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Research Grant PROPOSAL

Document Status: With Owner
MRC Reference:

Research Boards May/Jun 2016 Submissions
Molecular & Cellular Medicine Board Structural Biology

Organisation where the Grant would be held

Organisation	MRC Prion Unit	Research Organisation Reference:	Blood Test
Division or Department	MRC Prion Unit		

Project Title [up to 150 chars]

Development of enhanced methods for the screening and diagnosis of prion disease

Start Date and Duration

a. Proposed start date: 01 April 2017

b. Duration of the grant (months): 48

Applicants

Role	Name	Organisation	Division or Department	How many hours a week will the investigator work on the project?
Principal Investigator	Dr Graham Jackson	MRC Prion Unit	MRC Prion Unit	37.5

Objectives

List the main objectives of the proposed research in order of priority

Objectives

We have established the principle of diagnosing a neurodegenerative disease from blood using biochemical means. The methodology of steel capture provides 100% specificity opening up the possibility of such a test being used for screening applications. In parallel we have been developing methods for the amplification of disease-associated material using a PMCA based approach and recombinant PrP amyloid replication in vitro. The ability to capture disease-associated material and remove any potential inhibitors or degradative enzymes provides an ideal input material for amplification assays which will significantly extend the detection limit and hence sensitivity of diagnostic assays. We have also demonstrated a related approach can diagnose sporadic CJD in urine samples. This provides an exciting opportunity to isolate by fractionation and enrichment the disease-associated species detected in our assays, a strategy previously impossible due to the scarcity and biological complexity of blood samples. These species are more abundant than conventional PrPSc and infectivity and may represent a novel disease-associated isoform that can be rationally targeted for diagnostic applications. We therefore propose the following specific objectives:

1. Extend the detection limit for prion disease by coupling matrix capture to PMCA and amyloid seeding in vitro.

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We have established that abnormal PrP binding to austenitic steel is a highly specific phenomenon distinct from general protein adsorption. The selectivity exceeds that afforded by antibodies directed against PrP and offers the advantage of being stable to environmental conditions, captured material being irreversibly bound. The selectivity of matrix capture for disease-associated PrP provides an ideal input material to amplification assays where in combination the detection limits for prion infectivity will be significantly enhanced.

2. Isolate the molecular target detectable in sporadic CJD patient urine.
Using an adaptation of our blood assay for vCJD infection in blood, we have been able to demonstrate the presence of disease-specific abnormal PrP in the urine of patients with the more common sporadic CJD. This has an unparalleled advantage of large sample volumes being available for isolation of circulating disease-specific PrP. This permits development of pre-enrichment protocols to significantly extend the detection limits for detection and detailed molecular characterization of the isolated species.

3. Characterise the matrix captured disease-specific PrP species.
Currently it is not clear what molecular species are captured by the solid-sate matrix on which our previous assays are based. Evidence from rodent models indicates that in blood this is a much wider ensemble of conformers than prion infectivity alone. Material isolated from urine will be characterised with respect to its heterogeneity and composition of primary and secondary structure, aggregation state, morphology, post-translational modifications, stability and antibody epitopes. Knowledge of what these reactive species are will facilitate greatly enhanced methods for enrichment and detection.

4. Establish a panel of 'gold standard' positive controls for assay validation.
Our ability to detect vCJD infection in blood currently has an analytical sensitivity of a 1010 fold dilution of infected brain tissue which provides diagnostic sensitivity of 71%. There is uncertainty if the inability to detect infection in all patients is due to a limitation of the analytical sensitivity of the assay or reflects the variability of prionaemia as a feature of disease. We will establish a panel of samples comprising blood, blood fractions, urine and CSF from patients with CJD as true positives by multiple testing. These will be classified with reference to defined analytical sensitivities enhanced from the activities of aims 1 to 3, anticipated to be equivalent to a 1015-fold dilution of infected brain.

Summary

Describe the research in simple terms in a way that could be publicised to a general audience. If awarded, this will be made publicly available and applicants are responsible for ensuring that the content is suitable for publication.

Molecular Diagnostic Strategies in Prion Disease

The majority of the adult UK population and a lesser proportion of continental Europe and the rest of the world have been exposed to mad cow disease or BSE contaminated foods and this has resulted in the new human disease variant CJD (vCJD). Although the number of patients has remained small, the number of people infected but without any signs of illness is thought to exceed 30,000 in the UK alone. Infected individuals may never show any signs of disease or this can take over 50 years to develop. However, they are themselves infectious and there is a risk of spreading the disease through the contamination of medical and dental instruments; the use of contaminated blood for transfusion and the transplantation of infected organs and tissues. All of these problems could be avoided if simply tests were available to detect vCJD infection in the blood of carriers.

We were the first to demonstrate it is possible to detect vCJD infection in patient blood samples (Edgeworth et al, Lancet 2011). However, critical to the usefulness and benefit of a test for vCJD is the accuracy of the assay as any significant number of wrong results would outweigh the benefits of detecting the disease. By testing thousands of blood samples from healthy blood donors and hundreds of samples from patients with other dementias we subsequently established that our blood test is 100% specific for CJD (Jackson et al, JAMA Neurology 2014).

Despite being able to detect infection in about 7 out of every 10 patients with vCJD our blood test is not yet sensitive enough to identify all patients and can't currently be used to diagnose the more common sporadic form of CJD (sCJD). CJD is defined by a characteristic change in one of the body's proteins and in order to improve our assays and detect all cases of CJD we are trying different ways of detecting this rare altered protein or prion which accumulates in patients and carriers. One approach is to copy the chain reaction the prion uses to reproduces itself in the human body during disease. This allows us to 'amplify' the very small amounts of prion in patient samples into large amounts that can be easily detected using conventional laboratory tests.

This new approach uses the basis of our previous test to capture prions from infected blood onto microscopic steel spheres and then use these 'infectious' spheres to start a chain reaction in a test tube that produces larger quantities of the prions that we can easily detect. By amplifying the amount of material for detection in this way we will increase the accuracy of the test and reduce the possibility that a patient who has CJD will give a false negative test result.

An additional approach will be based upon the exciting new finding that we can use the capture of prions on steel microspheres to detect the presence of sporadic CJD in urine samples. This will now allow us to purify the prions found in urine and enable us to study their structures and composition which will in turn assist us in designing new and better ways to enrich and detect prions both in urine and in blood.

Technical Summary

Describe the research in a manner suitable for a specialist reader. If awarded, this content will be made publicly available and applicants are responsible for ensuring that the content is suitable for publication.

The deposition of aggregated conformational isoforms of a host's normal prion protein (PrPC) is the archetypal marker of infection with absolute specificity for prion disease. Whilst abundant in the tissues of the central nervous system and lymphoreticular tissues in cases of vCJD, the concentration of infectivity, and by inference other abnormal forms of PrP, is very low in blood and CSF.

We have made substantial progress in the detection of abnormal PrP, resulting in the ability to diagnose vCJD infection with a blood test, but very considerable challenges remain. Not least is the extension of detection limits to ensure detection of pre-clinical or sub-clinical carrier states and encompass the anticipated lower levels of disease-associated material in the blood of patients with sporadic CJD. An additional demand is that any methodology for detection should provide a dynamic range sufficient for the quantitative detection of infection capable of staging disease progression and monitoring therapeutic efficacy.

A conceptually obvious approach to improve the detection of abnormal PrP is to exploit the innate propensity of amyloid to self-propagate. This approach has been adopted in a variety of formats but is associated with significant of false positive reactions when the extreme conditions required to amplify ultralow prion concentrations are applied in addition to false negative results caused by reaction inhibition from complex biological analytes such as blood. We are exploiting the ability to present enriched abnormal PrP in a stable immobilised form bound to steel microparticles, free of inhibitory contaminants which has the potential to both overcome the requirements for extended amplification regimes thereby reducing the frequency of false positive reactions and to reduce false negative reactions. Concomitant with lowered detection limits are improved diagnostic sensitivity and increased confidence in the ability to detect sub-clinical infections.

Academic Beneficiaries

Describe who will benefit from the research

Academic beneficiaries

The proposed research will benefit a wide range of academics both interested in prion biology and across different disciplines by informing on methods to enrich and amplify disease-associated amyloid conformers from body fluids with potential relevance to a range of protein-misfolding disorders including Alzheimer's disease. The ability to couple the isolation of abnormal PrP to methods for the replication and amplification in vitro will substantially lower the detection limits and hence increase the sensitivity of such hybrid assays. The increased confidence of the resulting true negative results

coupled to the previously demonstrated absolute specificity could stimulate prionaemia prevalence studies both in the UK and abroad.

The transposition of prion disease paradigms to Alzheimer's disease has already been used to demonstrate that stainless steel matrices can very efficiently capture and deliver an ABeta amyloidosis in experimental animals (Eisele et al, PNSA 2009) and to develop amplification methods for the detection of disease-associated abnormal ABeta in vitro (Salvadores et al, Cell Report 2014). Our results and findings are likely to have direct relevance to attempts to devise diagnostic approaches for other neurodegenerative disease including Alzheimer's from blood and urine.

We will ensure academics can benefit from our results by publishing our results in open access journals as well as making them available through Europe PubMed Central, our institutional repository UCL Discovery and the Prion Unit web site to ensure the widest possible dissemination and benefit. We will also present our work at national and international conferences in relevant fields to reach as many potential academic beneficiaries as possible.

Within the Prion Unit several PhD studentships exist for which specific funding isn't requested in this application. They will have the opportunity to participate in our research where they will receive training not only in the specific techniques we are developing but also in the safe and ethical handling of human tissue and fluids.

Communications Plan

Please outline your plans for engagement, communication and dissemination about your research and its outcomes with the research community and, where appropriate, with potentially interested wider audiences

Communication Plan

The aim of this proposal is to extend the detection limit of prion disease diagnostic assays for prevalence screening and sCJD diagnosis. This will generate research findings and resources that will have impact for clinicians, academics and policy makers in the field of neurodegeneration and public health. Our research will also impact and be of interest to the wider community. To ensure the most widespread and effective dissemination of our research we will adopt the following plan:

Publications
The main method of communicating our research findings will be through publishing our work in peer-reviewed journals with associated press releases through the MRC Press Office. This will be done in a timely manner and we will aim for high-impact journals. These will be accessible electronically and searchable through standard methods including PubMed. To ensure the broadest possible access and in-line with MRC and University regulations, these will all be made freely available through open access arrangements as well as being available through Europe PubMed Central, our institutional repository UCL Discovery and our departmental web site. Details of publications will also be accessible through the UCL Institutional Research Information System (IRIS) and Research Gate.

Website
In addition to providing links to our latest scientific publications, our research is highlighted on our Unit web site. This is aimed at a general audience in order to broaden our impact as widely as possible. Details of Dr Jackson's research interests are also accessible through the UCL Institutional Research Information System (IRIS) and Research Gate.

Resources
During the course of this project, we will generate protocols, reagents and quality control samples that will be of interest to other academics and clinicians wishing to establish diagnostic or prevalence screening assays for prion disease. Following publication, these will be made publically available to interested parties through an appropriate MTA.

Presentations

A key part of our communication plan is for Dr Jackson and post-doctoral researchers to convey our findings as poster and oral presentations at both national and international scientific meetings. In addition to formal presentations, conferences and symposia provide informal opportunities for communicating our research through networking with other scientists.

Public Engagement
To communicate our research to the public we will participate in the MRC Prion Unit and National Prion Clinic open days hosted for families affected by prion disease. In addition, Dr Jackson will continue to provide media interviews in response to published work. Where appropriate we will work with MRC Press Office and UCL Communications and Marketing to publicise our findings to the wider public through a range of electronic and traditional media.

Impact Summary

If awarded, this content will be made publicly available and applicants are responsible for ensuring that the content is suitable for publication.

Impact Summary

The work outlined in this proposal will benefit academics by informing on methods to enrich disease-associated amyloid conformers from body fluids with potential relevance to a range of protein-misfolding disorders including Alzheimer's disease. However, this research programme will have an impact beyond the conventional academic environment impacts upon clinicians, policy makers and public health bodies in the UK and elsewhere. It will also generate know-how and intellectual property of potential value. The need for prion disease diagnostic tests applicable to routine use and screening applications was highlighted by the UK House of Commons Science and Technology Committee in 2014. The Unit and Dr Jackson are in regular communication with ACDP TSE Risk Assessment Sub Group, NHSBT through UK Blood Services Prion Working Group and the European Medicines Agency, all of whom would benefit from the ability to test for prion infection with enhanced sensitivity.

A significant impact of this research will also be upon patient groups and 'at risk' individuals directly. Extending the detection limits for prion infection to eliminate false negative results can provide reassurance that exposed individuals testing negative are free of infection. For individuals testing positive the extension of detection limits permitting the quantitation of abnormal PrP will allow correlation with clinical information collected by the National Prion Clinic and Prion Unit as part of the Nation Cohort Study which could provide not only confirmation of infection, but prediction of, if, and when clinical disease will develop.

The extension of abnormal PrP detection limits should also permit the diagnosis of sCJD in addition to vCJD. Significant progress within the Prion Unit in developing both conventional small molecule therapeutics and immunotherapies for prion disease is leading to imminent first-in-human (FIH) clinical trials in the near future. Should these prove successful the identification of infected individuals predicted to develop sCJD, vCJD or iatrogenic forms of CJD will offer the possibility of early treatment which could prevent the development of any symptoms of disease.

Summary of Resources Required for Project

Financial resources

Summary fund heading	Fund heading	Full economic Cost	MRC contribution	% MRC contribution
Directly Incurred	Staff	705052.00	564041.60	80
	Travel & Subsistence	8000.00	6400.00	80
	Other Costs	216000.00	172800.00	80
	Sub-total	**929052.00**	**743241.60**	

Summary of staff effort requested

	Months
Investigator	0
Researcher	96
Technician	96
Other	0
Visiting Researcher	0
Student	0
Total	192

Directly Allocated	Investigators	0.00	0.00	80
	Estates Costs	34400.00	27520.00	80
	Other Directly Allocated	0.00	0.00	80
	Sub-total	**34400.00**	**27520.00**	
Indirect Costs	Indirect Costs	445552.00	356441.60	80
Exceptions	Travel & Subsistence	0.00	0.00	100
	Other Costs	0.00	0.00	100
	Sub-total	**0.00**	**0.00**	
	Total	**1409004.00**	**1127203.20**	

Other Support

Details of support sought or received from any other source for this or other research in the same field.
Other support is not relevant to this application.

Classification of Proposal

(a) Grant Type

Research Grant	x								

Proposal Classifications

Qualifier:

Qualifiers are terms that further describe the area of research. Please ensure you complete this section if relevant. To add or remove Qualifiers use the links below.	
Type	**Name**
Project Engagement by Sector	Academic Users
Project Engagement by Sector	Business Sector
Project Engagement by Sector	Central and Local Government
Project Engagement by Sector	NHS Health Prof & Social Serv

Staff

Applicants

Role	Name	Post will outlast project (Y/N)	Contracted working week as a % of full time work	Total number of hours to be **charged** to the grant over the duration of the grant	Average number of hours per week **charged** to the grant	Rate of Salary pool/banding	Cost estimate
Principal Investigator	Dr Graham Jackson	Y	100	0	0	65497	0

Travel and Subsistence

Destination and purpose		Total £
Outside UK	Attendance at an international meeting each year by the PI and one of the postdoctoral researchers. Specifically; Prion 2017, Prion 2018, Prion 2019 and Prion 2020.	8000
	Total £	8000

Other Directly Incurred Costs

Description	Total £
£15,000 per annum for the postdoctoral researchers and £12,000 per technician for standard molecular biology costs for all lab consumables; plasticware, antibodies, immunological reagents, molecular biology reagents, biochemical and minor equipment.	216000
Total £	216000

Research Council Facilities

details of any proposed usage of national facilities
Research Council Facilities are not relevant to this application.

Human Biological Samples

Does your work involve human Biological samples: research which involves laboratory studies on human material which are specifically designed to understand or treat a disease / disorder? NB: basic biomedical research remote from application to a disease / disorder, such as the use of immortalised human cell lines in model biological systems, is excluded. Yes

Technology Development

Does your work involve Technology development for clinical use: development or adaptation of technologies for diagnosis or therapy, e.g. instrument development for diagnostic or surgical use; development of new techniques, such as photodynamic therapy, for clinical use. Yes

(b) Research Setting

Based on direct patient contact, indicate whether the research involves a particular medical setting such as primary care or secondary care.

None	x								

(c) Stem Cells

Does the research involve the use of Stem Cells or regenerative medicine?

No	x

(d) Developing Countries

Will the research involve a substantial component in developing countries? If so select those that apply.

(ii) Keywords

Prion	CJD
Diagnosis	Blood
Amyloid	

Human Participation

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Would the project involve the use of human subjects?		Yes	No✓
	If yes, would equal numbers of males and females be used?	Yes	No✓
Would the project involve the use of human tissue?		Yes✓	No
Would the project involve the use of biological samples?		Yes✓	No
Would the project involve the administration of drugs, chemical agents or vaccines to humans?		Yes	No✓
Will personal information be used?		Yes	No✓
	If yes, will the information be anonymised and unlinked?	Yes	No✓
	Or will it be anonymised and linked?	Yes	No✓
	Will the research participants be identifiable?	Yes	No✓

Please provide details of any areas of substantial or moderate severity:

As the primary objective of the proposal is to develop an enhanced methodology for ultra-sensitive detection of prion infection in human tissues and fluids the use of authentic human samples is essential. A range of tissues and fluids will required for the study including brain, CSF, blood, blood fractions, and urine. All experimental protocols are approved by the Local Research Ethics Committee of UCL Institute of Neurology/National Hospital for Neurology and Neurosurgery and comply with the code of practice specified in the Human Tissue Authority licence held by UCL Institute of Neurology. All samples are obtained with specific informed consent for their use in these studies.

Some aspects of the proposed research can utilise exogenously 'spiked' whole blood containing varying amounts of homogenised prion-infected brain tissue. Similarly artificial CSF (aCSF) can be used as a control along with 'spiked' aCSF. The quantities of brain tissue required are 1-5g. However, even rigorous disruption of a bolus of brain homogenate into CSF or blood does not produce material with identical physical and biochemical characteristics of endogenous patient CSF and blood samples. Ultimately authentic patient samples must be used.

In the case of vCJD blood and CSF samples these are scarce and will only be ultilised for assay validations requiring 10-100ul. The majority of the research will use samples obtained from patients with sCJD which are considerably more abundant.

For the isolation of abnormal PrP from urine this will be performed from samples obtained from patients with sCJD. For this element of the research several litres of sample will be required.

Animal Research

Would the project involve the use of vertebrate animals or other organisms covered by the Animals (Scientific Procedures) Act?		Yes	No✓
	If yes, what would be the maximum severity of the procedures?	Mild or non-recovery	
		Moderate	
		Severe	
	Please provide details of any areas which are Moderate or Severe:		

Animal Species

Does the proposed research involve the use of non-human primates?	Yes	✓No
Does the proposed research involve the use of dogs?	Yes	✓No
Does the proposed research involve the use of cats?	Yes	✓No
Does the proposed research involve the use of equidae?	Yes	✓No

Please select any other species of animals that are to be used in the proposed research.

Fish	Sheep
Rabbit	Rat
Amphibian	Poultry
Cow	Mouse
Reptile	Guinea Pig
Pig	Other Rodent
Bird	Other Animal

Genetic and Biological Risk

Would the project involve the production and/or use of genetically modified animals?		Yes	✓	No
If yes, will the genetic modification be used as an experimental tool, e.g., to study the function of a gene in a genetically modified organism?		Yes	✓	No
And will the research involve the release of genetically modified organisms?		Yes	✓	No
And will the research be aimed at the ultimate development of commercial or industrial genetically modified products or processes?		Yes	✓	No
Would the project involve the production and/or use of genetically modified plants?		Yes	✓	No
If yes, will the genetic modification be used as an experimental tool, e.g., to study the function of a gene in a genetically modified organism?		Yes	✓	No
And will the research involve the release of genetically modified organisms?		Yes	✓	No
And will the research be aimed at the ultimate development of commercial or industrial genetically modified products or processes?		Yes	✓	No
Would the project involve the production and/or use of genetically modified microbes?	✓	Yes		No
If yes, will the genetic modification be used as an experimental tool, e.g., to study the function of a gene in a genetically modified organism?		Yes	✓	No
And will the research involve the release of genetically modified organisms?		Yes	✓	No
And will the research be aimed at the ultimate development of commercial or industrial genetically modified products or processes?		Yes	✓	No

Implications

Are there ethical implications arising from the proposed research? No

Provide details of what they are and how they would be addressed [up to 1000 characters]

Approvals

Have the following necessary approvals been given by:			
The Regional Multicentre Research Ethics Committee (MREC) or Local Research Ethics Committee (LREC)?	Yes✓	No	Not required
The Human Fertilisation and Embryology Authority?	Yes	No	Not required✓

The Home Office (in relation to personal and project licences, and certificates of designation)?	Yes	No	Not required✓
The Gene Therapy Advisory Committee?	Yes	No	Not required✓
The UK Xenotransplantation Interim Regulatory Authority?	Yes	No	Not required✓
Administration of Radioactive Substances Advisory Committee (ARSAC)?	Yes	No	Not required✓
Other bodies as appropriate? Please specify.			

OTHER INFORMATION

Reviewers

1	Name	Organisation	Division or Department	Email Address
Dr Vincent Beringue		INRA	Infections Research Team	vincent.beringue@GRO-C GRO-C
Area of Expertise				
Relationship with Reviewer				
Reason for Reviewer		Dr Beringue conducts a similar programme of research in France and is therefore familiar with the current state of the art, challenges faced and the time and resources required to achieve our aims.		

Reviewers

2	Name	Organisation	Division or Department	Email Address
Professor Glenn Telling		Colorado State University	Microbiology Immunology and Pathology	Glenn.telling@ GRO-C GRO-C
Area of Expertise				
Relationship with Reviewer				
Reason for Reviewer		Recognised expert on prion disease with extensive knowledge of all areas of endeavour, including diagnosis of infection.		

Reviewers

3	Name	Organisation	Division or Department	Email Address
Professor Joerg Tatzelt		Ludwig Maximilians University Munich	Adolf-Butenandt Institut-Molekularbiolo	joerg.tatzelt@ GRO-C GRO-C
Area of Expertise				
Relationship with Reviewer				
Reason for Reviewer		Prion disease expert with specialist knowledge of protein misfolding and its relevance to prion replication and detection.		

Staff

Directly Incurred Posts

			EFFORT ON PROJECT							
Role	**Name /Post Identifier**	**Start Date**	**Period on Project (months)**	**% of Full Time**	**Scale**	**Increment Date**	**Basic Starting Salary**	**London Allowance (£)**	**Super-annuation and NI (£)**	**Total cost on grant (£)**
Researcher	Dr Elizabeth Sawyer	01/04/2017	48	100	4b	01/04/2017	32648	4777	9072	187484
Researcher	Dr Connie Luk	01/04/2017	48	100	4b	01/04/2017	33567	4777	9295	192088
Technician	Mrs Samantha Jones	01/04/2017	48	100	5s	01/04/2017	29954	4777	8419	173988
Technician	Mrs Claire Thomas	01/04/2017	48	100	5b	01/04/2017	25463	4777	7330	151492
									Total	705052

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