1. Project title: **Detection and characterization of rare strains of sporadic Creutzfeldt-Jakob Disease using a suite of novel biological and biochemical tools.**

2. Project objective: Human prion diseases are diverse and have varying rates of disease progression, symptoms and pathology. These strain characteristics are encoded in the shape of the misfolded prion protein that accumulates during disease. Our objective was to develop new laboratory methods to detect these differences in the misfolded human prion protein, especially those that are linked to rare disease strains.

3. Summary of accomplishments to date: We have analysed over 50 Canadian cases of CJD by semi-quantitative seeding assays with various pairings of prion seed and substrates. The majority of cases produced similar data in terms of the efficiency of amplification however a number of cases were clear outliers. Analysis of the misfolded proteins in these cases revealed them to have either unusual molecular weights, glycosylation, or sensitivity to proteinase K.
In addition to using classical Western blotting we developed a method using a capillary-based immunoassay that is able to separate multiple protein samples reproducibly so that they can be quantified. This requires smaller amounts of protein than Western blotting and so more tests can be done on each human tissue sample. In addition, proteins can be visualized using multiple detection reagents to simultaneously identify types, and is also highly sensitive for the detection of small molecular weight protein fragments that are sometimes characteristic of rare strains. A manuscript describing this innovative technology, "Development of an Automated Capillary Immunoassay to Detect Prion Glycotypes in Creutzfeldt-Jakob Disease" has been accepted for publication in the journal Laboratory Investigation and is currently in press.
Analysis of the outliers identified in the seeding assay revealed two cases of CJD characterized by the protease sensitivity of prions except for small fragments of misfolded protein that were proteinase K resistant. Correlation with the case pathology and relatively long duration of disease confirmed these to be novel cases called variably protease-sensitive prionopathy (VPSPr). The ability of the capillary electrophoresis system to separate very small proteins made this method particularly useful to detect this rare strain. Two other cases identified by slower seeding were found to exhibit unusual 20KD protease K resistant misfolded proteins; a molecular weight that is in between the majority of CJD cases that are either 19KD or 21KD after protease treatment. This type has rarely been noted in the literature and may represent a novel strain of human prion. A further outlier type also identified is unusually stable at high levels of chemical denaturants and temperatures. Manuscripts describing these findings are under preparation.

4. Key findings and implications for the prion disease field: Our key findings are that heterogeneity in human prion diseases can be characterized by variations in the seeding of misfolding as well as altered sensitivity to proteases. Innovations in methodology such as the semi-quantitative seeding assay, and the capillary-based immunoassay we have developed have utility in identifying rare strains of human prions that can be missed by other methods. This will ensure accurate surveillance includes not only the diagnosis of all cases, but also the frequency of different strains. Chronic wasting disease (CWD), a prion disease of deer, is reaching epidemic proportions in parts of North America leading to increasing human exposure, development of assays to identify unusual strains that could result from infection are important.

5. Next steps in your work (or other work you're doing in the field, if you'd like to share it): Given the utility of our seeding assay and capillary immunoassay, we have incorporated them into the protocol

used to characterize Canadian CJD cases in support of national surveillance. In addition, we have received 30 tissue samples from VPSPr cases from a number of international CJD centres to validate the detection and characterization specificity of the capillary immunoassay. We are also determining the relationship between PRNP codon 129 genotype and the amounts of relative amounts of protease resistant prion protein in a cohort of Canadian CJD brain tissue samples.

Over the past few years we have developed a new animal model in *peromyscus* mice that are susceptible to human and deer prions. We are using this model to study the rare strains that we have identified biochemically. These include those with novel seeding and protease sensitivities. Differences in clinical signs and disease progression in these mice will be used to confirm new strains.

One further acknowledgement should go to our PhD student Jennifer Myskiw, who was partially funded by this grant. Jennifer is nearing completion of her graduate studies program and is currently writing her thesis and manuscripts. Jennifer benefited hugely from the support of the CJD Foundation and is hoping to embark on a career in science research towards finding treatments for neurodegenerative diseases.