Project title: Elucidating the factors required for propagating human CJD prions.

Project objectives:

The overall aim of the project is to identify the cellular factors that are required in addition to the cellular prion protein for propagating human Creutzfeldt-Jakob disease (CJD) prions. This will be done by identifying changes in the genome and gene expression that are associated with the increase in susceptibility to both variant (v) and sporadic (s) CJD prions in the unique linear cell models that we have developed. Validation of the most promising candidates by over-expression or silencing expression will enable us to determine if they have a causative role in prion propagation and thereby identify direct targets for blocking it.

Summary of accomplishments to date:

To confirm that the cells susceptible to vCJD are propagating bona fide prion infectivity, wild type FVB/N and Tg (HuPrP129M$^{+/+}$*Prnp*$^{0/0}$)-35 (Tg35) mice that overexpress by 2-fold the human prion protein with methionine at amino acid 129, were inoculated with cell homogenates prepared from CAD5-PrP$^{-/-}$HuPrP M129-1D12 cells that had been challenged with T4 MM vCJD infected human brain homogenates and serially passaged to dilute out the inoculum. A proportion (attack rate: 3/20 and 9/20) FVB/N mice inoculated with cell passaged material succumbed to clinical disease. In contrast, almost all (attack rate: 17/20 and 17/18) Tg35 mice inoculated with cell passaged material succumbed to clinical disease. Histological analysis of clinically infected end stage terminally sick FVB/N and Tg35 mice showed that abnormal deposition of PrP was identical between cell passaged material and the prions present in the patient infected brain homogenates. Moreover, florid plaques were detected in the cortex of Tg35 mice inoculated with cell passaged material further confirming that these cells are propagating *bona fide* T4 MM vCJD prions.

To identify the cellular factors that are required in addition to the cellular prion protein for efficient propagation of human CJD prions, we will use the linear cell models of increasing susceptibility to vCJD and sCJD that we have developed - shown below.

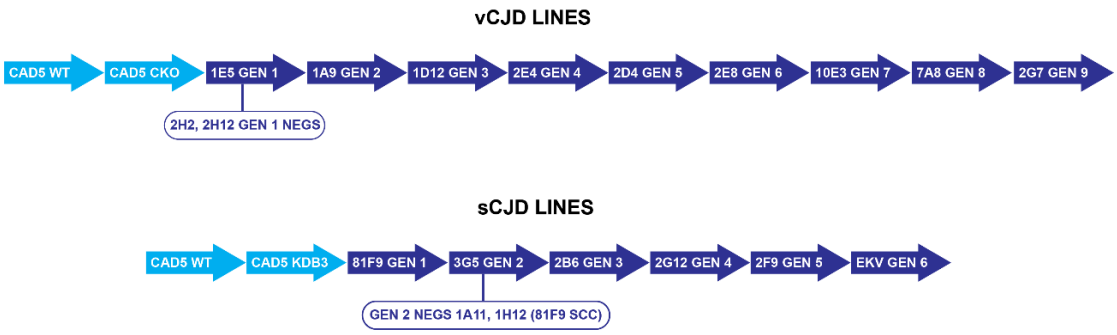

Figure: Development of cell lines with increasing susceptibility to human prions.

To identify changes at the genomic level we have extracted genomic DNA from all of the susceptible vCJD and sCJD cell lines. Genomic DNA was also extracted from the parental CAD5 cells as well as the CAD5 cells in which the endogenous mouse *Prnp* gene had been silenced by RNA interference (CAD5-KDB3) or CRISPR/Cas9 gene editing (CAD5-PrP$^{-/-}$, CAD5-CKO). We also extracted genomic DNA from two control cell lines each for vCJD and sCJD from the initial reconstitution or rounds of single cell cloning that did not exhibit an increase in susceptibility. A total of 22 genomic DNAs have been sequenced (>30x depth) using the most cost-effective technology provided by UCL Genomics facility, presently Illumina's short-insert paired-end sequencing by synthesis. Data analysis is now underway to determine if increases in prion susceptibility correlate with DNA mutation or changes in copy number particular loci.

To identify processive changes in gene expression that correlate with increasing prion susceptibility, total RNA has been extracted from the 22 cell lines in quadruplicate and RNA-seq undertaken by the UCL Genomics facility. RNA-seq has been carried out in quadruplicate to ensure the data is highly reproducible. Initial pipeline analysis of the RNA-seq data is highly encouraging. The principle analysis plot of the RNA seq- data for the vCJD and sCJD cell lines shows that both cell models show processive changes in RNA expression as the cells become more susceptible.

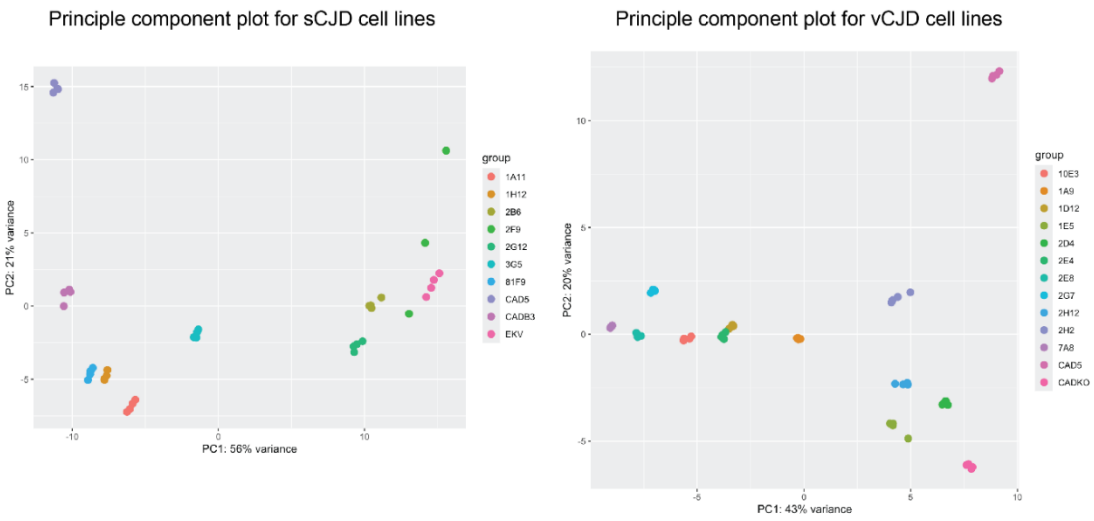

Figure: PCA plots for vCJD and sCJD

Hierarchical clustering of the RNA-seq data presented as dendrograms indicate that the first generation of susceptible cells cluster closely with the parental CAD5 and CAD5 cells in which *Prnp* has been silenced or edited. The control cells also cluster with these cell lines. As the cells become more susceptible, they form separate clusters.

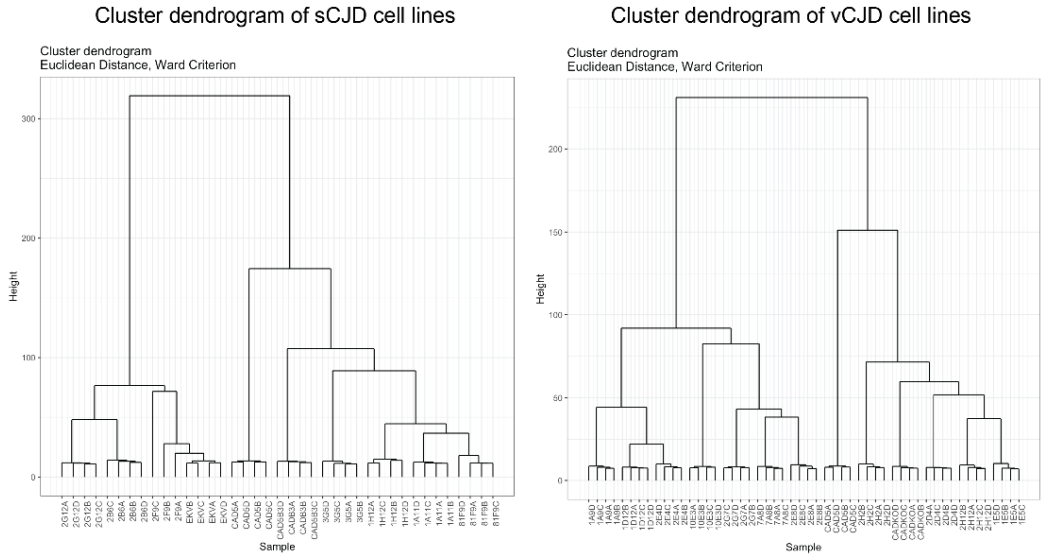

Figure: Cluster dendrograms

Next steps in the work:

Bioinformatics analysis of these data sets is now underway to identify changes in gene expression that correlate most strongly with increases in susceptibility to CJD prions. One approach will be to model increases in cell susceptibility as a quantitative trait and analyze parameters by linear regression with covariates. Another approach can be to treat the increasing susceptibility as a time course. These are underway. These changes should not be observed in the negative control cell lines that do not have altered susceptibility, but increase processively as the cells become more susceptible. This analysis will be undertaken in parallel for the vCJD and sCJD cell lines, and combinatorially.

Our aim is to identify the shared changes in the genome and/or gene expression, genes or pathways/gene sets between mice and human that are the most promising candidates for determining if they play a causative role in increasing prion propagation.

Key findings and implications for the prion field

- Developed cells that stably and reproducibly propagate T4 MM and MV vCJD prions but not sCJD prions.
- Developed cells that can stably propagate T3 MV and VV sCJD prions but not T4 MM vCJD prions.
- These cells propagate *bona fide* vCJD and sCJD prions as demonstrated by mouse bioassay.
- Developed human prion assays for T4 MM and MV vCJD and T3 VV and MV sCJD.
- Developed cells that are chronically infected with sCJD - retain infectivity upon freeze/ thaw.
- Developed cells that are chronically infected with vCJD - retain infectivity upon freeze/ thaw.
- Cells chronically infected with vCJD and sCJD can be cured of infectivity upon treatment with the anti-PrP^{C} monoclonal antibody ICSM18.