Report for ***CJDSGN Memorial Award in memory of Ross Glasscock, Robert Craig, Carmelo Tripoli, Arthur Schinck and Arlene Hamilton*** and ***CJDSGN Memorial Grant – City2Sea 2015, in memory of Sandra Kernahan, Stephen (Jake) O'Hara, Catherine Heagerty, Grasso family, Victoria Larielle, Barbara Childerhouse, Marilyn Hart and Pamela Thomas***.

In mid 2015 I was fortunate enough to have been awarded a CJDSGN memorial award. This award, for $20,000, allowed me to purchase essential consumables and laboratory supplies to carry out the various experiments in my research project. In 2016 I was awarded a CJDSGN memorial grant, for $25,000, which contributed towards my salary for 2016 and with that enabled me to not only continue on with my own research project, but also allowed me to continue in my responsibilities as a co-supervisor for three PhD students. I would therefore like to thank the families and the CJDSGN for their continued and extremely generous support of prion research in Australia.

My main area of research in the time frame of these awards has been investigating the role of a specific family of proteases, the matrix-metalloproteases (MMP), in prion protein processing (cleavage), and how this might link to the prion disease process. We know that the normal cellular prion protein is required and is likely to interact with disease associated forms of the prion protein, in order for the normal protein to continue to misfold and prion disease to eventually develop. Logically then, any process affecting the normal prion protein and its ability to interact with disease associated prion proteins, including protein cleavage where the protein is cut in various places producing different fragments, may ultimately impact the misfolding of the protein and the prion disease process. I therefore firmly believe this is a very important area of normal prion protein biology that needs to be better understood, as currently reported data is highly contested with little consensus reached as to the precise protease/s and mechanism/s involved. In addition I have been investigating a prion gene polymorphism (codon 127V) recently discovered in the Fore people of Papua New Guinea that appears to have arisen to provide resistance for these people to the Kuru epidemic which has affected them over last century. I have been interested in trying to understand what is it about this normal prion protein variant that appears to give it the resistance to Kuru, and in particular whether this resistance is linked to its cleavage by MMPs or other proteases.

Over the course of the last 18 months, I have carried out several laboratory techniques in order to mutate and then produce various recombinant prion proteins (made in bacteria) as well introduce them such that they are made in mammalian cells in culture. I have so far identified three different members of the MMP family as capable of cleaving the normal human (including codon 129M, codon 129V and codon 127V variants) and mouse prion proteins (including the mouse equivalent of

the codon 127V human “resistant” variant). Interestingly, I’ve found that these proteases appear to cleave the prion protein with different proficiencies and/or at slightly different sites depending on the protease/normal prion protein variant combination, which may prove to be relevant to disrupting any interactions with misfolded prion proteins. Preliminary results also indicate prion infected cells treated with some of these MMPs may result in a reduction in the propagation of misfolded prion proteins, though whether this is a prion strain specific observation or because of an interference between the normal and disease associated proteins, or because of a direct cleavage effect on the misfolded proteins remains to be determined. All of these results are completely novel, with nobody else working on these MMP-prion protein links (as far as I’m aware), and I hope therefore once the data sets are completed, will be highly publishable and of interest to the prion research community worldwide.

Along with my own laboratory-based research, I have been actively involved in the projects of my PhD students, recently graduated Dr Matteo Senesi, Mr Simote Foliaki and Mr Taufiq Islam. These three student have had similarly aligned projects, investigating the nature of the most toxic prion species in both animal behaviour (*in vivo*) and sophisticated electrophysiological (*in vitro*) paradigms. This are important projects, as we and others believe that the misfolded prion proteins that are responsible for prion disease transmission/spread are unlikely to be the same species responsible for the actual brain cell death (i.e. the toxic species) and ultimately the symptoms which occur. In my role as co-supervisor, I’ve been able to provide feedback and advice in the design of experiments and data interpretation, as well as help with writing up some of the results and observations ready for publication. Also, with Simote I have carried out several experiments, and been able to show that partially protease resistant misfolded prion species that cause significant toxicity in his “ephys” paradigm, have by comparison, substantially reduced infectivity in cultured cells. This is a very interesting observation, further lending support to the theories that the transmissible and toxic prion species are different, and will form the basis of our next grant applications with national/international funding bodies. Fingers crossed we have some success with these applications in order to continue this body of work and elucidate more specifically what the actual differences are in these misfolded proteins. Any findings in this research area will be highly important when it comes to the design of potential drugs against prion diseases, as we want to make sure that the correct/most relevant disease associated prion proteins are targeted.