**Project title:** Examining copy number variants as a possible phenotype modifier in prion disease**.**

**Project objectives:**

1) Are there unrecognized genetic factors that influence age of disease onset in Creutzfeldt - Jakob disease sporadic forms sCJD and VV1?
2) What genetic determinants influence these differences in phenotype and age of onset and penetrance in genetic prion diseases?

**Summary of accomplishments to date:** Genome wide DNA single nucleotide polymorphism (SNP) arrays was performed on frontal cortex, occipital cortex and cerebellum tissue with age and sex matched controls. Of the 44 cases, blood sample was available in seven cases. Out of the 44 cases, 33 were NORMAL (absence of rare copy number variants). The remaining 11 cases were ABNORMAL and showed a gain of the X chromosome in genetic prion disease patients. The sporadic CJD cases, sFI (n=five, all males) and VV1 (n=eight, two females and six males) did not show a gain of the X chromosome. The negative of non-prion disease cases (n=10, three females and seven males) did not show again of the X chromosome.
In the genetic prion disease cohort;

- Genetic CJD, E200K cases (n=11, 4 females and 7 males), 3/11 abnormal
- Fatal Familial Insomnia (FFI) (n=5, 1 female and 4 males), 4/5 abnormal
- Gerstmann-Straussler Scheinker (GSS) (n=5, 2 females and 3 males), 4/5 abnormal

The 11/44 abnormal cases were all from the genetic prion disease cohort and included the following:

**1)** Of the three E200K- 129 MM, two females and one male showed a gain of the X chromosome
**2)** Of the four GSS cases, one female and three males showed a gain of the X chromosome
**3)** Of the four FFI cases, one female and three males showed a gain of the X chromosome

**Key findings and implications for the prion disease field:**
Gain of the X chromosome was limited to the genetic subtypes and not seen in sporadic CJD or non-prion disease cases. In the FFI and GSS cohort, a gain of the X chromosome was seen in 75% of the males respectively. In the E200K patients, it was predominantly seen in females (66%). The age of onset was earlier in these diseases as compared to normal males and normal females in the each genetic cohort.

Gain of X chromosome can be an age-related phenomenon in females. Loss of the Y chromosome can be an age-related process in men, yet no loss of Y was seen in males in this cohort. Gain of the X chromosome could increase expression of X-linked genes in both sexes, while the over expression of X-linked genes may cause early disease onset.

**Next steps in your work (or other work you're doing in the field, if you'd like to share it)**

The percentage of gain of the X chromosome between the different regions of the brain varied. Fluorescence in situ hybridization (FISH) test will be performed to evaluate the proportion of gain of X chromosomes in different regions of the brain using a centromeric probe for chromosome X and chromosome Y. The next phase of this research includes whole genome sequencing using third generation sequencing methods (Nanopore technology) to explore pathogenic variants in the genome. A targeted next generation sequencing approach will be utilized to identify mutations (aka pathogenic variants) in the genes on the X chromosome. Proteins encoded by genes identified by sequencing methods will be assessed for reduced or overexpression using immunohistochemistry and Western blot analyses and correlated with patient outcome data.