Profiling serum derived exosome-associated miRNA as a diagnostic tool for prion disease

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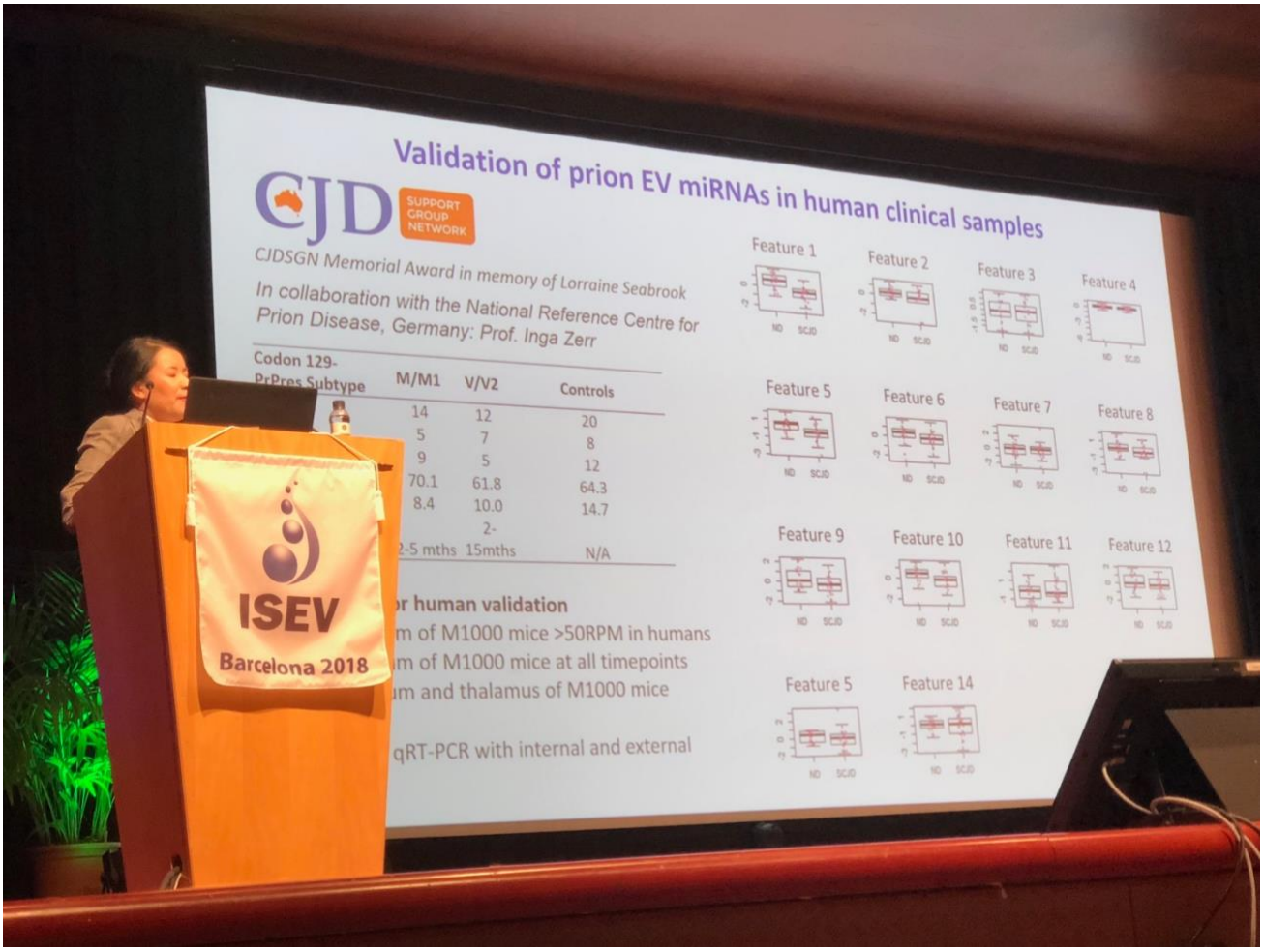

Principle Investigators: Lesley Cheng, Andy Hill (La Trobe University, Australia)
Collaborators: Inga Zerr (Germany)

Background: The detection of infectious prions in the blood has been difficult to reproduce in the field most likely due to sensitivity issues with current technology. Our hypothesis for this project is that sensitivity and detection could be improved by isolating exosomes from blood to enrich for a diagnostic indicator associated with prion disease. Exosomes are extracellular membrane vesicles which are secreted by cells and are packaged with very specific cargo including defined panels of protein and RNA, in particular, miRNA. miRNA are a small non-coding RNA species involved in gene regulation. Currently, there are more than 2500 known miRNA species in humans and approximately 1500 miRNA species are found in exosomes. Upon secretion, exosomes can circulate in the body where they can be isolated from bodily fluids such as blood. We have demonstrated that serum exosomes are enriched with miRNA making them a useful source of disease indicators. Furthermore, exosomes could be harbouring pathological prions and associated miRNA species.

Previous work in the Hill laboratory identified a prion associated exosomal miRNA panel of indicators using a Prion mice model. This mice model involves intracerebrally injecting mice with M1000 prions or normal brain homogenate (NBH) which we use to understand various stages representing pre-clinical and clinical stages of prion disease. We found 20 miRNA species in M1000 infected mice which changed in levels throughout the pre-clinical and clinical phases of this mice model. Using these indicators, our aim was to observe whether they could be used to predict the diagnosis of human CJD cases. Our collaborator from the National Reference Centre for Prion Disease, Germany, Prof. Inga Zerr provided the Hill Laboratory with

exosomal RNA isolated from CJD patients genotyped with M/M1 and V/V2 subtypes including control samples.

Aims: There are 3 main objectives for this project:

1) Process human clinical samples with CJD and controls for exosomal RNA analysis
2) Detect the 20 prion associated miRNA species in the clinical samples
3) Perform statistical analysis and develop an algorithm to classify patients

Status of project: This project commenced in September, 2017 with the team in Germany processing the serum samples (CJD, n = 16 and controls, n = 20) and isolating the exosomal RNA. These samples were then sent to the Hill Laboratory in Melbourne. Upon receipt, the samples were checked for quality and the 20 prion associated miRNAs were used to screen both the CJD and control sample groups. The raw data was analysed, samples unblinded from the Germany group and results were provided to the Statistical Platform, Department of Mathematics, LTU, to develop a predictive model. The current predictive model provide 70% accuracy using the 20 miRNA indicators to predict for CJD using blood. This completes the 3 objectives for the project. This is the first time that anyone has examined exosome associated RNA's for prion disease in human blood samples.

While the project is largely completed, we wish to increase sample size and observe whether exosomal miRNA can be used to predict CJD cases with the future in validating the prion blood indicators in high risk patients. This was at the suggestion of the statistician who said we need to screen more clinical samples to increase the power of the study. This will form an extension to this project.

Other Outcomes: Dr Lesley Cheng presented this work and acknowledged the CJDSGN Memorial Award in memory of Lorraine Seabrook at the International Society for Extracellular Vesicles Conference in Barcelona (2-6 May). Her talk was awarded a 'Featured Abstract' presentation as it was judged to be of the highest quality.