Final Report: 2016 Brain Foundation Research Gift

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Date: 14/12/18

***Title of Project**:* Identifying the critical neuronal signatures of epigenetic modifier complexes of Alzheimer's disease initiation and progression

***Summary**:*
Aging constitutes a time-dependent decline in cellular integrity and function leading to impairments and failures in bodily systems. As we age the incidence of cancers and neurodegenerative disorders including Alzheimer's disease (AD), both increase. A focus on characterising epigenetic aberrations in cancers has resulted in substantial advances in understanding their aetiology and new treatments. By contrast, the impact of AD on our health system is only now coming to the fore and relatively little is known about the role of epigenetic drivers of AD initiation and progression. The genome is exquisitely controlled by epigenetic mechanisms (e.g. DNA methylation and histone modifications) that determine transcriptional output of cells and underpin normal cellular processes such as learning and memory. Indeed, the importance of proper epigenetic programming has been well established in aging and cancers (CI Taberlay). Existing knowledge of epigenetic changes in AD is extremely limited, lacks cell type specific analysis and "genome-wide" assessments are incomplete.

This project aimed to identify the epigenetic alterations that occur in nerve cells in early- and late-stage sporadic Alzheimer's disease (AD) compared to healthy aging. We have requested and obtained fresh frozen human brain tissue (inferior temporal gyrus) from the Banner Sun Health Tissue Bank (California, USA) with ABC pathological staging of not/low (n=10), intermediate (n=10) and high (n=10) AD pathological change. The advantage of sourcing our tissue from Banner Sun Health is the low post-mortem intervals (all <5.3 hours) and extensive medical history of the cases.

The progress on this project has slowed due to CIA Taberlay returning from full-time maternity leave in September 2018, and CIB Woodhouse being on part time maternity leave (0.4 FTE) for all of 2018. We have recruited a PhD student (Thalia Perez Saurez) in mid-2018 to work full time on this project. While the timing of our project has altered, this will not impact on the expected outcomes and we will still be aiming to submit our original research publications on these data between August-October 2019. Notably this work will still be the first ChIP-seq data from purified human neurons in healthy aging and AD cases.

The novelty of this grant, both nationally and internationally, hinged on the separation of a pure population of neuronal nuclei from human brain. We have successfully optimised this protocol in mouse brain; however, so that we remain at the forefront of the field (which is rapidly developing), we are also optimizing a fluorescent activated cell sorting protocol to purify the neuronal nuclei from only

excitatory neurons (excitatory neurons are the vulnerable neurons that degenerate and die in AD) in our human brain samples. Optimizing this FACS protocol to purify excitatory neurons from human brain with improve the design of our study and lead to the production of an incredibly valuable dataset in the field. We have performed immunohistochemistry to determine the plaque and neurofibrillary tangle load in the ITG (in fixed tissue sections) from the same set of cases that will be used for the ChIP-Seq experiments so that we can cross-correlate pathology load with the ChIP-Seq datasets. In 2018 we have also invested a large amount of time optimizing a bespoke bioinformatics pipeline to analyse biological ChIP-Seq data from purified neurons. This is not a trivial task, as current bioinformatics pipelines for ChIP-Seq data are optimised for data obtained from cell lines with very limited variability in the data. This bioinformatics pipeline is now optimised and ready for use with human neuronal ChIP-Seq data.

We anticipate that sample processing will begin (via FACS) in February 2019, ChIP-Seq experiments will be performed in April 2019 and sequencing data will be obtained for analysis in June 2019. Manuscript/s describing the data will be submitted in August-October 2019.

Finally, we would like to highlight that this grant along with a Judith Jane Mason and Harold Stannett Williams Memorial Foundation National Medical Program grant has enabled us to secure an NHMRC project grant to commence in 2019 (APP1161768; “Delineating the epigenetic evolution of neurons in human sporadic Alzheimer’s disease.“) to extend our work investigating epigenetic alterations in neurons in human sporadic AD and healthy aging.

Hypothesis vs Findings

Hypothesis: The neuronal epigenome is reprogrammed early in sporadic AD, driving continued epigenetic evolution that leads to neuron dysfunction and degeneration in late sporadic AD cases.

Findings: As detailed above the progress on this project has been delayed due to both CIA and CIB taking maternity leave in 2018. While we haven’t yet completed the human studies, we have detected dramatic epigenetic alterations (H3K27ac and H3K4me3 ChIP-Seq data) in neurons purified from an AD mouse model very early (at 3 months of age) which show evolution across disease progression (up to 12 months of age). These data strongly support our hypothesis and will be used to support the human study supported by the Brain Foundation that is in progress.

Unanswered Questions

As detailed above the progress on this project has been delayed due to both CIA and CIB taking maternity leave in 2018. Thus, the unanswered questions relate to the Aims of our project and are:

1) What are the patterns of epigenetic modifying proteins that distinguish neurons subject to normal aging from those in sporadic AD brains?
2) Does the epigenetic signature of neurons evolve across the course of disease progression in sporadic AD? If so, which epigenetic changes in neurons change in tandem with disease progression?

What these research outcomes mean

The data generated by this project will identify critical epigenetic events that distinguish neurons in sporadic AD from those in healthy aged cases. Importantly we will detect the epigenetic alterations that are modified in tandem with pathology load, and whether the epigenetic landscape in neurons evolves across disease progression. Of particular interest is determining the epigenetic hallmarks of neurons in early sporadic AD that may drive neuronal dysfunction and degeneration.

Thus, the outcomes of our study will be at least two-fold; providing information on epigenetic dysregulation in neurons as well as predicting roles for many novel genes and pathways in sporadic AD. In the long term this will provide the opportunity to therapeutically target the epigenome or pathways of neuronal dysfunction early in sporadic AD. Obtaining this new information is a major outcome in its own right and represents a critical first step in understanding the complexity of epigenetic reprogramming of neurons in sporadic AD. Completion of this work will provide an ideal platform for future work to validate the function of novel epigenetic pathways and genes in vivo, and then demonstrate causation.