A. **Project Title:** Systematic evaluation of the zoonotic potential of different CWD isolates.
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B. **Objectives:** (as stated in the original proposal) *The objective of this proposal is to assess, in a detailed and systematic manner, the direct and indirect (by co-existing animal species) zoonotic potential of different Chronic Wasting Disease (CWD) isolates.* While no evidence of natural CWD transmission to humans has been reported, *in vitro* and *in vivo* data suggest that zoonotic transmission of cervid prions may be possible under certain circumstances. This experimental evidence has been restricted to certain PrP polymorphic groups in both humans and cervids, neglecting the virulence/susceptibility of polymorphic variants (strains) of the agent or host. In the same line, CWD has been described to transmit to other animal species, including some used for human consumption. The ability of these new PrP<sup>Sc</sup> isolates to misfold human PrP has not been tested. In this proposal, we aim to test the efficiency of different CWD isolates (harboring different PrP polymorphisms) to misfold human prions by PMCA. Same will be done for CWD isolates passaged in other animal species including cattle, sheep and pigs.

C. **Summary of accomplishments:** We are grateful for the support that the CJD Foundation provided to start this exciting project. We are happy to report that all originally proposed objectives were successfully completed. Below, I provide a summary of the results generated for each specific aim:

**Specific Aim 1. Evaluation of the zoonotic potential of polymorphic variants of CWD prions:** For these experiments, we considered prions from a single animal species susceptible to CWD: white-tailed deer (WTD). The most common polymorphic variation in the prion protein of this animal species involves the presence of either glycine (G) or serine (S) at position 96. While animals carrying the former PrP variant are associated to increased risks for CWD, the latter is thought to provide resistance (in just a proportion of the WTD population). In this aim, we modeled the zoonotic potential of PrP<sup>96G</sup> and PrP<sup>96S</sup> CWD prions using an *in vitro* replication assay (Protein Misfolding Cyclic Amplification or PMCA). As starting materials for these experiments, we used CWD prions collected from brains of both a terminally sick PrP<sup>96GG</sup> WTD and a presymptomatic PrP<sup>96SS</sup> animal. Due to the possible instability of CWD isolates present in animals (due to inter-species or inter-polymorphic prion infections), we stabilized each class of prions by performing 15 PMCA rounds in either homologous or heterologous substrates. Estimations suggest that 15 PMCA rounds allow a complete elimination (by dilution) of the original inoculum and resulting materials correspond just to *de novo* generated infectious particles. Electrophoretic profiles (glycosylation pattern and mobilities after PK digestion) suggest that all four resulting materials corresponded to different prion strains. Different dilutions of these *in vitro* generated CWD prions were used to seed the conversion of human PrP harboring either methionine (M) or valine (V) at position 129. Our results show that only homologous prion adaptation (PrP<sup>96G</sup> → PrP<sup>96G</sup> and PrP<sup>96S</sup> → PrP<sup>96S</sup>) were able to template human PrP<sup>129M</sup>. No conversion was detected when human PrP<sup>129V</sup> was used as PMCA substrate.

**Specific Aim 2. Zoonotic potential of bovine-, porcine- and ovine- adapted CWD prions.** In light of the results obtained in SA1, this aim tested only CWD prions adapted in homologous substrate. These materials were used to template the conversion of bovine-, porcine- and ovine- PrP in a single PMCA round. Our results show that 96S CWD prions adapted in PrP<sup>96S</sup> substrate were able to template the misfolding of ovine and bovine PrP. All other PrP<sup>C</sup>/PrP<sup>Sc</sup> combinations tested were negative for prion conversion. All resulting materials generated in the previous set of experiments were used to template the misfolding of human PrP *in vitro* (both polymorphic groups at position 129). Here, we observed that ovine- and bovine- adapted 96S CWD prions were able to induce misfolding of human PrP<sup>129M</sup>. All results were negative when the human PrP<sup>129V</sup> variant was used as PMCA substrate. In order to discard that the previously described results were due to the presence of original CWD prions present in the inocula, we used ovine, bovine and porcine PMCA products generated with a higher dilution of deer-derived PrP<sup>Sc</sup>. Our results were very similar to the ones obtained in the previous experimental set, confirming our results.

D. **Key findings and implications**
- Serial *in vitro* adaptation of polymorphic CWD variants resulted in different prion strains.
• Different CWD prion strains have different zoonotic potentials as assessed by PMCA.
• PrP 96S prions (adapted in vitro in homologous substrate) were able to template the misfolding of bovine and ovine PrPc. In turn, these materials induced PrPc → PrPSc conversion of human PrP 129M.
• Overall, our results suggest that a plethora of CWD prions may exist in nature. Each isolate may have distinct potentials to misfold the normal prion protein of other animal species.

E. Next steps: The data set obtained on this project will be used to prepare a manuscript that will be submitted to an appropriate journal. This data is also being used to apply for additional finding (NIH) that will allow to expand these experiments to animal models (bioassays). These studies will provide us with better tools to analyze the effect of CWD prions in other animal species. At the same time, in vivo assays will allow us to characterize strain-specific pathological features of the different prion isolates generated in this experiment.

F. Published papers and points of interest: As mentioned, we are currently preparing a manuscript using the data discussed above. Results derived from this project have been presented (oral format) in the following instances:

• “Bases molecular de las proteopatías transmisibles: el caso de las enfermedades priónicas y de Alzheimer (Molecular bases of transmissible proteopathies: the case of prion and Alzheimer’s diseases)”. Centro de Geronciencia, Salud Mental y Metabolismo (GERO)/Universidad Mayor. Santiago, Chile. September, 2019.
• “Implications of protein misfolding strain variation in neurodegenerative diseases”. Seminar series for The Mitchell Center for Alzheimer’s Disease - The University of Texas Medical Brach at Galveston (UTMB). Galveston, Texas, USA. October, 2019.