Title: Strain-specific pathology and spread in prion organotypic slice culture assay infected with different strains of Creutzfeldt-Jakob Disease.

Objectives:

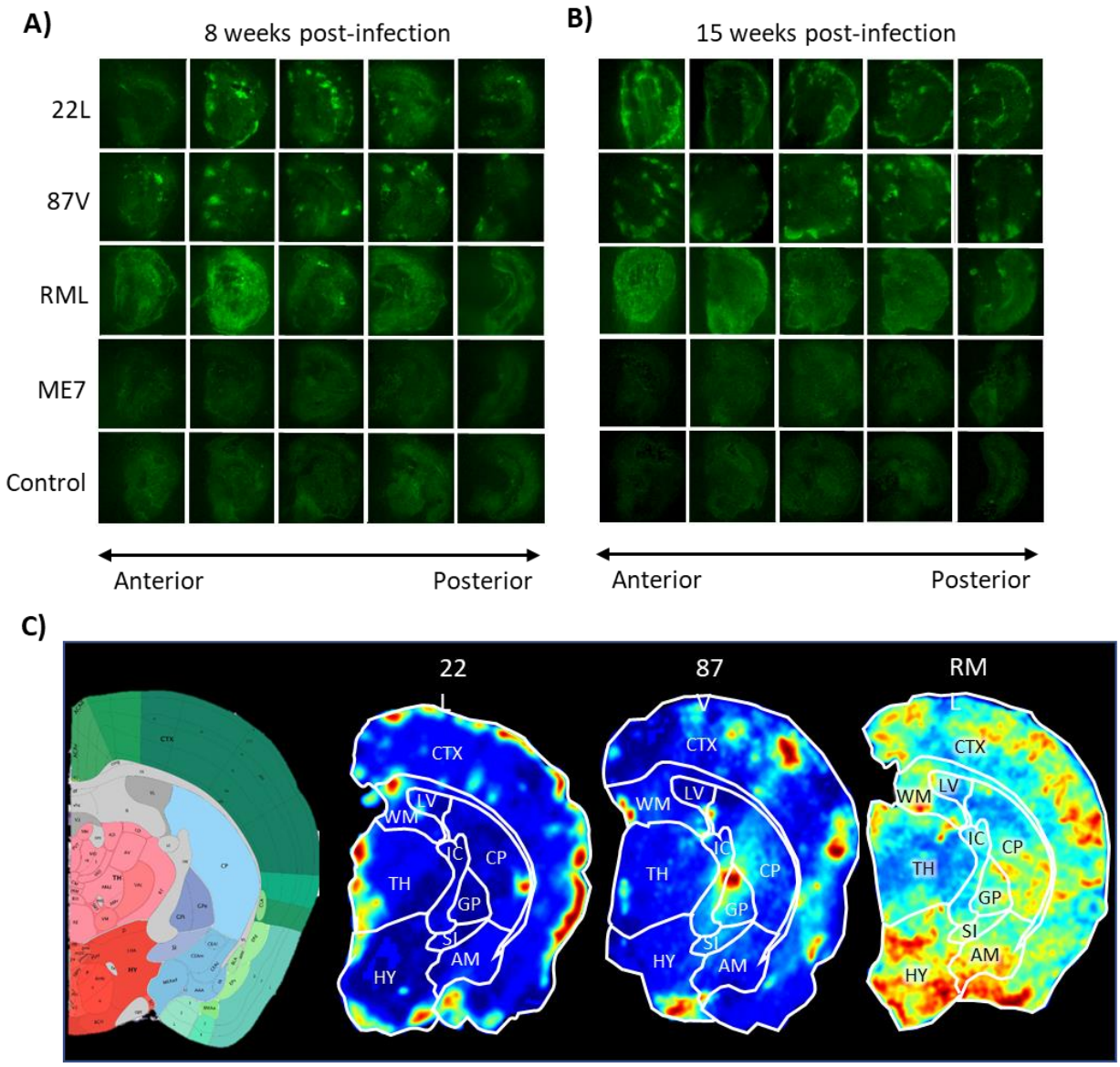
1. Propagate multiple strains of human prion disease in prion organotypic slice culture assay (POSCA), quantifying regional differences in pathology, using a combination of immunoblot, confocal microscopy and texture analysis.
2. Assess strain-differences in spread and clearance, through use of targeted wire inoculation and microglial suppression.

Summary of accomplishments to date:
We set out to infect brain slice cultures, taken from different areas of brain, with different strains of human prion disease, based on preliminary data we had showing detection of prions in brain slice cultures infected with MM1 strain human prions from sporadic CJD. We were already able to culture different brain areas, and reliably infect them with different types of prions, but we noticed some inconsistencies with human prion strains. Through many subsequent control experiments, we came to realize that what we thought were newly formed prions in infected brain slice cultures were, at least in part, actually leftover prions from the initial infection. Lowering the amount of infectious prions or adding wash steps helped us avoid this, but then we did not get consistent infection to the level that we could detect by our prion Western blotting method. For this reason, we turned to non-human prion strains to address our objectives, specifically looking at how different brain areas are affected in slice culture, both in terms of prion accumulation and neuronal loss. Our results reveal a disconnect between regions of prion accumulation and neuronal loss, in strain-specific way. These results are the basis of my MD/PhD student's defense, planned for summer, 2023. We are also preparing two manuscripts from the data.

We already know that different prion strains can accumulate in different brain areas, but it is not clear why. One possibility is that a given prion strain is only able to spread to certain areas of the brain. Another possibility is that some strains just self-propagate more easily in some brain regions. The great thing about using brain slice culture, is we can test this by exposing the entire brain slice to the same amount of prions all at once (removing the need for prions to "spread" anywhere). If the reason that a prion strain winds up in certain brain areas is because it can only "reach" some areas because of limited spreading ability, we should be able to remove that constraint by bathing the whole slice in prions. We would then predict that the brain slice would accumulate prions in all areas, regardless of strain. On the other hand, if it is the specific brain region that influences the accumulation / self-propagation of specific strains of prions, then bathing the whole slice in prions won't affect the strain differences. In fact, we observed the latter to be true (see Figures).

For Figure 1, mouse brains were sliced coronally from anterior to posterior, infected with prions and cultured for 8 or 15 weeks, then histoblots were prepared, digested with protease to remove other proteins, leaving a "fingerprint" of the pattern of prion accumulation in each slice. The accumulation of prions (PrP^{Sc}) can be seen in green in panels A and B. The pattern / area of accumulation varies with each strain, 22L, 87V, RML and ME7. For ME7 there actually is no detectable level of PrP^{Sc} (the signal is comparable to the uninfected control) suggesting that this strain may not infect this culture easily, or may not accumulate protease-resistant prions. By comparing the slice anatomy with the atlas of mouse brain anatomy, a heat map of where each prion strain accumulates in the brain culture can be created (panel C).

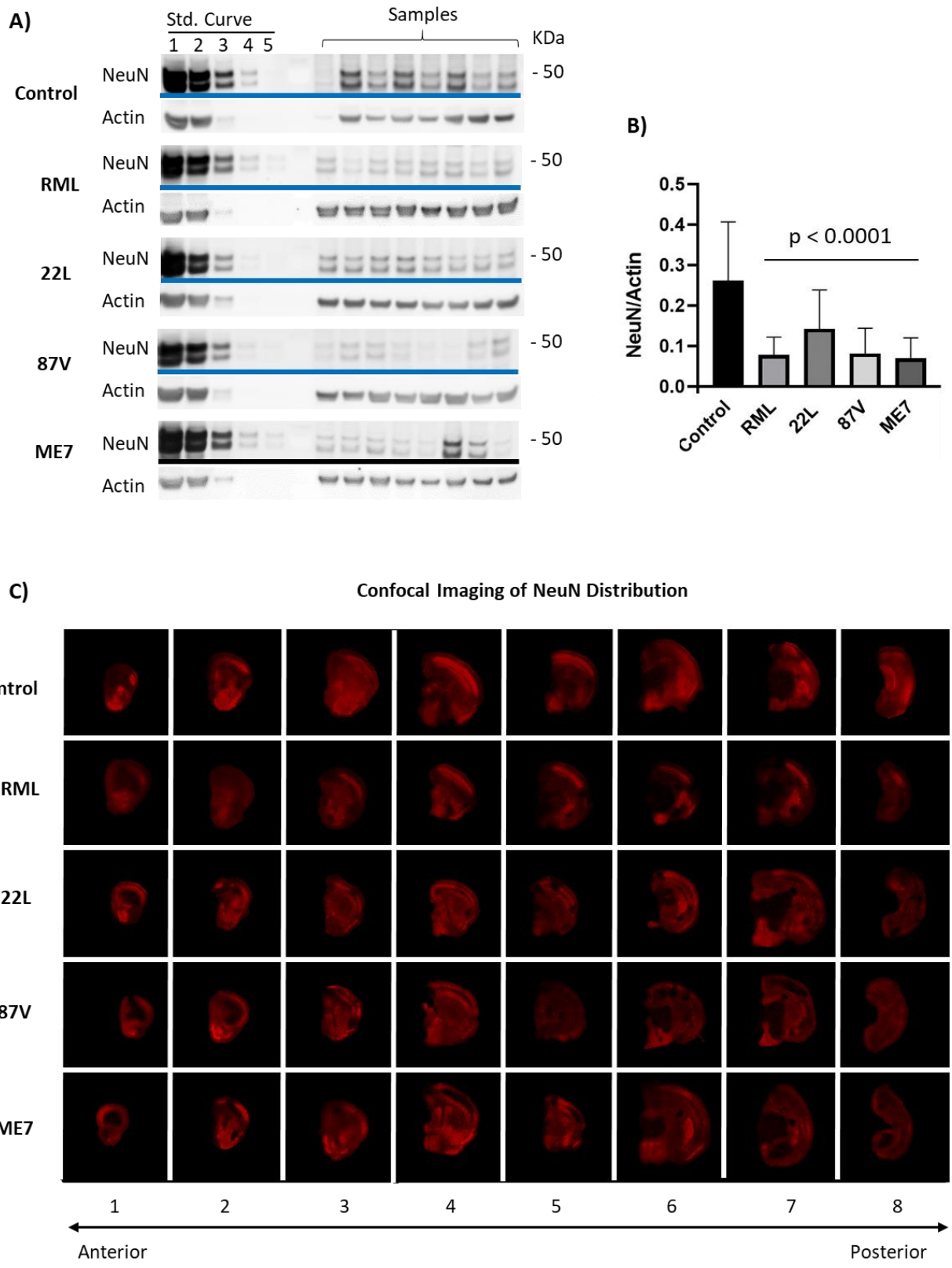
Figure 1: Histoblots show strain-specific patterns of PrP^{Sc} deposition



AM = Amygdala, CP = Caudoputamen, CTX = Cortex, GP = Globus Pallidus, HY = Hypothalamus, IC = Internal Capsule, LV = Lateral Ventricle, SI = Substantia Inominata, TH = Thalamus, WM = White Matter (various tracts)

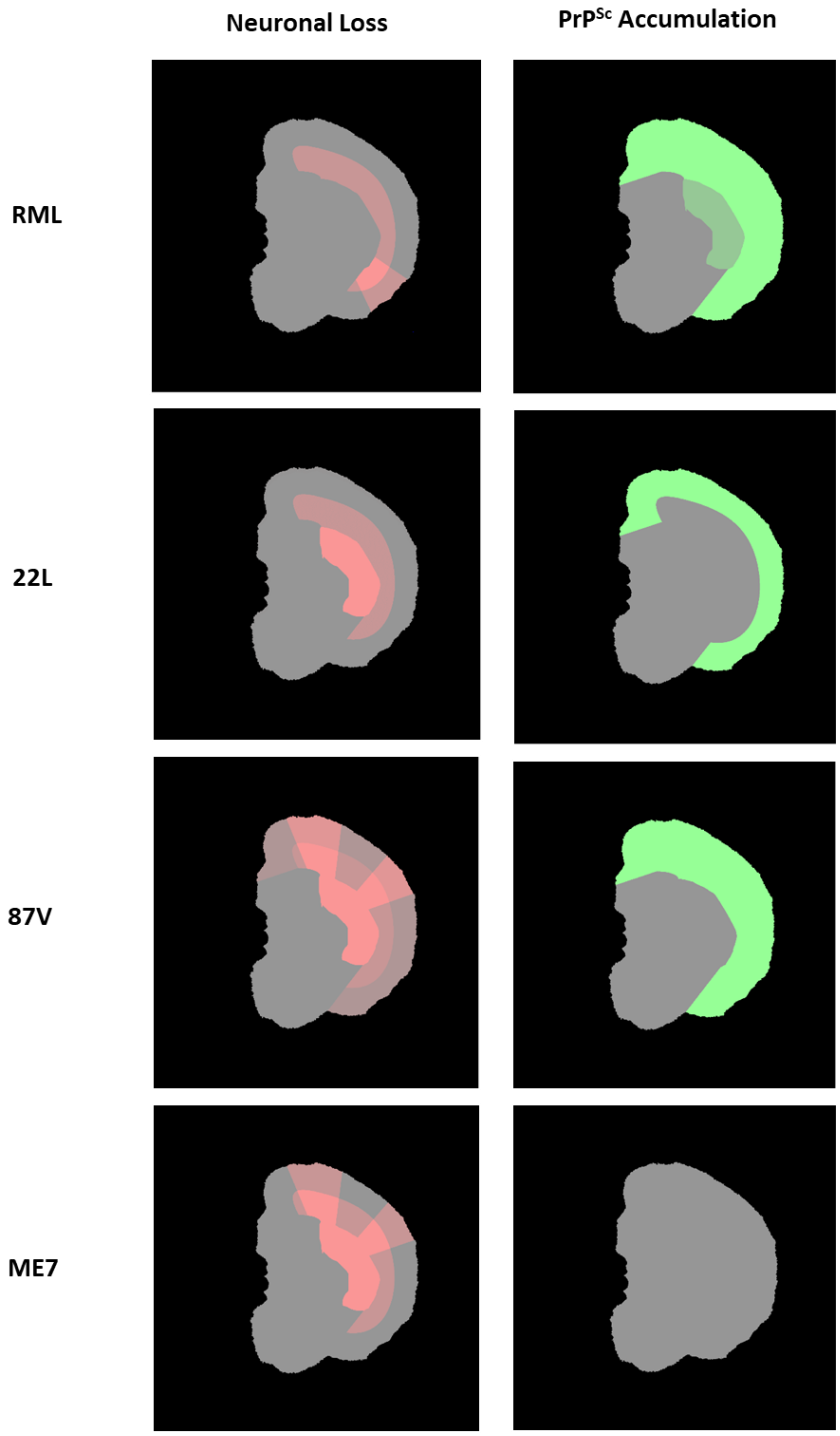
The next questions were whether death of neurons could be seen in the cultures, whether the pattern of cell death varied by strain, and whether cell death directly correlated with areas of prion accumulation. Slices were cultured and infected as before, then harvested, homogenized, and the amount of NeuN was measured by immunoblot (Fig 2A, B). NeuN is a marker of living neurons. We compared infected and uninfected cultures and discovered that there was significant loss of neurons in all infected cultures, including ME7 infection, even though we could not see the accumulation of protease-resistant prions in ME7 strain infection. Then we repeated the experiment and labelled slices with NeuN for imaging by confocal microscopy, and looked for differences in regions of neuron loss depending on which strain was used (Fig 2C).

Figure 2: Neuronal Loss in scrapie-infected whole brain slice cultures



My student then developed a computer program to compare and analyze 7 areas of brain anatomy across the slices, to quantify any differences in NeuN signal (neuronal loss) in an unbiased manner, compared to uninfected controls. What she found was striking. Each infected strain underwent loss of neurons, including ME7 despite it not producing detectable levels of protease-resistant prions. For ME7, we interpret this to mean that smaller forms of ME7 prions, which are digested by our proteases, must still be present and causing neuronal loss. Furthermore, the areas of neuron loss were different for each strain and did not directly match the areas where PrP^{Sc} accumulated (Fig 3).

Figure 3: A comparison of neuronal loss and PrP^{Sc} accumulation in scrapie-infected coronal whole brain organotypic slice cultures



Key findings and implications for the prion disease field:
We have demonstrated that whole brain slice cultures can discriminate prion strains based on region of prion accumulation and neuronal loss, and that prion accumulation and neuronal loss do not have to occur in the same region. Further, ME7 infection can cause neuronal loss without protease-resistant prion accumulation, highlighting the importance of looking at smaller protease-resistant oligomers as important in causing neuronal loss, perhaps more than where the large prion aggregates build up. This has implications for treatment targets, as targeting the smaller, less resistant oligomers, may be more feasible and be more likely to affect neuronal death.

Next steps:
We are now analyzing our brain slice culture system with attention to microglia, which is a cell type that may be able to clear prions from the brain. We can identify microglia in our cultures, and they appear to respond differently to different prion strains in culture. We are also culturing microglial cells alone, and testing how they respond to different prion strains. We are looking at how they may become activated to ingest certain prions, and are also asking whether the size of the prion may influence their ability to do so. For example, in our slice cultures, the 87V infection produces large aggregates in culture, and is associated with more microglia activation, compared to 22L infection which produces small aggregates. Using another technique in our lab, asymmetric flow field-flow fractionation, we are separating different sized prion particles from human prion disease brains, and will test whether the strain and / or size of human prion particle influences how the microglia respond.