristol, BS10 5ND, UK.

Professor Daniel Altmann,
Human Disease Immunogenetics Group, Department of Infectious Diseases, ICSM, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK.

Professor Helen Saibil
Department of Crystallography, Birkbeck College and Institute of Structural Molecular Biology, University of London, London, UK.

Proteome Sciences plc,
Coveham House, Downside Bridge Road, Cobham Surrey, KT11 3EP, UK.

National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG, UK.

Dr Stuart Hassard (CSO),
deltaDOT Ltd, Bessemer Building (RSM), Prince Consort Road, London, SW7 2BP, UK.

External Grants
HLA polymorphisms and resistance to variant Creutzfeldt-Jakob disease, an investigation of molecular mechanisms. MRC (Clinical Research Fellowship) awarded to Dr Jeremy Isaacs, Dr G.S Jackson principal supervisor; £137,411, 2003-2006.

The development of an effective treatment for prion infection of humans. Co-applicant with Profs. J. Collinge, Anthony Clarke and GlaxoSmithKline. Department of Health; £2,356,848 for three years, 2005-2008.

Development of pre-symptomatic blood test for vCJD. MRC Technology, Development Gap Fund. Principal investigator. £98,000 for 12 months. 2008-2009.

The Development of an Effective Treatment for Prion Infection of Humans. Co-applicant with Profs. J. Collinge, Anthony Clarke and GlaxoSmithKline. Department of Health, £4,882,120.00 for three years. 2009-2012.

Professional Activities
Dr Graham Jackson

Scientific advisory panels
Chair of the Department of Neurodegenerative Disease and MRC Prion Unit Safety Committee. (2006 to Present)

Member of the Department of Neurodegenerative Disease and MRC Prion Unit Tissue Management Committee. (2007 to Present)

Member of the Department of Health (DoH) working group on the decontamination of surgical instruments. (2004 to 2007)

Member of the Health Protection Agency (HPA) Expert Advisory Group on the Laboratory Testing Strategy for Large Scale Abnormal Prion Prevalence Studies. (2006 to Present)

Postgraduate and undergraduate teaching
Internal and external examiner for the degrees of MSc, MD and PhD.
Honorary Lecturer and principal PhD supervisor, Department of Neurodegenerative Disease, UCL Institute of Neurology. (2003 to Present)

Other academic activities
Referee of research grant applications for the Wellcome Trust, the Biotechnology and Biological Sciences Research Council, the Medical Research Council, the UK Department of Health, DEFRA, BRACE and the Scottish Hospital Endowments Research Trust.

Ad hoc referee for : Biochemistry, Biochemical Journal, EMBO Journal, Journal of Molecular Biology, Lancet; Nature Structural Biology, Nature Medicine, Science, Trends in Biochemical Sciences, Trends in Microbiology, Protein Science, Journal of Biological Chemistry, Journal of General Virology, Journal of Animal Science, FEBS Letters.

Contributor to MRC codes of practice for safe working with prion-infected materials and provider of specialist training for ACDP containment level III laboratories (2003 to present)

Significant invited reviews
Isaacs, J.D., Jackson, G.S. and Altmann, D.M. The role of the cellular prion protein in the immune system. Clinical and Experimental Immunology **146**:1-8 (2006).

Edgeworth, J.A. and Jackson, G.S. Detergent-enzyme reagents for the sterilisation of vCJD contaminated surgical instruments. Private Hospital Healthcare in Europe (2008)

Significant oral presentations
2004: Joint funders TSE workshop, University of York
2005: Invited lecture, Banbury Centre Meeting "Prion Biology: Puzzles and Paradoxes" New York, USA.
2006: Department of Neurology, University of Texas Medical Branch, Texas, USA.
Joint funders TSE workshop, University of Warwick.

2007: CSC Annual Conference 2007, Manchester, UK.
2008: Joint Funders TSE Workshop, University of Warwick, UK.
2009: Informa Life Sciences, Transmissible Spongiform Encepahalopathies 2009, Cologne, Germany.

FUTURE PROPOSALS

Abstract

Although the number of vCJD patients remains small, the number of people infected but clinically silent is unknown and could be very significant. The latency from infection to disease can be prolonged spanning several decades. In the absence of a diagnosis infected individuals pose a risk of iatrogenic transmission via contaminated medical and dental instruments, the donation of contaminated blood for transfusion and the transplantation of infected organs. The deposition of PrP^{Sc}, a conformational isoform of the hosts normal prion protein, PrP^{C} is a characteristic marker of infection. The concentration of infectivity, and by inference, PrP^{Sc} is very low in blood and is difficult to detect against the large background of excess PrP^{C} present in both healthy and infected individuals. One of our main approaches to overcoming these problems is to develop rapid and sensitive methods for *in vitro* prion replication and amplification. By defining efficient systems for prion amplification we will also gain crucial insights into the fundamental mechanisms of prion replication that are likely to involve factors other than PrP alone. Such co-factors are alluded to in several genetic studies and may offer novel targets for diagnostic detection, and therapeutic intervention.

Rationale

Prions are transmissible agents causing invariably fatal neurodegenerative diseases of humans and other mammals. Animal prion diseases include scrapie, a worldwide endemic disease of sheep and goats, chronic wasting disease of elk and deer and transmissible mink encephalopathy, and, since the mid-1980's, bovine spongiform encephalopathy (BSE). The occurrence of variant CJD (vCJD), and the confirmation that it originates from exposure to bovine spongiform encephalopathy (BSE)[49,50], has sparked a plethora of epidemiological models and prevalence estimates. A retrospective study of archived surgical lymphoreticular specimens estimated a prevalence of infection in the UK of 237 per million (95% confidence interval 49-692 per million)[51], higher than the number of clinical cases would so far suggest. Other phenotypes of clinical disease resulting from infection with BSE prions are also to be anticipated which may or may not be detectable by the methods used for prevalence estimates. The incubation period from infection to clinical disease can be extremely prolonged and variable, particularly when crossing a potential zoonotic transmission barrier. Ongoing studies within the Unit under Programmes 1 and 2 have identified several genetic modifiers of both susceptibility to BSE infection and the incubation time for development of clinical vCJD.

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In the meantime, we are faced with the certainty that a proportion of the population may be incubating this disease and that they might pass it on to others via blood transfusion[54,55], tissue and organ transplantation and other iatrogenic routes[56,57]. Concern regarding possible iatrogenic transmission of vCJD has already had widespread effects on public health policy in the UK and in other countries. The demonstration of sub-clinical carrier states of prion infection in animal models is also relevant to public health, both with respect to prion zoonoses and iatrogenic transmission of human prions[41,58-61]. The report of a further case[62] resulting from red-cell transfusion has confirmed that transfusion poses a clear and significant risk. As the clinically silent incubation period of prion infections in humans is known to be long, in some cases exceeding 50 years[63], and the prevalence of asymptomatic infection is therefore unknown[64], the extent of future transfusion-transmission of vCJD cannot be estimated and have caused major disruption to blood services world-wide with significant economic impact in the UK from the sourcing of plasma from the USA and the leukodepletion of donated blood. Whilst the number of known recipients at risk of vCJD from transfusion of packed red cells numbers are small, around 7000 recipients of contaminated plasma products have previously been identified and notified of their at-risk status[65]. Concern for this cohort has recently increased following post-mortem evidence for infection with vCJD in the spleen of a person with haemophilia[66].

Further risks to public health are posed by the ability of prions to resist conventional sterilisation techniques[67-69] and to adhere rapidly and avidly to metal or plastic surfaces from which they can efficiently transmit infection in experimental models[70,71]. Classical CJD has been transmitted iatrogenically by surgical instruments[72] and there is evidence to suggest at a proportion of sporadic CJD may be linked to surgery[57,73]. Until new sterilisation methods are widely introduced into the NHS, and/or blood screening tests to identify infected patients are developed, there will be continued distress and significant costs from quarantine and destruction of instruments including fibreoptic endoscopes used on patients subsequently recognised to have a prion infection or disease. The immediate solution to all of these problems is to develop a sensitive pre-symptomatic blood-based diagnostic test for vCJD infection that can be utilized for the screening of donated blood and organs or patients before surgery.
Deposition of PrP^{Sc} is the only validated surrogate marker for prion disease[74-76] and is to date 100% specific for infection. Detection of disease-associated PrP in CNS and lymphoreticular tissues correlates widely with presence of the disease and with the presence of prion infectivity[30,74-76]. Although the quantities of PrP^{Sc} deposited in neural tissues is sufficient during the symptomatic phase of illness for detection by conventional immunoassays such as western blot and ELISA, levels in peripheral tissues are significantly lower[29,74-76]. Transmission studies, however, have demonstrated that prions although at much lower concentrations, are present in peripheral tissues, including lymphoid organs and blood, and from early stages of the disease during the pre-symptomatic period[77,78]. The Unit has previously developed a clinical diagnostic test for vCJD using biopsied tonsillar tissue[29]. The pioneering use of this test on patients referred to the National Prion Clinic has led to it's validation and inclusion in the WHO's international diagnostic criteria for vCJD[79]. However, this assay requires invasive surgery under general anaesthesia is not suitable for screening applications.

There are a number of challenges associated with developing novel assays for the presence of abnormal PrP in blood. PrP^{Sc} and related disease-associated isoforms are conformers of a host encoded protein PrP^{C} with identical amino acid sequence, which do not contain nucleic acid and do not elicit a host humoral immune response. Conventional diagnostic methods such a tissue culture, antibody detection and polymerase chain reaction are therefore, not applicable[29,74-76]. GRO-B GRO-B GRO-B the Unit has a unique and unparalleled collection of patient samples for assay development and validation. However, with approximately only 200 vCJD patients reported to date, and suitable research samples obtained from only a proportion of these, the numbers of blood samples are lower than what would normally be used for assay validation. To assist with development and validation we have access to endogenous blood samples from various wild-type and transgenic mouse models of prion disease (see Programme 3). Such models offer the additional advantage of providing blood samples from various time points in the pre-clinical phases of infection giving confirmation that any nascent assay can detect infection in pre-clinical as well clinical stages.

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Aims

1. Detection and characterisation of blood-borne infectivity.
2. Prion replication *in vitro.*
3. Seeded conversion of recombinant PrP.
4. Combined immunoassay for PK-sensitive and PK-resistant PrP^{Sc}.
5. Detection and discrimination of abnormal PrP isoforms by High Performance Capillary Electrophoresis (HPCE)

Experimental plans

(1) Detection and characterisation of blood-borne infectivity
Future studies will encompass three main areas of research which will run in parallel.

(i) *Characterisation of blood pathogenesis in a rodent model system.*
(ii) *Transmission of blood and blood fractions from CJD patients*
(iii) *Characterisation of the biochemical properties of blood-borne infectivity*

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(i) Characterisation of blood pathogenesis in a rodent model system.
All the available evidence suggests that infectivity and therefore abnormal PrP is present at very low levels in blood, even during the clinical phase of prion disease (see above). The distribution of infectivity appears roughly even throughout both plasma and cell components[38,38-40] although buffy coat fractions report a higher titre due to the their concentration during isolation. Overall the data available is limited due to the low infectivity titres and the imprecision of rodent bio-assay which can only report differences of more than a log with any reliability.
Significant advances within Programme 8 have seen a cell-based assay for RML prions (Scrapie Cell Assay or SCA)[81] become robotised with a throughput of over 600 complete assays per week. In addition to speed and throughput this novel assay can determine infectious titres with high accuracy and is capable of detecting differences in titre as low as two-fold.

It is proposed to collect various tissue samples; including brain, spleen and blood (whole and fractionated) from CD-1 mice challenged with RML prions and culled at various time points throughout the disease incubation period. Although the volume of blood available from an individual mouse is small (<200µl), pooling of blood samples will provide sufficient material for fractionation and enrichment. Both precipitation with sodium phosphotungstic acid and immunoprecipitation provide methods for the enrichment of infectivity and abnormal PrP which will be utilised to further extend the sensitivity of detection. Samples will be analysed for the presence of PrP^{Sc} by high sensitivity western blotting, ELISA for PK-sensitive forms of abnormal PrP and for the presence of infectivity using the SCA. It is of fundamental importance for epidemiological modelling and the risk assessment of exposed individuals as well as essential for diagnostic strategies to

determine with accuracy at what point following infection prions accumulate in the blood and to what levels. Additionally, the RML model of prion disease is known to have levels of infectivity and PrP^{Sc} that correlate very closely as has now also been shown to be the case for vCJD prions[30]. Thus by quantifying the infectious titre in blood using SCA, we can calculate the expected levels of PrP^{Sc} and the increase in sensitivity required to detect PrP^{Sc} in blood using immunoassays at any particular point in the incubation period.

(ii) Transmission of blood and blood fractions from CJD patients

The absence of transmission barrier-free models for human prions has limited the ability to perform end-point titrations of vCJD material and to detect low titres of infectivity that maybe associated with blood GRO-B GRO-B GRO-B we have concluded that if any PrP^{Sc} is present in vCJD blood it is at levels far below those found in secondary lymphoid organs[29]. Reliable detection of such low titres of infectivity requires that either large volumes of blood fractions are inoculated requiring substantial groups of experimental animals (several hundred or thousands) or that all of the infectivity be captured and enriched before inoculation. GRO-B GRO-B GRO-B The unique resource of samples available to the Unit through the National Prion Clinic and the Unit's clinical research (Programme 11) means sufficient quantities of whole blood are available from patients.

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Transgenic modelling of human prion disease has proved successful in the last quinquennium and advances made in Programme 3 have provided suitable host lines for inoculation with enriched blood fractions. GRO-B

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(iii) Characterisation of the biochemical properties of blood-borne infectivity

The biochemical properties of blood-borne prion infectivity and associated abnormal PrP are unknown. It is largely assumed that such material will behave similarly to that found in CNS and lymphoreticular tissues. GRO-B

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(2) Prion replication *in vitro*

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(i) Identification of crucial cofactors for the replication of prion infectivity

The first description of an *in vitro* prion conversion reaction was made over 14 years ago[84] and despite significant advances made by the development of PMCA[33] 7 years ago, brain homogenate remains the only substrate capable of supporting high levels of prion GRO-B

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(3) Seeded conversion of recombinant PrP

Mechanisms for prion replication involving linear polymerisation have been proposed for several years[104]and have gained credence from the ability to detect the presence of infectivity by seeding the formation of amyloid fibrils[43,44] although the replication of infectivity by such reactions has only been reported by one group[105]. We have been pursuing seeded-polymerisation assays as a means to detect vCJD prions in blood and have to date focused upon achieving sensitive detection in an mouse model system using RML prions and recombinant murine PrP.

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There is a Unit wide contribution to identifying potential cofactors required for prion replication and the formation of infectivity; Programmes 1 and 2 are identifying genetic modifiers of susceptibility and incubation period respectively. Purification of infectivity within Programme 6 is leading to the identification of potential co-factors associated with PrP and Programme 7 is attempting to determine what ligands interact with PrP^C *in vivo.* All of these activities can inform on what may be required in addition to recombinant PrP to support prion replication *in vitro* and attempts to generate synthetic prions in Programme 8.

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(4) Combined immunoassay for PK-sensitive and PK-resistant PrP^{Sc}

There is a dearth of information available about the biochemical nature of disease associated PrP in blood, in particular uncertainty remains about whether PrP^{Sc} in blood is protease resistant. A large body of evidence is accumulating that challenges the belief that all disease-associated PrP is resistant to proteolysis. Indeed, it has been shown in number of different studies that the majority of disease associated PrP may well be sensitive to proteolytic digestion with proteinase K (PK)[21-26,28,107,108]. GRO-B

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ETHICS AND RESEARCH GOVERNANCE

All of the work performed within Programme 9 has ethical and health and safety approval and is conducted under the highest standards of research governance. Storage and biochemical analysis of human tissue samples is performed with consent from relatives, with approval from the Local Research Ethics Committee of the Institute of Neurology/National Hospital for Neurology and Neurosurgery, and complies with the code of practice specified in the Human Tissue Authority licence held by UCL Institute of Neurology. All procedures carried out in ACDP microbiological containment level III facilities are performed with strict adherence to safety protocols that are specified in formal risk assessments and SOPs. Work with animals is performed under licence granted by the Home Office and conforms with UCL institutional guidelines.

RESOURCES

The infra-structure and key collaborations required for conducting the future work of Programme 9 are already established. Programme 9 requests level funding to maintain its existing resources and an additional Investigator Scientist to lead with work on seeded fibrillisation of recombinant PrP. One item of significant capital investment is requested; GRO-B

Staff

Dr Graham S Jackson Band 2 Programme Leader (100% MRC)
Main responsibilities: To lead and co-ordinate the research efforts of Programme 9 and administer research governance, ethics and health and safety policies for all aspects of the work. To set priorities for experimental research, design research strategies and allocate resources and staff to specific projects. To conduct and supervise research, perform experimental analysis, collate and interpret data, and write manuscripts. To recruit, line manage and appraise staff and provide technical leadership and support the day-to-day activities of staff, and to act as principal PhD supervisor for a Clinical Research Fellow to be appointed (UCL funded).

Dr M. Howard Tattum Band 4 Investigator Scientist (100% MRC)
Howard is an experienced postdoctoral researcher with a background in molecular biology and biochemistry who contributes to all aspects of the programme's activities. It is intended that Dr Tattum will be primarily responsible for the definition of essential components of *in vitro* prion replication as typified by PMCA and the use of such reactions in detecting blood-borne infectivity.

Requested Band 4 Investigator Scientist (100% MRC)
We request an additional post-doctoral fellow with a proven track record in biochemistry or enzymology. The post-holder will be responsible for developing seeded fibrillisation

reactions for the detection of infected tissue and blood analytes. The post-holder will be required to work closely with Programme 6 (Professor Anthony Clarke) to produce and characterize mutant forms of prion protein as idealized substrates for conversion and polymerisation.

Mrs Samantha Jones Band 5 Research Support Technician (100% MRC)
Samantha is an experienced research technician with expertise in protein purification, western blotting and ELISA. She is essential to provide continuing technical support to the programme and to take greater responsibility for minor projects including the immunoassay of infected samples for the presence of PK-sensitive and PK-resistant PrP^{Sc}.

PhD Studentship
As part of the Units' mission for career development and capacity building we plan, at the Director's discretion, to appoint a new UCL-funded Clinical Research Fellow during the next quinquennium.

Consumables
We request level funding for consumables per member of staff per year equivalent to the support received during the past quinquennium. In addition we require new funding for consumables for the requested Band 4 Investigator scientist and three years consumables for the incoming Clinical Research Fellow.

Programme leader Band 2: GRO-B per year
Investigator Scientist Band 4 (x2): GRO-B per year
Research Support Technician Band 5: GRO-B per year
PhD Student : GRO-B per year (3 years only).

Capital equipment costs
We require GRO-B in the first year only for the purchase of discounted instrumentation for our collaboration with GRO-B

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GRO-B Funds are requested within this programme and are not costed elsewhere.

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STAFF ASSOCIATED WITH PROGRAMME 9 IN THE LAST QUINQUENNIUM

Name	Status	Date from	Date to	% time on programme	Type of appointment	Source of funding
Dr Graham S Jackson	Programme Leader (Band 2)	March 2007	Present	100	Established	MRC
Dr Graham S Jackson	Programme Leader Track (Band 3)	April 1999	February 2007	100	Fixed term contract	MRC
Dr M. Howard Tattum	Investigator Scientist (Band 4)	August 1999	Present	100	Established	MRC
Mrs Samantha Jones	Research Technician (Band 5)	June 2000	Present	100	Established	MRC
Dr Suvankar Pal	Clinical Research Fellow	October 2004	September 2007	50	Ring-fenced fellowship	MRC
Mrs Mohini Kaneri	Research Technician (Band 5)	June 2006	January 2008	100	Part-Time (18 hours)	MRC
Dr Jeremy Isaacs	Clinical Research Fellow	March 2004	March 2007	50	Personal clinical fellowship	MRC

STAFF ASSOCIATED WITH PROGRAMME 9 IN THE FUTURE QUINQUENNIUM

Name	Status	% time on programme	Source of funding
Dr Graham S Jackson	Programme Leader (Band 2)	100	MRC
Dr M. Howard Tattum	Investigator Scientist (Band 4)	100	MRC
Mrs Samantha Jones	Research Technician (Band 5)	100	MRC
Requested	Investigator Scientist (Band 4)	100	MRC
To be appointed	Clinical Research Fellow	100	UCL

Programme 11

CLINICAL RESEARCH STUDIES IN THE UK AND PNG

These comprise UK based studies described in section A below and field studies on kuru from our research base in Papua New Guinea described in section B.

11A: UK CLINICAL RESEARCH (IN ASSOCIATION WITH NATIONAL PRION CLINIC)

Dr Simon Mead and Professor John Collinge

Background to programme and contribution to Unit mission
The Unit was established on the firm principle of combining basic and clinical research: many of the key contributions towards understanding the basic biology of these diseases have come from observations of their clinical and neuropathological aspects. Such close interaction between clinical and basic work has been, and will continue to be, crucial to advances in this field and requires a stable long-term facility to attract and provide adequate training for academic physicians. The Unit aims to provide an attractive training environment both for basic scientists and for clinical scientists including academic neuropathologists. The Unit has a key role in developing the infrastructure and patient base, in partnership with the NHS, to facilitate the development of early diagnostic markers for these diseases and future therapeutic trials. The Unit is embedded in the Department of Neurodegenerative Disease at the Institute of Neurology and adjacent to the National Hospital for Neurology and Neurosurgery where the NHS National Prion Clinic (NPC) is located and led by Unit staff providing a seamless integration of our translational research. The Unit, together with the NPC and MRC Clinical Trials Unit has successfully conducted the UK's first clinical trial in prion disease and is about to formally launch the National Prion Monitoring Cohort, both funded by the Department of Health. The Cohort study will provide the overarching structure to facilitate much of the Unit's clinical research and the ongoing collection of biological samples crucial for many of the Unit's programmes. The NPC is obviously fundamental to our clinical research programme but is also crucial to the molecular genetic studies of Programmes 1 and 2, biochemical and biological studies of human prion strains in Programmes 3 and 6, and the molecular diagnostic and biomarker research of Programme 9.

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PROGRESS REPORT

Outline and continued development of the National Prion Clinic
In 1997, we established the Specialist Prion Disease Clinic at St. Mary's Hospital with

funding from Kensington, Chelsea and Westminster Health Authority. This was designated the NHS National Prion Clinic (NPC) in March 2001 by the UK Department of Health with central funding from the National Specialist Commissioning Advisory Group. In August 2004, following the last stage of relocation of Unit activity from Imperial College to UCL, the NPC relocated to the National Hospital for Neurology and Neurosurgery at Queen Square, part of the University College London Hospitals NHS Foundation Trust. The NPC is constituted as a specialist tertiary referral service within the Trust. The NPC was established to be, and remains, closely integrated with the MRC Unit and this relationship is of fundamental importance to the Unit's mission. The NPC continues to receive central funding from the Department of Health and receives UK-wide and international referrals of all forms of suspected prion disease. It is internationally unique and has developed unparalleled experience in the clinical management of prion disease.

In addition to providing a national NHS centre of expertise for the management of all forms of human prion disease, the clinic was established to facilitate research in both diagnostics and therapeutics including the organisation of clinical trials. The Health Services Director at the NHS Executive wrote to all hospitals to inform them of the clinic, and encourage referral to it[1]. The importance of supporting the ongoing clinical research programme at the MRC Unit was particularly emphasised. The clinic liaises closely with the National CJD Surveillance Unit in Edinburgh (NCJDSU) to ensure that both centres are aware of all referred cases. A UK-wide national referral protocol was agreed in 2004 by the Department of Health, MRC Head Office, NPC/MRC Prion Unit and NCJDSU to ensure clear arrangements for national referral to support the responsibilities and research activities of both Units and monthly meetings of clinical leads ensure effective co-ordination of respective functions.

Since its inception, clinical activity by the NPC has steadily risen; in the financial year 2006-07, activity was 323 patient episodes comprising inpatient, outpatients, hospital or domiciliary visits. This increased to 385 patient episodes in 2007-08, including 132 new referrals with suspected prion disease. Our success in achieving high patient referral rates is partly a result of the implementation of the National Agreement with the NCJDSU, and partly through increasing awareness of our services amongst Consultant Neurologist colleagues and the patient community. In addition, we continue to receive many direct referrals of at-risk individuals from families with inherited prion disease. With some of these large pedigrees we have had a clinical relationship for around two decades since mutations in the prion protein gene (*PRNP*) and their use in diagnosis and genetic counselling were first reported from 1989[2-4]. Through our collaboration with the Health Protection Agency we receive referrals from individuals at high-risk of vCJD through whole blood transfusion.

Aims and services of the NPC

The clinic staff comprise consultant neurologists Professor John Collinge and Drs Simon Mead and Peter Rudge and consultant neuro-radiologist Harpreet Hyare. Permanent NHS staff include Michele Gorham, a lead nurse; Liz Ford, a clinical nurse specialist; Clare Morris, a clinical counsellor; and part-time input from a senior clinical psychologist. A project manager and three mobile teams of clinical nurse specialists and clinical research fellows are supported by the Department of Health funded National Prion Monitoring Cohort. Simon Mead was appointed as the lead clinician of the NPC in May 2008. He has worked with Professor Collinge for over 10 years and has sub-speciality interest in cognitive disorders. Also in 2008, the NPC was very fortunate to recruit Dr Peter Rudge, a renowned and highly respected senior clinical neurologist and former member of the UK Government Spongiform Encephalopathy Advisory Committee (SEAC).

The aims and services include:

- To provide inpatient and outpatient services for the comprehensive assessment of all suspected prion disease (sporadic, acquired and inherited). The service includes rapid

clinical assessment and diagnosis including, neuroimaging, neuropsychology, neurophysiology, molecular genetics, cerebral and tonsil biopsy (including biochemical analysis of prion strain type), CSF markers and the co-ordination of subsequent management of patients and their families, in liaison with local healthcare professionals.

- Information, advice, support and counselling for patients and their families, both during investigation, on diagnosis, and long term follow up. We aim to work closely with and provide information and support for professionals working with registered patients in their local area.
- To provide expert advice and support from a clinical nurse specialist, counsellor and consultant staff for patients, families, carers and health professionals including a telephone help-line and email advice. We have established close liaison with the principal UK patient support groups: the Human BSE Foundation and CJD Support Network. Both the NPC and the MRC Prion Unit hold annual open days, in addition to regular topic-related study days, for patients and their families and carers and health professionals to explain our clinical research strategy, to report progress, and, importantly, to obtain feedback. The NPC and MRC Prion Unit greatly values its liaison and with patient and public groups. In the design stages of the PRION-1 trial we conducted an in-depth consultation with patients, families and carers[5].
- We provide an internationally recognized centre of expertise for prion disease diagnosis and staff training. We are also helping to develop national standards for patient management and infection control in hospitals, and provide clinical and scientific input to the activities of several government appointed national committees.
- To provide the infrastructure to enable UK clinical trials in prion disease and to facilitate the clinical and other research of the MRC Prion Unit and its collaborators.

Interaction with MRC Unit programmes

The NPC is obviously fundamental to our clinical research programme and has enabled the MRC PRION-1 trial, the first UK clinical trial in prion disease which was conducted at the request of the Chief Medical Officer and successfully completed during the past quinquennium. The NPC is central to our ongoing longitudinal study of prion disease which is laying the foundation for PRION-2 and other trials. However, the NPC is also vital to many other Unit programmes. All patients attending the NPC are invited to participate in Unit research studies. The large majority do so and donate blood or other tissue samples for our research. Many consent to autopsy and donation of tissues for research. Rapid autopsy with full CJD precautions is performed by Professor Sebastian Brandner, former Unit Programme Leader and now Head of the Department of Neuropathology, who is consultant neuropathologist to the Unit. Samples collected under various research protocols at the NPC are crucial to the molecular genetic studies of Programmes 1 and 2, biochemical and biological studies of human prion strains in Programmes 3 and 6, and the molecular diagnostic and biomarker research of Programme 9. Patient samples are also made available to other investigators worldwide subject to appropriate patient consent and ethical approval. Under a sample sharing agreement, samples are routinely shared with our colleagues at NCJDSU and aliquots are being collected and stored for transfer to the National CJD sample collection once their ethical and logistical arrangements are in place.

Research governance and ethics

The last quinquennium has been a time of huge change in the regulatory environment surrounding clinical and healthcare research. Since 2003, in UK legislation alone, we have seen the introduction of the Human Tissue Act 2004; the UK Medicines for Human Use (Clinical Trials) Regulations 2004 and Clinical Trials Amendment Regulations 2006; and the Mental Capacity Act 2005. In addition, as a MRC Unit, we must also ensure that we are fully up to date and compliant with all MRC Governance Policies, such as the MRC Policy on Clinical Trials Regulations; Research Governance Frameworks; Consent from Adults Without Capacity; and Use of Human Tissue Guidelines. Given the large scale and highly multidisciplinary nature of the National Prion Clinic / MRC Unit's clinical research portfolio,

involving many of the highest profile and regulated areas of biomedical research (prions, handling and shipping category 3 pathogens, use of human tissues, human genetic testing, genetic manipulation, animal experimentation, clinical research, clinical trials, human stem cells). This increasing regulatory load meant that appointment of a Research Governance and Quality Officer (RGO) was essential.

Governance of Clinical Trials
In 2004 the UK Medicines for Human Use (Clinical Trials) Regulations came into force, bringing into law the standards that had formerly been Good Clinical Practice guidelines. The PRION-1 clinical trial that commenced in 2004 was therefore subject to these regulations. The Unit formed a partnership with the MRC Clinical Trials Unit (CTU) for the management and coordination of this study. PRION-1 was recently selected for inspection by the Medicines and Healthcare products Regulatory Agency (MHRA) as part of their inspection of the MRC CTU. The Unit was therefore required to ensure all documents pertaining to the study were available for inspection. This inspection is currently on-going as the MHRA further selected PRION-1 for a site visit to be carried out in January 2009, and has involved liaison with the MHRA Inspectors, MRC CTU and ourselves to ensure the inspection was dealt with as smoothly as possible.

Mental Capacity Act
Implementation of the Mental Capacity Act 2005 required us to amend all of our protocols, information sheets and consent forms to incorporate the requirement to refer to a nominated consultee where potential patients do not have the capacity to consent for themselves to take part in research. This was a huge task for our Unit, managed by the RGO, as due to the nature of our research, these changes were required for the majority of our studies. As a Unit leading in the field of neurological studies involving adults that may not initially have, or may lose, capacity during the study, we were one of the first to go through this process. This therefore required close liaison with our local research ethics committees by our governance team to ensure we complied with the Act, while still keeping the care and treatment of our patients at the forefront of our work.

The MRC PRION-1 trial
Using prion-infected cultured mouse cells, several compounds have been shown to block production of the abnormal form of the prion protein (PrP^{Sc}), including quinacrine[6,7]. Since longstanding clinical experience in the treatment of malaria and rheumatoid arthritis had shown that orally administered quinacrine was relatively safe and could cross the blood-brain barrier, it was proposed as a potentially useful treatment for human prion disease[7]. This report generated wide interest and patient requests for immediate access to quinacrine. The Chief Medical Officer asked the MRC to prepare a protocol for a clinical trial in prion disease and determine any therapeutic potential of quinacrine.

In 2007 the trial was successfully completed and we have established and proven the national relationships and infrastructure to allow future evaluation of therapeutic agents[8]. Primary efficacy end points were death or evidence of a response based on neurological and functional rating scales. As the largest prospective study with longitudinal assessments carried out in human prion disease to date, PRION-1 demonstrated that national recruitment and retention to such studies is feasible and acceptable to patients and their carers. However, PRION-1 highlighted the difficulty of undertaking randomized controlled trials in human prion disease. Few patients or their carers agreed to randomisation. As a consequence, PRION-1 was essentially an observational study of patients choosing to take quinacrine or not.

107 participants were recruited in total; forty-four sporadic, 2 iatrogenic, 19 variant and 42 inherited CJD patients. Choice of therapy was strongly associated with severity of disease, with those of the least or most disease severity choosing not to receive quinacrine. 78

(73%) of the patients died and diagnosis was confirmed in all patients proceeding to autopsy. Although unadjusted mortality was lower in those choosing quinacrine, there was no difference in length of time between first symptom and death, and adjustment for severity at entry resulted in no difference in mortality between groups. Only 2 serious adverse events were thought to be drug related. Thus we did not find any evidence that oral quinacrine at a dose of 300mg a day prolonged survival in human prion disease.

The principal objective of PRION-1 was the evaluation of quinacrine as a therapeutic agent in human prion disease and these and other headline findings are now in press[8]. However, much more has been learnt from this trial, in particular, regarding the development of clinical and other outcome measures to evaluate future treatments in these diseases, the assessment of MRI and other surrogates of disease progression, the development of a tissue bank for validation of putative diagnostic blood tests, and informing the development of a staging system for prion disease together with the National Prion Monitoring Cohort (NPMC). Further statistical analysis of PRION-1 data is ongoing and additional publications are planned. As randomisation to placebo is unlikely to be acceptable, given the lack of alternative therapies and invariably fatal outcome, we consider that assessment of future therapies will be against historical longitudinal disease progression data and this is the core objective of the NPMC (see future proposals).

Investigation of possible new BSE prion-related human disease and the emergence of secondary (iatrogenic) vCJD

All patients with vCJD identified to date (~200), have been of the *PRNP* codon 129 methionine homozygous (129MM) genotype[9-11]. Precedents set by kuru (see this programme part B) and growth-hormone associated iatrogenic CJD, suggest that all genotypes may eventually be affected, but with differing incubation times[12-16]. Transgenic experiments with humanised mice have been used to model the transmission of BSE to heterozygous (129MV) and valine homozygous (129VV) genotypes; the results are complex (see Programme 3) with evidence for subclinical transmission of infection, and novel histopathology and PrP^{Sc} phenotypes analysed by Western blot[17,18] suggesting that BSE infection of these genotypes may result in clinicopathological phenotypes distinct from that of vCJD. In 2007, we reported the molecular investigation of a young woman with a clinicopathological phenotype atypical for sporadic CJD and a 129VV genotype[19]. PrP^{Sc} analysis revealed unique Western blot findings with some similarities to the type 4 (London classification) seen with typical vCJD[20,21] suggesting a BSE-related aetiology was a possibility. In 2008, the National Prion Clinic was referred the first patient with a clinical diagnosis of vCJD and 129MV (manuscript in preparation). Clearly it is difficult to draw conclusions from small numbers of cases; it will be important to see if other similar cases emerge in BSE-exposed populations.

Four cases of prion infection from a cohort known to have received blood transfusion from a donor who subsequently developed vCJD are now known[22-24] (further manuscript in preparation). Importantly, analysis of autopsy tissues revealed typical prion colonisation of lymphoreticular tissues including tonsil, confirming that tonsil biopsy, developed at the Unit for diagnosis of vCJD[25,26], could, contrary to suggestions in earlier published work[22] also be used in secondary vCJD diagnosis. That each of these patients has only received a single Unit of implicated red cells and that one of the patients described in 2004 (who was found to be infected at autopsy but died of an unrelated cause) was of the MV genotype associated with relative resistance to prion disease[22], argues that the risk to other recipients is substantial[24]. These individuals have or through their GPs, will be offered specialist follow-up and investigation by the NPC. A number are under long follow up at the NPC and are participating in ongoing research and have kindly agreed to serial blood sample donation which may be of great value in validation of blood tests and understanding the evolution of prionaemia should they be in fact infected.

Around 7000 individuals, predominantly patients with haemophilia, have now been informed that they have received blood products prepared from pools containing blood from a vCJD implicated donor. The recent finding of PrP^{Sc} in an autopsy spleen sample from an elderly haemophiliac highlights a real risk of vCJD transmission in this cohort and emphasises the need for an effective blood screening test (see Programme 9) and effective secondary prophylaxis or therapy (see later sections this Programme). While tonsil biopsy allows early and pre-symptomatic diagnosis in other iatrogenically exposed individuals at high risk, as in those with primary infection with bovine spongiform encephalopathy prions[24] we are developing ultra-sensitive techniques to detect low levels of PrP^{Sc} in blood samples (see Programme 9). The participation of patients and individuals at-risk of iatrogenic vCJD in clinical research studies and their contribution of large volume blood samples will provide a unique collection for validation of such a test developed at the Unit or elsewhere through our planned contribution to the CJD resource centre at the National Institute for Biological Standards and Control (NIBSC).

Neuroimaging studies

The clinical and research neuroimaging activities of the National Hospital for Neurology and UCL Institute of Neurology are internationally renowned. Notable resources for collaboration include multidisciplinary research teams at the Wellcome Trust Centre for Neuroimaging, the Lysholm Department of Neuroradiology and UCL Dementia Research Centre (see collaborators).

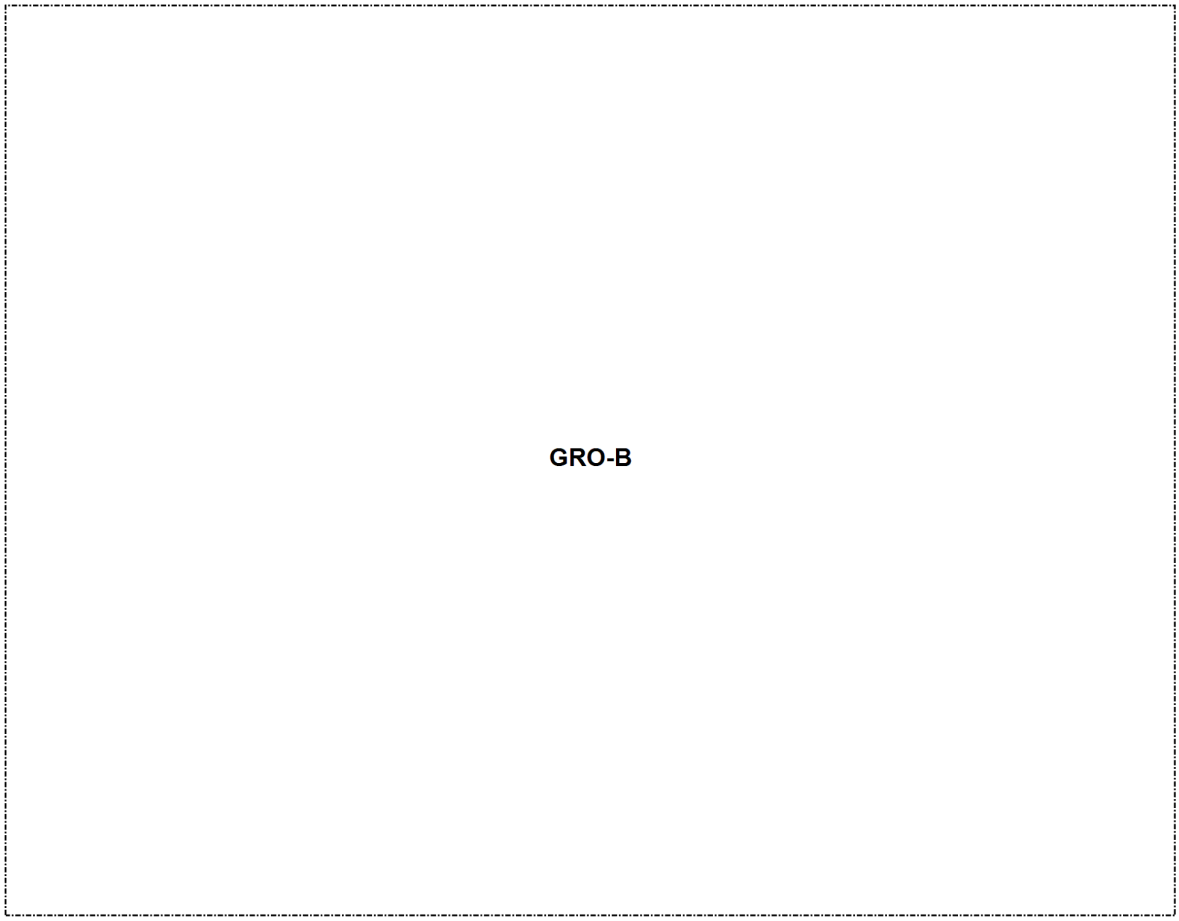
There are no useful disease progression biomarkers of human prion disease and we need to improve the sensitivity and specificity diagnostic prion neuroimaging. A good surrogate of progression is a major goal of translational research across neurodegeneration[27-30]. Major international imaging studies are being conducted in neurodegeneration, for example, the $64 million Alzheimer's Disease Neuroimaging Initiative[31]. The most common category of human prion disease, sporadic CJD, has a median survival of five months[32], which would be the natural choice for a primary outcome measure in a clinical study. However, our experience of PRION-1 informs us that many participants in future studies will have less rapidly progressive forms of the disease, for example, some of the inherited prion diseases, earlier onset patients and those with genotypes associated with a longer course (129MV)[8]. Further, the precise time of death does not necessarily reflect the rate of disease progression and may instead relate to the degree of supportive care, feeding or treatment of terminal infections. The development of a progression biomarker is therefore a significant goal of clinical research in prion disease. A useful neuroimaging biomarker would not only be an indirect measure of neurodegeneration but could be used to monitor a relevant aspect of disease pathophysiology, correlate with treatment-induced changes and assist in identifying responders to a specific treatment[30].

Over the last few years a number of advanced MRI techniques have been developed with the aim of detecting subtle pathological changes in brain tissue and indirectly reflecting microscopic aspects of tissue damage. These techniques provide the opportunity to develop surrogates for prion disease pathology (spongiform change, gliosis, neuronal loss, PrP^{Sc} deposition) *in vivo*. The techniques of proton magnetic resonance spectroscopy (^{1}H–MRS), diffusion weighted imaging (DWI), magnetization transfer imaging (MTI) and volumetric MRI have been applied to neurodegeneration[33]. As nested studies in the PRION-1 trial, we investigated many of these advanced MRI imaging techniques as markers of disease activity and in many cases correlated the MR measures with prion disease pathology. Several reports of this imaging work are currently in press, in submission or in final stages of preparation[34-39]. Focused hypothesis testing based on these pilot studies, with modified protocols, are planned for the NPMC (see future proposals). Pilot/PRION-1 studies are described briefly below,

- We have investigated the ante- and post-mortem cerebral magnetisation transfer ratios (MTR), measured by MRI, as a surrogate for prion disease pathology. Our data suggests

that MTR correlates with severity of spongiform change[38,39] and has potential utility as a therapeutic biomarker in human prion disease as this pathology is known to be reversible in animal models[40,41].

- Diffusion weighted imaging (DWI) has emerged as a sensitive diagnostic technique in cases of sporadic CJD[42-45] but few studies have quantified Apparent Diffusion Coefficients (ADCs) which provide quantitative estimates of regional and whole brain water mean diffusivity and are thought to be sensitive to histopathological abnormalities. In PRION-1 we found significant positive associations of ADC with disease severity, the strongest association, between an ADC measure and clinical neurological status, is consistent with the known distribution of prion disease pathology and therefore shows promise as a quantitative pathological biomarker in inherited prion disease. Our diffusion weighted imaging data, specifically measures of frational anisotropy in the cerebral cortex and pulvinar, also suggests potential for the detection of early histopathological changes of disease and for monitoring disease severity in human prion diseases[46].
- Proton Magnetic Resonance Spectroscopy allows non-invasive detection of cerebral metabolites known to be sensitive to underlying cerebral pathologies[47-53]. Our ^{1}H-MRS data at short echo time, demonstrated a reduction in [N-acetyl-aspartate] and elevation in [myoinositol, MI] at baseline with continued elevation of [MI] on follow up studies, suggesting that [MI] a postulated marker of astrocytic proliferation could be a sensitive marker of disease activity[54].
- Atrophy rates measured by MR were significantly higher in symptomatic prion disease patients than in control patients with mean whole brain atrophy rate 1.3% higher than controls in inherited prion disease (IPD) patients. In addition, several clinical measures were predictors of brain atrophy and cerebellar impairment at baseline was a predictor of cerebellar atrophy.



GRO-B

PUBLICATIONS, AWARDS, COLLABORATIONS AND PROFESSIONAL ACTIVITIES

Publications

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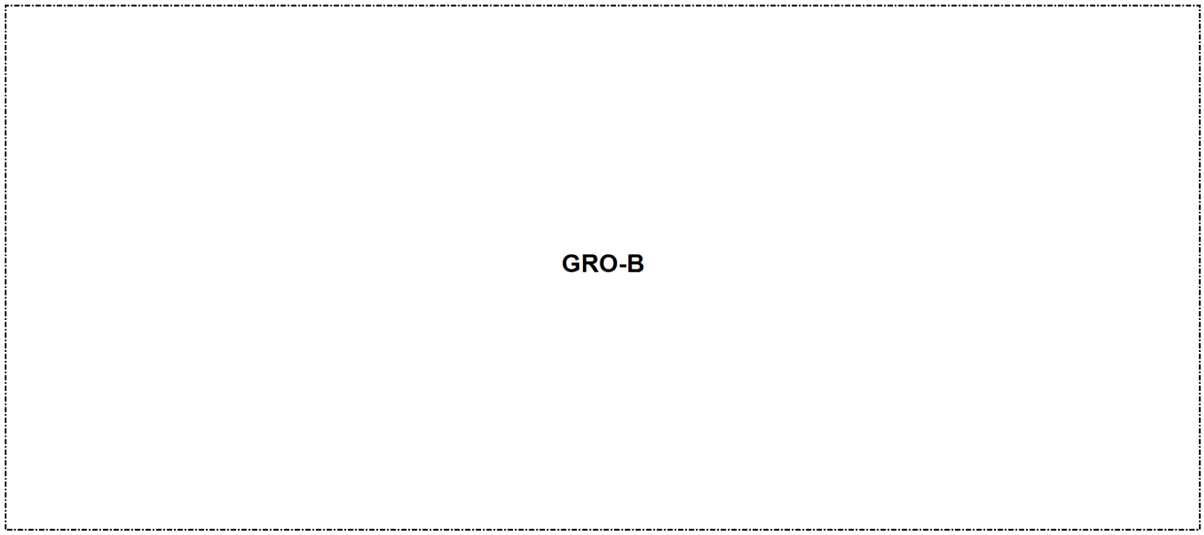

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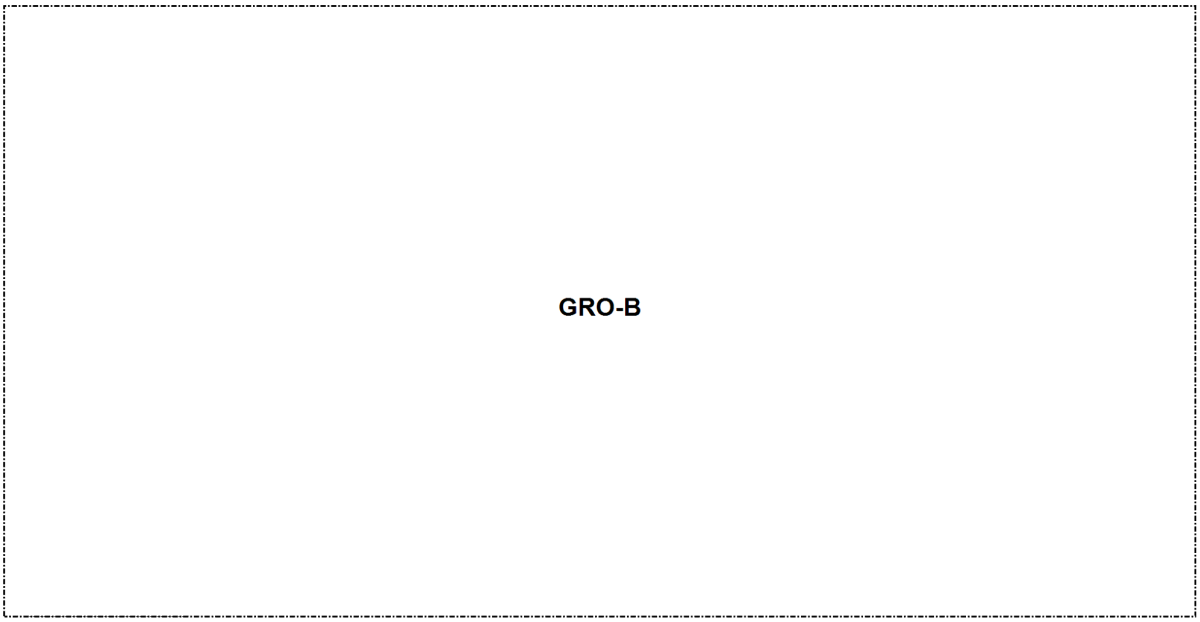

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GRO-B

FUTURE PROPOSALS

Abstract

The principal aims of the Clinical Programme are to facilitate the translational agenda of the Unit - the development of early diagnostics and effective therapeutics for human prion infection. While the majority of the UK population will have been exposed to BSE prions, the number of clinical cases has been modest to date. However, the extent of clinically silent infection remains unclear and secondary transmission of vCJD is now a reality. These public health uncertainties will persist for many years and present a major ongoing annual cost to the NHS (£M hundreds) in risk reduction measures of uncertain necessity or efficacy. There is a clear imperative in the UK to follow through on research to provide effective solutions to existing problems and to anticipate any further BSE-related human disease waves: such research remains a strategic priority. Furthermore, proof of principle for effective therapeutics for prion disease has been achieved in animals, and early pathology and clinical features shown to be reversible. We consider human therapeutics based on effective targeting of PrP to now be achievable: two parallel programmes to achieve this are funded. Leaving aside arguments about disease prevalence and public health uncertainties, success in our aim that prion neurodegeneration becomes the first neurodegenerative disease to have a curative treatment would have an impact that would completely transcend "orphan status" issues. We anticipate much will be learned of wider relevance in dementia by understanding the capacity of the brain to recover or functionally adapt by curative intervention to clear prion infection: essentially abruptly halting an established neurodegenerative process. Effective clinical testing of such therapeutics is challenging but much has been learned from the PRION-1 trial that will now be developed under the Department of Health funded National Prion Monitoring Cohort. The Cohort study will provide the infrastructure for the development of effective disease staging, selection of outcome measures and design of future trials, in addition to facilitating the diagnostics, biomarker, neuroimaging and other translational research of the Unit. Patients at-risk because of their family history or exposure will be recruited allowing clinical or molecular diagnostic insights into the earliest stages of disease when we hope to start therapies. Although all these studies look forward to progress in our immunotherapy and small molecule therapeutic programmes, we also plan clinical studies designed to provide robust evidence for the use of symptomatic therapies available to clinicians now, and other ways to ameliorate some of the distressing symptomatology of prion diseases. These studies will capitalise on the wide expertise at The National Hospital for Neurology and Neurosurgery and UCL Institute of Neurology within which the Unit is embedded, as well as other national and international collaborations. The clinical programme will continue to work closely the Human Genetics programme to identify novel causal and disease-modifying mutations, make new genotype-phenotype correlations and understand the impact of prion infection on the peripheral blood transcriptome.

AIMS

(1) To successfully conduct the National Prion Monitoring Cohort to determine the natural history and of prion disease and its clinical and investigation features at clinical onset
(2) To develop a staging system for prion disease
(3) To develop imaging biomarkers of prion disease
(4) To initiate PRION-2 based on (1), (2) and (3)
(5) To improve the clinical evidence for symptomatic prion disease therapies
(6) Provide samples for validation of blood or CSF tests including key samples for the CJD Resource Centre
(7) To progress genotype-phenotype studies with Programmes 1, 2

GRO-B

The effectiveness of symptomatic therapies

Although there is no prion disease modifying therapeutic in UK clinical trials at present, as specialist physicians, we make many recommendations for management. These recommendations are based on low quality clinical evidence, such as personal experience and case reports (see below for examples). We plan initially to look at three major clinical problems: the treatment of disabling myoclonus, behavioural/psychiatric disturbance and swallowing difficulties.

- *Myoclonus*, observed as a sudden, brief jerk caused by involuntary muscle activity is a frequent and problematic clinical sign in CJD patients, with several possible therapies[66-68]. Our study aims to monitor the clinical course of myoclonus in CJD by video recording and blinded assessments by clinicians, and carer reports. Additionally, where feasible, portable surface electromyogram recordings will be made to provide quanitification of the response to therapy. Therapeutic decisions by the NHS clinical team will be recorded.
- *Behavioural disturbance/hallucinations*, visual hallucinations and delusions are a recognised feature of many forms of prion disease, although the frequency and nature of these events have not been characterised systematically[69,70]. We will record the type, nature and severity of hallucinations as part of the clinical history and administer the Brief Psychiatric Rating Scale (BPRS) to assess delusions. Patients experiencing hallucinations will also, with appropriate consent, undergo a digital recording of their description of the event and clinical assessment. Therapeutic decisions made by the visiting clinician will be

recorded. Where appropriate a witness, will be asked to grade the severity of the behavioural disturbance on a simple rating scale.

- *Swallowing difficulty.* Prion diseases frequently involve severe swallowing dysfunction, with certain categories of disease developing this sign early, prior to terminally severe intellectual dysfunction (e.g. A117V IPD). In this circumstance the clinician, the multidisciplinary team and the family/carers are faced with a decision about artificial feeding. We plan to investigate this question within the NPMC by interviewing family/carers and the global and functional outcome data in the cohort. We will also collect data on the complication rate of interventions to achieve artificial feeding in our patient group and weight change.
- Fatal familial insomnia (FFI), associated with *PRNP* D178N mutation, is characterised by insomnia and autonomic hyperactivity[71,72]. However, sleep disturbance also occurs in a substantial number of other types of prion disease including sCJD and there is no satisfactory therapy for this[73]. There have been few studies of FFI in a sleep laboratory and none reported in other forms of CJD. We propose to study patients of all types of CJD who have a history of sleep disturbance in the sleep laboratory at the National Hospital for Neurology. These studies will involve polysomnographic and video recording throughout the sleep cycle to characterise sleep onset, assess sleep stages and determine any sleep movement disorder.
- Alternative cohort studies might focus on other management problems tractable to clinical research such as pain, autonomic dysfunction, and extrapyramidal abnormalities such as dystonia, chorea and bradykinesia, and management of dysphasia.

Blood and tissue biomarkers

The ideal diagnostic biomarker for prion disease should reflect an important aspect of the fundamental pathogenesis, have a high sensitivity and specificity, be reliable, reproducible, noninvasive, simple to perform, and inexpensive. Such an investigative tool would greatly help with screening of donors to protect the UK blood supply, presurgical screening to prevent iatrogenic transmission and improved early diagnosis and voluntary testing of at-risk groups[74,75]. A biomarker may also correlate with severity of illness, providing an opportunity for an objective measure of progression when used serially, termed a progression biomarker. Further, if change in a biomarker was predictive of a clinically meaningful response to a therapeutic intervention, this would have utility in trials[30]. Embedded within the NPMC are several studies linked with MRC Prion Unit programmes, aiming to discover biomarkers with one or more of these properties.

The distribution of PrP^{Sc} and investigation of strain properties

Work at the Unit and elsewhere to define the tissue distribution of abnormal prion protein deposition has been critical to the public health response to vCJD[76-78]. Some outstanding questions remain, such as the risk of dental tissues in vCJD[79]. Studies to address this are planned should appropriate consent for a vCJD autopsy become available. We also plan transmission studies into wild-type and a range of transgenic mouse models that would enable the rapid characterization of novel phenotypes of human prion disease which might develop in the future (see Programmes 3 and 6).

Development of a prion disease blood test

One of the critical challenges for achieving effective therapeutics of prion disease is making an early diagnosis. A large proportion of the NPC workload involves patients presenting in advanced clinical stages when it is highly unlikely that disease modifying therapies will be able to make a clinically meaningful benefit. Although several practical issues may speed referral to specialist NHS care, a simple blood test would allow rapid triage of patients as soon as the disease is considered by local doctors, followed by confirmatory testing under NPC care.

The risk of secondary transmission of vCJD via contaminated blood donations and medical and surgical procedures is unquantifiable at present, causing major disruption to the blood supply nationally and internationally and concern about the contamination of surgical instruments[80]. All of these risks can be managed by the application of a sensitive and specific pre-clinical blood test. Such a test can be routinely applied to pre-surgical screening and the screening of tissue and blood donations thereby substantially reducing if not eliminating any iatrogenic transmission risk. In addition to the direct public health benefits there are likely to secondary benefits as a result of major financial savings to the NHS. The cost of attempting to protect the public from iatrogenic CJD is estimated at several hundred million pounds per annum. GRO-B

GRO-B

GRO-B It will be essential to participate in external assessments of assay sensitivity and specificity and we plan large scale testing of normal blood samples in conjunction with the National Blood Service and CJD Resource Centre at NIBSC (CJD-RC). The NPMC study includes regular ~50ml blood samples to be requested. Our present collection is one of the worlds largest available for protein or RNA based studies of blood components in vCJD, and probably the largest collection of all human prion disease. Prospectively, within the NPMC we plan to send aliquots of plasma to the CJD-RC for this purpose. The sampling of at high-risk individuals, who have received blood transfusions from donors who went on to be diagnosed with vCJD and who are seen or will be contacted though our collaboration with the HPA, is one of the critically important roles for the NPMC.

GRO-B

GRO-B

Imaging biomarkers

GRO-B

GRO-B

Determination of Clinical Onset in At-Risk Individuals

There is a realistic prospect that effective therapeutics for CJD will become available in the next few years. To be most effective they would need to given as early as possible before significant neurodegeneration had occurred. Diagnosis of prion disease is typically late in the clinical course and a blood test allowing early diagnosis would be essential to enable real benefit from any therapeutic, before extensive irreversible CNS damage has ensued. However, the NPC has several opportunities to record early clinical signs through its involvement over two decades with two of the worlds largest IPD families; and by collaboration with the HPA and working with patients at high-risk of vCJD following implicated blood transfusion. By documenting early clinical signs and investigations in those at-risk, the NPMC aims to identify sensitive clinical or investigation markers that could aid early diagnosis.

Neurophysiology of prion disease

The P102L variant of Gerstmann-Straussler-Scheinker syndrome (GSS) has evidence of a peripheral neuropathy in most patients but this has been little studied objectively[57,96]. By collaboration with Professor Martin Koltzenburg, techniques of assessment of large and small nerve fibre function will be used to identify the origin of the motor and sensory abnormalities found in this form of GSS and to assess patients with other types of CJD who have evidence of a neuropathy. All neurophysiological studies will be conducted in the Clinical Neurophysiological Department of the National Hospital, Queen Square.

GRO-B

Programme 11

CLINICAL RESEARCH STUDIES IN THE UK AND PNG

11B: KURU FIELD STUDIES IN PAPUA NEW GUINEA

Professor John Collinge and Professor Michael Alpers

Background to programme and contribution to Unit mission

Kuru is a fatal subacute neurodegenerative disease restricted to the Fore people and their immediate neighbours in the Eastern Highlands of Papua New Guinea (PNG). It was the first of the human prion diseases shown experimentally to be transmissible. Its natural route of transmission was oral, through the consumption by extended kin of infective brain and other material from dead patients at mortuary feasts. Research into kuru began in 1957 and, following the work of many investigators, by the mid-1980s the research responsibility had devolved entirely on the Papua New Guinea Institute of Medical Research. Collaborative studies on kuru commenced in 1996, initially funded by Wellcome Trust support to Professor Collinge, and this research was then incorporated into the Unit's programmes at its inception in 1998 and kuru field studies were expanded and developed. Kuru provides the principal experience of an epidemic human prion disease, and one that is now reaching its end. In addition to its intrinsic interest, it provides an opportunity to learn much of relevance to understanding variant CJD, also thought to be caused by dietary prion exposure. Indeed vCJD gave kuru a new global relevance. In particular the Unit has explored the remarkable range of possible incubations in orally acquired human prion infection and performed detailed studies of genetic susceptibility to acquired prion disease. These studies continue to be of major importance to the Unit's genetic research (Programmes 1 and 2) and in understanding the role of prion strains and route of exposure in determining prion pathogenesis (Programme 6). Our recent finding of a powerful novel genetic resistance factor is impacting on transgenic modelling of prion disease (Programme 3) and understanding its structural biology (Programme 7). These studies therefore are of major importance to the Unit's mission both with respect to our fundamental studies of prion biology and to inform ongoing public health concerns posed by vCJD, BSE and other emerging, potentially zoonotic prion diseases. Kuru is also of wider importance to the history of medicine and human society, and the Unit is documenting cultural practices and traditions that will otherwise soon otherwise be lost. The interaction of the Unit with our collaborators at the PNG Institute of Medical Research, with the Fore and other communities affected by kuru and with Papua New Guinean society more generally has enormously enriched the life and work of the Unit and National Prion Clinic at many levels.

PROGRESS REPORT

Introduction

The transmissible spongiform encephalopathies or prion diseases are a closely related group of neurodegenerative conditions which affect both humans and animals[1]. They are transmissible experimentally both within and between mammalian species by inoculation with infected tissues and sometimes by dietary exposure. The prototypic disease is scrapie, a naturally occurring disease affecting sheep and goats, which has been recognized for over 200 years and is present in many countries world-wide. The human prion diseases have been traditionally classified into Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS) and kuru. The human prion diseases occur in inherited, acquired and sporadic forms. Around 15% are inherited as an autosomal dominant condition and all are associated with coding mutations in the prion protein (PrP) gene. Acquired prion diseases include kuru, iatrogenic and now variant CJD. Recognised iatrogenic routes of transmission of classical CJD are treatment with human cadaveric pituitary-derived growth hormone or gonadotrophin, dura mater and corneal grafting and

use of inadequately sterilized neurosurgical instruments.

While scrapie has been thought not to pose a risk to human health, the large-scale epidemic of a bovine prion disease, bovine spongiform encephalopathy (BSE) in the UK and to a lesser extent other countries, led to major public health concerns with the arrival of variant CJD and the experimental confirmation that it was caused by the same prion strain as that causing BSE in cattle[2-4]. The number of cases of vCJD confirmed to date (around 200) is thankfully small, given the massive population exposure to zoonotic BSE prions. While clinical case numbers have been decreasing, the number infected in the population remains unclear, as human prion disease incubation periods are known to span decades and major genetic effects on incubation period have been identified at the Unit and elsewhere, such that further waves of disease in other genotypes are possible[5,6]. Indeed, anonymous screen of discarded surgical tissues suggests a much higher silent infection prevalence than these case numbers would suggest[7]. Subclinical carrier states of prion infection in animal models are now well recognised[8]. Significant public health concerns will persist for some time and have been heightened by the arrival of secondary vCJD during the current quinquennium in individuals exposed to blood (and now blood products) from asymptomatic donors who went on to develop vCJD[9-11] (Wroe et al, submitted).

By far the largest experience of an acquired human prion disease comes from the kuru epidemic in Papua New Guinea (PNG)[12]. Kuru had reached epidemic proportions amongst the Fore and some adjacent linguistic groups in the Eastern Highlands Province in the 1950s. Kuru was transmitted by exposure to prion-infected tissues during the practice of transumption of the dead at endocannibalistic feasts. It was the practice in these communities for individuals to consume their deceased relatives as the preferred means of disposal of the dead. The disease largely affected adult women and children as males after the age of about seven years rarely participated in such feasts. Since the cessation of transumption in the late 1950s the disease has gradually declined in incidence, but a few cases have occurred in the South Fore since 2000, with both males and females, all aged over 50 years, affected, which is consistent with childhood transmission and incubation periods of more than 40 and, in some cases, more than 50 years as we have recently reported[13].

Kuru was a devastating epidemic amongst the Fore, at its peak affecting 200 people annually amongst a small, relatively isolated, population; an annual mortality of over 2% was recorded in some Fore villages[14]. The disease had a profound psychological and cultural impact on the Fore, which continues in the area of highest incidence to the present. However, kuru has also been of immense importance historically to an understanding of this group of diseases. In addition to providing by far the most extensive clinical experience of an acquired human prion disease, the experimental transmission of kuru to chimpanzees was a crucial landmark, establishing the field of human transmissible spongiform encephalopathies[15]. The epidemiology of kuru clearly indicates the infectious, but non-contagious, properties of prions and provided strong evidence that vertical transmission from a pregnant woman to her child does not occur[12]. vCJD shows a number of striking similarities, both clinically and pathologically, with kuru[16]. As the kuru epidemic nears its end, it provides a unique opportunity to study the range of incubation periods possible in human prion infection, to characterize genetic susceptibility factors as well as to gain insights into the peripheral pathogenesis of orally acquired prion disease in humans.

Therefore compelling public health reasons, in addition to the fundamental scientific interest in understanding the molecular determinants of incubation period and susceptibility, provide clear reasons to continue our focus on maximising our understanding of the unique kuru epidemic[17]. Remarkably, and in sharp distinction to other infectious disease epidemics, we are able to study the entire epidemic, not simply sample it, thanks to the phenomenal kuru archive maintained by Professor Alpers and updated and refined in the current

quinquennium.

Working in rural PNG involves particular challenges and responsibilities and these logistical, political and ethical issues are briefly outlined in the next section.

Collaboration with the Papua New Guinea Institute of Medical Research

Close links with the Papua New Guinea Institute of Medical Research (PNGIMR) have been essential to the success of the kuru project[18].

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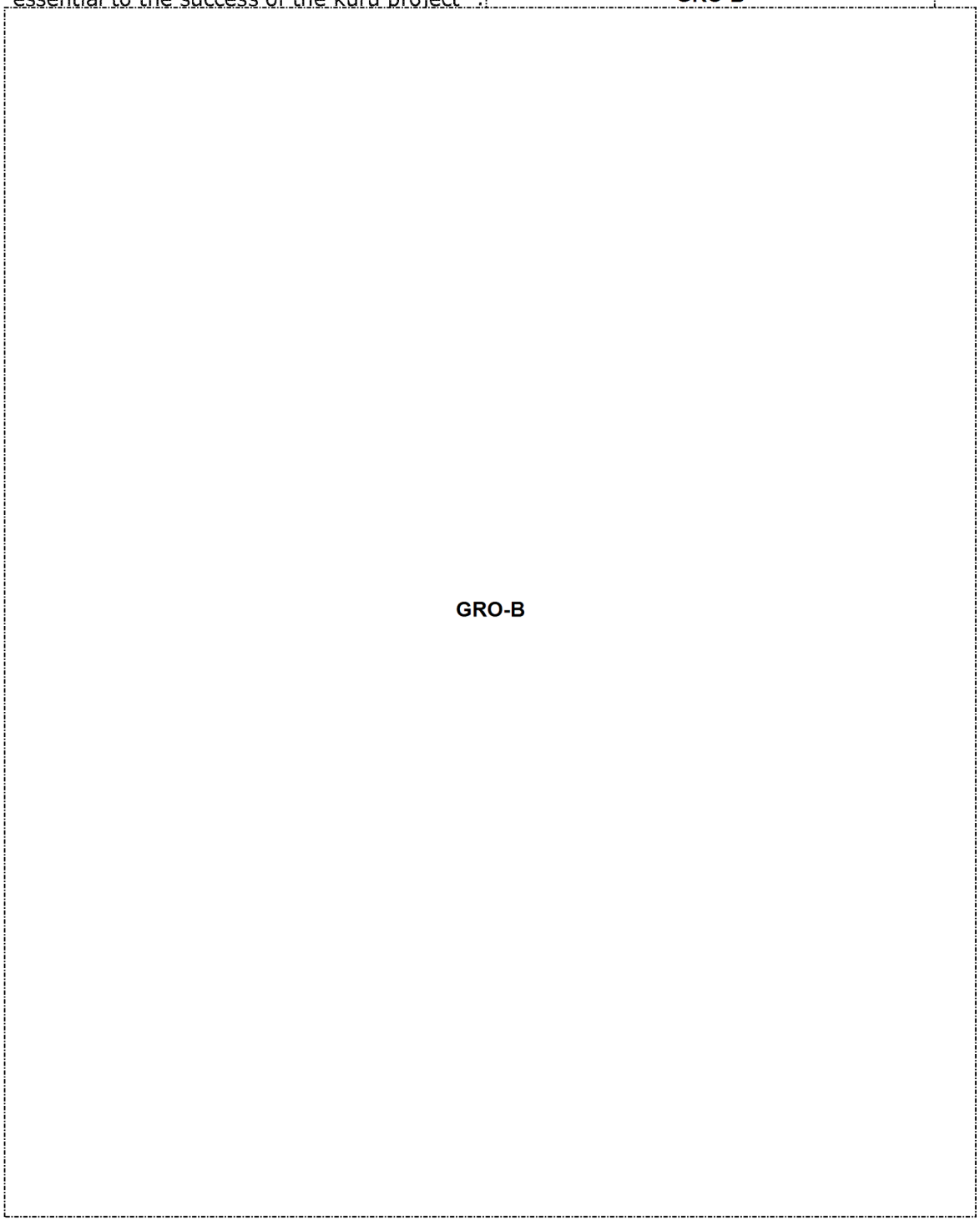

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Previous objectives of the programme

GRO-B

- GRO-B
- Study the clinical features of current kuru patients and compare clinical and other diagnostic features with other human prion diseases, notably iatrogenic and variant CJD.
- GRO-B

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FUTURE PROPOSALS

Abstract

The widespread exposure of the UK population to zoonotic bovine spongiform encephalopathy prions, and the potential consequences for public health, led to a renewed interest in kuru, the principal example of epidemic human prion disease and one that is nearing its end, allowing major insights and comparisons with vCJD in the UK.

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These studies have been integrated with the work of the Prion Unit on sporadic and variant CJD. This has a two-way benefit: the established techniques and expertise of the Unit have quickly produced new insights into kuru; the findings in kuru, which is essentially a completed epidemic, have important implications for the evolution and outcome of the vCJD epidemic.

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One of the major unknowns in the field of prion diseases is the existence and extent of a subclinical carrier state of human prion infection. A related question is at what stage after exposure, and in what tissues, such persons carry infectious prions. GRO-B

GRO-B

GRO-B This work will link closely with studies on other human prion diseases, especially vCJD, conducted by the Unit. GRO-B

GRO-B

GRO-B They significantly enhance these themes and provide power to better understand and predict the characteristics of the vCJD epidemic. Moreover, they are unique and, at this late stage of the epidemic, could only be conducted, in the field and laboratory, by the established research programme of the Unit.

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IRO

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(2) Obtain autopsies from elderly survivors of the kuru epidemic to investigate asymptomatic carrier states of prion infection

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GRO-B

GRO-B Indeed, the tissue distribution of infectivity is of considerable interest both in preclinical and subclinical states, not least since kuru infection, like classical CJD but in distinction to vCJD, does not appear to involve lymphoreticular prion colonisation. We will also investigate if prionaemia is present in such individuals if tests of sufficient sensitivity are developed by Programme 9.

GRO-B

GRO-B

(3) Undertake further experimental studies on material from the last autopsy and archival kuru samples with programme 6

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GRO-B

We compared the transmission properties of kuru prions with those from sporadic, iatrogenic and variant Creutzfeldt-Jakob disease and showed that kuru prions have the prion strain characteristics of classical (sporadic and iatrogenic) CJD prions and are distinct from variant CJD prions[41,42]. These studies will be integrated within Programmes 6 and 3 and will form an important part of the Unit's research examining peripheral pathogenesis states in human prion disease. Findings from this research will have direct translational benefit by informing risk assessment models that aim to protect public health by preventing iatrogenic transmission of human prions. These studies are costed under the relevant programmes. These studies may be replaced by a cell-based assay if this becomes feasible (see Programme 8).

Appendix 1

THE DEVELOPMENT OF PASSIVE IMMUNISATION FOR TREATMENT AND SECONDARY PROPHYLAXIS OF HUMAN PRION INFECTION

Background

GRO-B

Since the arrival of variant Creutzfeldt-Jakob disease (vCJD)[7], caused by the transmission of BSE-like prions[8-10], there have been concerns about iatrogenic transmission. There are now five known transmissions of vCJD in this way, four by whole blood and one by a blood product used in haemophilia, factor VIII[11-13] (and National Prion Clinic, unpublished); around seven thousand individuals have been informed that they have received implicated blood products. These developments have put new emphasis on the development of prion therapeutics. Further to targetting early symptomatic patients for treatment, those from at-risk groups are a particularly attractive target as immunotherapy is known to be effective at clearing prions prior to CNS penetration[6].

GRO-B

SECTION 3: RESOURCES AND VALUE FOR MONEY

INTRODUCTION

This section focuses on reviewing the current resource elements and financial performance of the scientific programmes and the support services utilised to deliver the research strategy of the current quinquennium and presents summaries of the future requested resources of both the scientific programmes and support services needed to deliver the next quinquennium.

3.1 BACKGROUND

Unit Establishment

In 2001 The Prion Unit was established on two floors at the UCL Institute of Neurology (IoN) Queen Square House (QSH) and the transgenic mouse facility on two floors in the Institute's Wakefield St premises. The capital cost of this move, including major enabling costs, was £12.5m and was part of a major Joint Infrastructure Fund (JIF) bid made by UCL. The JIF funds allowed provision of custom-designed laboratory, tissue culture and transgenic animal facilities, together with office and administrative space, shared by the MRC Prion Unit and the University Department of Neurodegenerative Disease (see below). There was no capital investment by the MRC in the establishment of the Prion Unit.

In addition to his role as Director of the MRC Prion Unit, Prof Collinge was appointed to be head of the new Department of Neurodegenerative Disease at the UCL Institute of Neurology. Many of the Department staff are co-located within the MRC Prion Unit and members of this university Department work very closely with those of the Unit. The Prion Unit is therefore embedded within the Department of Neurodegenerative Disease and the arrangements when the Unit was established recognised that certain management tasks and responsibilities would need to be shared between the two organisations. This is a symbiotic relationship with many unquantifiable intangible benefits. Both institutions have realised the advantages of this arrangement in scientific growth and developments particularly with shared access to facilities and equipment.

The Unit was due to complete its second quinquennium at the end of March 2009, however in February 2008 the Unit was notified by MRC Head Office Research Management Group that the quinquennial review would be postponed for 12 months pending the outcome of a Strategic Review of Neurodegeneration by the MRC. The Unit welcomed the strategic review and contributed a paper from Professor John Collinge. In June 2008 the Strategic Review report concluded that the Unit's research was a "UK scientific success story", that our scientific output and translational successes were impressive and that the Unit was "a paradigm of a focused research initiative". The Review recommended the Unit develops further to adopt a key role in building UK capacity in neurodegenerative disease and noted that its fundamental research, particularly with respect to protein misfolding, was highly relevant in understanding neurodegenerative diseases more widely, an area of major strategic importance.

It was already foreseen in 2001 that the Institute would need additional space to house a growing MRC Prion Unit and the newly established Department of Neurodegenerative Disease. This was because the remainder of John Collinge's laboratory team at Imperial College and those running the National Prion Clinic at St Mary's Hospital also needed to move to Queen Square, and the Institute needed to provide appropriate lab facilities for new appointments to Prof Collinge's new Department and in particular Professors Parmjit Jat and Elizabeth Fisher.

In 2006 the Prion Unit and Department of Neurodegenerative disease successfully applied to UCL and the MRC to jointly fund the refurbishment of an additional floor at QSH to provide urgently needed Laboratory space which included a Category III Containment laboratory, general laboratories and Tissue Culture facilities. A small office area and meeting room were also planned. Building commenced in September 2008 and will complete May 2009, at which time the Unit will take occupation.

The transgenic mouse facility has been operating on two floors of the Institute's Wakefield Street premises and it was envisaged that at some point a third floor would be required to support existing and planned research. This floor is now available and it is intended that this will be operational in March 2009.

Current Position

In addition to the Unit's successful development and continuing delivery of its scientific programmes as discussed in Section 2 significant changes and evolution of the Unit's operating environment has also occurred. There have been many internal as well as external forces impacting on the Unit (set out below) which have required a dynamic and flexible management of resources, changes to operating procedures and practices and the development of new systems. Whilst some changes were foreseen and planned for in the current quinquennium others were not and have impacted on the workload of the current management as well as the overall costs to the Unit. This is discussed more fully below. However the management team at the Unit proactively responded to all the challenges encountered and not only ensured the continuation of the science but re-engineered many practices in order to work smarter (see 3.2).

Corporate requirements/initiatives and external regulations introduced over the quinquennium have incurred costs most of which could not have been foreseen nor were they planned for in our last application. GRO-B

GRO-B

Nevertheless, prudent and pragmatic steps have been taken by the Unit's management team to ensure that the Unit operates efficiently and with the maximum possible weighting of scientific spend versus infrastructure/overhead cost.

3.2 UNIT ORGANISATION, ADMINISTRATION, GOVERNANCE AND MANAGEMENT RESOURCES INCLUDING SUPPORT SERVICES

The Unit management is organised into three main areas of responsibility namely Scientific Programme Direction, Administration/Governance and Technical Support, and Directors Office Management as shown in Figure 1. The three core members of the Management team being Professor Collinge the Director, The Business Director and The Director's Office Manager.

The Unit and university Department staff work to the same processes and procedures wherever possible which allows the day-to-day management of the combined entities to be streamlined. As such all staff report in through the core management team. There are two main areas where the procedures are necessarily different namely finance and purchasing and HR and these are managed separately but by the same management team. In addition intellectual property is managed either solely with IoN/UCL or MRC or both depending on the circumstances.

In addition to the core management team, a number of committees have been established with cross function expertise and Departmental as well as Unit representation to ensure consistent management across all the groups, ownership of the combined Unit/Department amongst all staff, uniform information dissemination and to ensure policy and procedures meet best practice. These committees convene regularly, are chaired by a senior member of staff reporting to the core management team and minutes formally documented. The committees include:

- Animal Research Scientific Committee
- Safety Committee
- Tissue Management Committee
- Radiation Committee
- Biological Safety Committee
- Senior Managers committee
- Staff/Stress Committee
- Business Continuity Committee
- Ethical Review Process Committee (Biological Services)
- Risk Committee

3.2.1 Scientific Programme Management

Professor Collinge is responsible for, and directs, the scientific programmes and scientific policy of the Unit. Each programme is led by a senior member of the scientific staff (Group Leader) who manages the day to day work of their teams. Professor Collinge himself also leads a team.

The programmes are managed through regular monthly meetings held by Professor Collinge with each of the Group Leaders and where appropriate their teams, where progress and scientific data are reviewed, strategy developed and future needs planned.

Throughout each year, the combined scientific staff from the Unit and Department meets weekly and present their research. This encourages the sharing of scientific information and stimulates cross fertilisation of ideas and potential developments. A weekly seminar with invited speakers is also held to which all staff are expected to attend.

Whilst our scientific staff are organized into scientific programme groups all Unit and Department staff are highly interdependent. We also have cross-programme virtual groups, usually chaired by the Director, where particular goals which critically rely on close coordination between multiple groups are project managed. These are formed and changed rapidly to respond to evolving needs and indeed new groups are rapidly formed to tackle new scientific challenges in a fast moving field. This template of a focused disease-oriented approach is used as a model by other researchers at UCL who can transfer methods and techniques we have established to "their" disease. The Unit's expertise across several of the disciplines crucial to neurodegenerative research provides a powerful catalyst and foundation for UCL's growing neurodegenerative disease portfolio.

3.2.2 Administration and Technical Support, Governance and Specialist Scientific Support Services

The Business Director deputises for the Unit Director in all aspects of the running of the combined Unit and Department, except the science and science policy. In addition to managing the day to day running of the Unit she has overall line management responsibility for the scientific support services (see below). She represents Professor Collinge at Professorial level and grant-agency committees and liaises with the host institutes on his behalf. She is also responsible for the management and coordination of the Department of Neurodegenerative Disease infrastructure and research programmes funded through grants and Unit Supplementary Council Awards and manages staffing and budgetary issues relating to these, as well as those of the core Unit programmes. The current Department grants are valued at £24 million with the overall quinquennium period grants being around £45 million. This has proved a successful appointment, enabling the Director to focus on the scientific direction of the Unit and maximizing his effectiveness in dealing with national and international government issues.

The Unit has the following administrative and technical support functions

- HR, Finance, IT, Reprographics, Research Governance and Quality Control, Laboratory Management and Health and Safety Management

and specialist scientific support services

- Histology
- Biological Services facility

At the start of this quinquennium the total Unit staff complement was 90 with 13 of those posts being within the management and technical services team. Over the quinquennium the Prion Unit has grown to a total of 113 posts.

GRO-B

	9	Molecular diagnostic strategies in prion disease	Graham Jackson	Current quinquennial financial data for this group reported as part of finance programme 6, future quinquennial financial data reported as finance programme 12	6 current qq 12 future qq

GRO-B

TABLE 3.8 Summary of Headcount by Activity

REVIEW OF LAST SIX YEARS		Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	Yr 6
HEADCOUNT							
	Programme 9*	5.00	5.00	7.00	6.00	7.00	7.00

GRO-B

GRO-B

The Unit demonstrates significant value for money over the current quinquennium not only in financial performance indicators already described in Section 3.3 but also in other measures of performance. These include 129 peer reviewed publications, external presentations (Section 4.3 Table D), training courses attended (Section 4.2 Table 1) and scientific/academic and industrial collaborations (Section 1) all of which have been achieved alongside successfully delivering our scientific objectives as described in Section 2 with notable advances in the current quinquennium including:

- Completion of a successful genome-wide association study in vCJD and the identification of multiple human prion susceptibility genes
- GRO-B
- Completion of our modelling of human susceptibility to BSE in humanised transgenic mice representing all three *PRNP* codon 129 genotypes
- GRO-B

- GRO-B
- Molecular explanation of why vCJD has occurred to date exclusively in *PRNP* codon 129 methionine homozygotes: human PrP V129 incompatible with vCJD prion strain conformation
- GRO-B
- Effective prion decontamination on metal surfaces using non-corrosive reagents
- First antemortem diagnosis of secondary vCJD (related to blood transfusion) and characterisation of pathogenesis and diagnostic features
- GRO-B

3.4

GRO-B

Table 3.11 presents the planned headcount profile for the new quinquennium. Discounting the Immunotherapeutics SCA programme 11 and studentships we are requesting net additional 5.25 scientific posts in year 1 rising to 8 in year 3 through to 7 at the end of the new quinquennium as compared to the planned 2009/10 headcount. The movements in headcount are as below:

- GRO-B
- Programme 9 (Programme 8 Section 2) – plus two posts - Research Technician (Band 5) year 2 onwards, Investigator Scientists (Band 4) years 3 onwards. One post transferred from Programme 4 Research Technician (Band 5)
- GRO-B

GRO-B

Table 3.11 Planned Headcount per Activity

			2010/11	2011/12	2012/13	2013/14	2014/15
HEADCOUNT							
	By function						
	Direct Scientific Staff						
		GRO-B					
		Programme 9*	8.00	9.00	10.00	10.00	10.00

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GRO-B Programme 9 and 10 request additional funding to support running costs associated with their additional staff. GRO-B

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3. Unit Technology Transfer Activities within the last quinquennium

a) Filings and Invention Disclosures

GRO-B

There has been one MRC patent filing which is currently in the international phase:

MRCT Reference and Patent Application Number	Patent Filing Date	Inventors	Invention
A813/2135, GB0718603.4	Sept 2007	John Collinge and Graham Jackson	Use of an antibody that specifically enriches for disease prion and its application in combination with a prion amplification and detection process in development of a sensitive test for detection of disease prion in human blood

There has also been one patent filing arising from a collaboration with Proteome Sciences (see below) that is jointly owned by MRC and Proteome Sciences:

MRCT Reference and Patent Application Number	Patent Filing Date	Inventors	Invention
A813/1946, WO06061609	7th Dec 2004	John Collinge, Graham Jackson et al	Diagnosing variant Creutzfeld-Jakob disease (vCJD) in a diagnostic sample of valid body tissue from human subject comprises detecting protein concentration in the diagnostic sample compared with sample of a control human subject

GRO-B

MRCT Reference Number	Unit PI	Duration and Start Date	Award	Research Program
A853/0090	Graham Jackson	June 2008 for 2 years	£98000	Development of an optimised disease prion enrichment process for use as the first capture step which will be an essential component of a sensitive and specific test to detect disease prion in human blood. Validation of patent claims for MRC patent filing A813/2135

GRO-B

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We would like to highlight our extensive and long-standing collaborations for knowledge sharing with the National CJD Surveillance Unit (NCJDSU) at the University of Edinburgh, both at a basic science and clinical level. The Unit works closely with the clinical and nursing staff from the NCJDSU regarding the care of, and research with, patients with suspected prion disease. We also form an integral part of the Department of Health National Referral Scheme in partnership with the NCJDSU; ensuring prion disease patients experience the highest quality of care in a clinic at the forefront of understanding of such diseases. Through such initiatives the Prion Unit has significantly contributed to maintaining high levels of public health protection in this field.

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GRO-B

Molecular Diagnostics **Dr Graham Jackson**	Professor Elizabeth Fisher, Department of Neurodegenerative Disease, Institute of Neurology, Queen Square, London	Studying the mechanisms of SOD1 induced pathogenesis in ALS. Full scientific collaboration to investigate SOD1 amyloidogenesis.
	Professor Claudio Soto, UTMB, Galveston, Texas, TX 77555, USA.	Collaboration to develop PMCA as a technique for the amplification of human prions in blood. Exchange of information and materials.
	Professor David Anstee, International Blood Group Reference Laboratory, Southmead Road, Bristol	Provision of blood samples and blood fractions for the development and validation of blood-based diagnostic tests.
	Professor Daniel Altmann,Human Disease Immunogenetics Group, Department of Infectious Diseases, ICSM, Hammersmith Hospital, London	Studying the role of PrP in the immune system. Full scientific collaboration to determine the function of PrP in memory T-cells.
	Proteome Sciences plc, Coveham House, Downside Bridge Road, Cobham Surrey.	Screening vCJD plasma samples for identifiable surrogate markers of disease.
	National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, UK.	Provision of blinded panels of blood samples and blood fractions for the development and validation of blood-based diagnostic tests.
	Dr Linda Greensmith, Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, London	Studying the mechanisms of SOD1 induced pathogenesis in ALS. Histopathological examination of murine tissues from transgenic mice expressing human SOD1 proteins.

Annex 4b: Prion Unit Future Planned Collaborations

Group/ Group Leader	Collaborator	Nature of Collaboration
GRO-B		
	National CJD Surveillance Unit Professors Bob Will and Richard Knight	Care of and research with patients with suspected prion disease.
GRO-B		

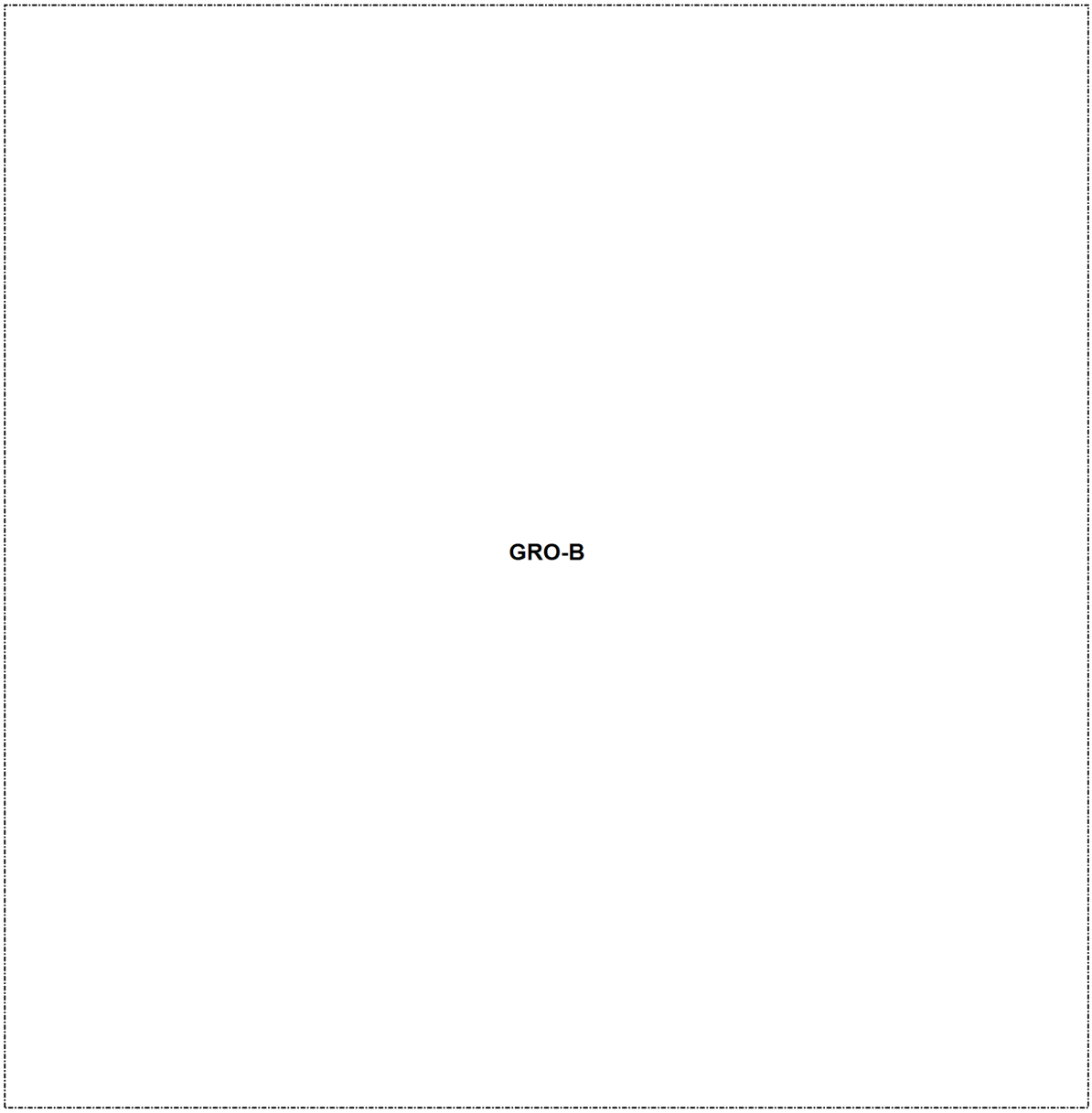
GRO-B

Molecular Diagnostics **Dr Graham Jackson**	Professor Elizabeth Fisher, Department of Neurodegenerative Disease, Institute of Neurology, Queen Square, London	Studying the mechanisms of SOD1 induced pathogenesis in ALS. Full scientific collaboration to investigate SOD1 amyloidogenesis.
	Professor Claudio Soto, UTMB, Galveston, Texas, TX 77555, USA.	Collaboration to develop PMCA as a technique for the amplification of human prions in blood. Exchange of information and materials.
	Professor David Anstee, International Blood Group Reference Laboratory, Southmead Road, Bristol	Provision of blood samples and blood fractions for the development and validation of blood-based diagnostic tests.
	Professor Daniel Altmann,Human Disease Immunogenetics Group, Department of Infectious Diseases, ICSM, Hammersmith Hospital, London	Studying the role of PrP in the immune system. Full scientific collaboration to determine the function of PrP in memory T-cells.
	GRO-B	
	National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, UK.	Provision of blinded panels of blood samples and blood fractions for the development and validation of blood-based diagnostic tests.
	Dr Linda Greensmith, Sobell Department of Motor Neuroscience and Movement Disorders, Institute of Neurology, London	Studying the mechanisms of SOD1 induced pathogenesis in ALS. Histopathological examination of murine tissues from transgenic mice expressing human SOD1 proteins.

6. Active Partnering With Stakeholders

As discussed at sub-section 3 above, UCL as our host University plays a pivotal role in supporting our students and all of our Programme Leader and Programme Leader Track staff currently hold honorary teaching positions within UCL.

As discussed in more detail in the Public Engagement section of this report the Unit maintains very strong links with patients groups. With representatives from the Unit regularly attending support group meetings and our hosting regular open days. The main CJD patients' group, the CJD Support Network, have also kindly provided a small annual bursary towards the travel and consumables costs of one of our students. This student and her supervisor have developed particularly close links with the Support Network and provide regular updates on their research and have attended and spoken at Support Network meetings.



4.3 PUBLIC ENGAGEMENT

1. The Unit's Communications Strategy is at Table A to this section.

2. vCJD/BSE continues to be a matter of considerable public interest and the Unit works hard to keep stakeholders fully informed regarding our research, developments in the field in general and the resulting benefits of that research to public health and to the wider scientific community. To this end considerable effort has been and continues to be expended to improve our channels of communication and we detail below some of our initiatives in this regard:

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8 December 2006
http://www.mrc.ac.uk/NewsViewsAndEvents/News/MRC003431
Scientists have confirmed that Variant Creutzfeldt-Jakob disease (vCJD) can be passed from person to person through blood transfusion.

GRO-B

Table A:

Communications Strategy 2008 – 2009

MRC Prion Unit

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3. Activities

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c) GRO-B

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g) The Director is a member of a number of Government, MRC or other Research Advisory Pannels these include the following:

DH CJD Tissue Management Steering Group

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Table C

Expert Scientific Committees and Advisory Bodies

Name	Scientific Committees/Advisory Bodies	Role	Membership Dates
Prof. John Collinge	Deutsche Forschungsgemeinschraft	Ad Hoc International Advisor	2004 - Ongoing
	International Advisory Board - Institute Pasteur de Lille	Member	2005 - Ongoing
	The Royal Society Research Grant Board F	Member	2006 - 2008
	The Royal Society Sectional Committee 10	Member	2007 - Ongoing
	DoH/DEFRA/FSA Spongiform Encephalopathy Advisory Committee (SEAC)	Member	2007 - Ongoing
	SEAC New Variant CJD Epidemiology Sub-Group	Member	1997 - 2005
	DoH/MRC Steering Group for Studies of Detectable PrPSc	Member	2002 - Ongoing
	DoH CJD Tissue Management Steering Group	Member	2002 - 2007
	DH CJD Therapy Group	Member	2002 - 2007
	MRC New Therapies Scrutiny Group	Member	2005 - Ongoing
	MRC Horizon Scanning Working Group	Member	2006 - Ongoing
	St Mary's and St Charles' Hospitals Acute Services Trust Medical Advisory Committee	Member	
	University College London Hospitals Medical Committee	Member	
	National Hospital Medical Committee	Member	
	Institute of Neurology Executive Committee	Member	
	University College London Academic Committee	Member	
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Dr Graham Jackson	The Department of Health working group on the decontamination of surgical instruments	Member	2005 - 2008

	UK Health Protection Agency Expert Advisory Group on the Laboratory Testing Strategy for Large Scale Abnormal Prion Prevalence Studies	Member	2006 - Ongoing

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Table D

Scientific Meetings and Conferences

Name	Date	Meeting Title	Role
Prof John Collinge	2004	The Centre for Neuroscience's 29th Woolsey Lecture - University of Wisconsin, USA	Invited Speaker
		Prion 2004 International Conference – Paris, France	Attended
		Keystone Symposium: 'Protein Misfolding, Amyloid and Conformational Diseases Conference' – Colorado, USA	Invited Speaker
		Foundation IPSEN Meeting – Paris, France	Invited Speaker
		Transmissible Spongiform Encephalopathies Joint Funders' Workshop – York, UK	Invited Speaker
		Banbury Center Annual Scientific Symposium – New York, USA	Invited Speaker
	2005	Keystone Symposium: 'Molecular Mechanisms of Transmissible Spongiform Encephalopathies' – Utah, USA	Invited Speaker
		Banbury Center Symposium: 'Molecular Mechanisms of Human Neurological Diseases' – New York, USA	Invited Speaker
		Scientific Meeting of EU Collaborators – Lech, Austria	Invited Speaker
		Prion 2005: 'Between Fundamentals and Society's Needs Conference' – Dusseldorf, Germany	Invited Speaker
		Scientific Meeting of EU Collaborators – Crete, Greece	Invited Speaker
		Cold Spring Harbor Laboratory and Wellcome Trust Conference: 'Functional Genomics of Mammalian Nervous Systems' – Hinxton, UK	Invited Speaker
		Papua New Guinean Institute of Medical Research's 41st Annual Symposium – Goroka, Papua New Guinea	Attended
		Banbury Center Meeting: 'Prion Biology: Puzzles and Paradoxes Conference' - New York, USA	Invited Speaker

	2006	Wellcome Trust Advanced Course: 'Molecular Neurology and Neuropathology' - Hinxton, UK	Invited Speaker
		International Congress on Occupational Health – Torino, Italy	Invited Speaker
		Transmissible Spongiform Encephalopathies Joint Funders' Workshop - Warwick, UK	Attended
		Prion 2006 International Conference – Torino, Italy	Invited Speaker
		Papua New Guinean Institute of Medical Research's 42nd Annual Symposium – Goroka, Papua New Guinea	Attended
	2007	Banbury Center Workshop: 'When is Amyliod Functional and When is Amyloidogenesis Pathological' - New York, USA	Invited Speaker
		Advances in Neurology Symposium - Liverpool, UK	Invited Speaker
		Health Protection 2007 Symposium - Warwick, UK	Invited Speaker
		Prion 2007 International Conference - Edinburgh, UK	Invited Speaker
		MRC Prion Unit Conference: 'The End of Kuru: 50 Years of Research Into an Extraordinary Disease' - London, UK	Organised & Chaired
		GlaxoSmithKline Protein Misfolding Symposium - Ware, UK	Invited Speaker
		Papua New Guinean Institute of Medical Research's 43rd Annual Medical Symposium - Port Moresby, Papua New Guinea	Attended
		EMBO-FEBS Workshop: 'Chaperones in Normal & Aberrant Protein Folding, Aging & Cancer' - Tomar, Portugal	Invited Speaker
		The Wellcome Trust Meeting: 'Logistics of the Genomics of Common Diseases' - Homerton, UK	Attended
	2008	Transmissible Spongiform Encephalopathies Joint Funders' Workshop - Warwick, UK	Attended
		Ringberg Symposium of the SFB 596: 'Molecular Mechanisms of Prion Diseases and Parkinson's Disease' - Ringberg, Germany	Invited Speaker

		Banbury Center Meeting 'Prion Strains: Origins, Mechanisms and Implications for Disease' - New York, USA	Invited Speaker
		Papua New Guinean Institute of Medical Research's 44th Annual Medical Symposium - Port Moresby, Papua New Guinea	Attended
		Liverpool University Structural Proteomics Symposia - Liverpool, UK	Invited Speaker
		Harvard Neurodiscovery Center 2008 Annual Symposium: 'Protein Folding & Neurodegeneration' - Boston, USA	Invited Speaker
		Prion 2008 International Conference - Madrid, Spain	Attended

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		GRO-B	
Dr Graham Jackson	2004	Transmissible Spongiform Encephalopathies Joint Funders' Workshop – York, UK	Invited Speaker & Presented Poster
		Banbury Center Meeting: 'Prion Biology: Puzzles and Paradoxes' - New York, USA	Invited Speaker
	2006	Transmissible Spongiform Encephalopathies Joint Funders' Workshop - Warwick, UK	Invited Speaker & Presented Poster
		British Society for Orthopaedic Anaesthetists' Annual Scientific Congress - Telford, UK	Invited Speaker
	2007	CSC Annual Conference 2007 - Manchester, UK	Invited Speaker
		Prion 2007 International Conference - Edinburgh, UK	Presented Poster
	2008	Transmissible Spongiform Encephalopathies Joint Funders' Workshop – Warwick, UK	Invited Speaker & Presented Poster

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