**Final Report**

Author: Dr Zac Chatterton

Qualification: PhD

Institution: The University of Sydney

Date: 04/11/2019

Title of Project: Peripheral Monitoring of Neurodegeneration in Frontotemporal Dementia using Cell-Free DNA methylation.

Summary: Frontotemporal Dementia (FTD) is a common early-onset neurodegenerative disorder with incidence of 3.5 per 100,000 in adults aged 45 to 64 years (Mercy et al, Neurology, 2008) however due to the early onset, FTD patients have the greatest loss of remaining life of all dementias (Brodaty et al, Int Psychogeriatr, 2012). FTD patients are subtyped into behavioral-variant frontotemporal dementia (bvFTD), that is clinically defined by changes in personality and behaviour (Johnson et al, Arch Neurol, 2005) and two forms of primary progressive aphasia (PPA) - Semantic dementia (SD) and Progressive non-fluent aphasia (PNFA). For accurate diagnosis of bvFTD, other medical conditions such as substance abuse and non-neurodegenerative neuropsychiatric diseases have to be excluded along with the presence of several behavioural symptoms and localized neurodegeneration in susceptible brain regions (frontal and/or temporal lobe). Notably the earliest tissue damage observed in bvFTD patients is found within the medial frontal and orbitofrontal regions, however MRI may appear normal in these early stages of disease (Perry et al, Dement Geriatr Cogn Disord, 2006). Language disturbances observed in PPA are correlated with neurodegeneration of the language-dominant hemisphere. The clinical assessment of a patient language issues are used to diagnose and differentiate PPA, however structural imaging (i.e. CT-scans) are typically performed to rule out cerebrovascular disease and brain tumours that can cause the same language issues. There are many conditions that require consideration in the diagnosis and differentiation of FTD (Arvanitakis et al, Neurologist, 2010). Behavioural symptoms represent the earliest indicators of FTD in which patients exhibit neuroanatomic-behaviour correlations. A cost effective, minimally invasive biomarker of the neurodegenerative process would enable diagnosis of FTD, FTD subtyping and circumvent the need to exclude other medical causes of behavioural symptoms while offer monitoring of disease progression in real-time.

Cell-free DNA (cfDNA) derived from brain tissue has been identified within the peripheral bloods of patients diagnosed with neurological diseases including glioblastoma (Salkeni et al, J Neurooncol, 2013), traumatic brain injury (Lehmann-Werman et al, Proc Natl Acad Sci, 2016) and multiple sclerosis, revealing the presence of an amplifiable (PCR) signal (DNA) of neurological cell death within the bloodstream. By characterizing DNA methylation profiles of the human brain (Lister et al, Science, 2013) and comparing these to DNA methylation profiles of blood and several other tissue types we have created a targeted epigenetic sequencing platform capable of rare detection of brain-derived cfDNA within blood. With support from the prestigious Brain Foundation Awards, we aimed to investigate the utility of cfDNA within FTD by;

1. Characterising DNA methylation of brain cells commonly affected by FTD (frontal and temporal lobe neurons) within post-mortem human brain tissue from healthy control.
2. Profiling brain-derived cfDNA within FTD, Motor Neurone Disease (MND) and Healthy Controls (HC), and
3. Develop new brain-region specific epigenetic sequencing assays that can be used for cfDNA analysis to identify the brain-region undergoing atrophy.

Key outcomes of the study

1. We have extended scope of experiments to characterise DNA methylation brain cells (bulk neurons) in light of new collaborations and technology advancement’s. Specifically, we have extended the analysis 2 brain regions susceptible to neurodegeneration in FTD from 2 patients though to12 rare human post-mortem healthy control patient samples from 13 brain regions susceptible to neurodegeneration (extended to include cerebellum, motor cortex, hippocampus etc.) in collaboration with the National Institute of Health (NIH, USA). Furthermore, we have now developed technology to profile DNA methylation of every single-cell as opposed to bulk neurons in a collaboration with the Recombinant Products Facility of the University of New South Wales. The ability of single-cell techniques to resolve cell-subtype DNA methylation expands DNA methylation characterisation from bulk neurons to neuron subtypes. These studies are currently ongoing and we hope to generate the worlds-first single-cell DNA methylation profiles of the human frontal and temporal lobes, motor cortex and cerebellum in late 2019/ early 2020.
2. To investigate the utility of brain-derived cfDNA within FTD/ MND we extracted cfDNA from 99 blood plasma samples from 70 FTD/MND patients and 29 Healthy Controls (HC). The cfDNA was bisulfite treated and analysed using our targeted epigenetic sequencing platform to investigate the presence of brain-derived cfDNA. We identified the presence of Dorsolateral Prefrontal Cortex (DLPFC) Neuron cfDNA within ~13% of FTD/MND patients and Healthy Controls at levels of ~1:10,000 molecules. However, we did not find any difference in the amount of brain-derived cfDNA between FTD/MND patients and controls. We performed intraindividual longitudinal structural imaging (MRI) within the same individuals and measured the amount of DLPFC neurodegeneration of specific brain regions. Notably, we observed some loss of the DLPFC in several HC. Importantly, only individuals (FTD/MND or HC) with loss of the DLPFC reported the presence of DLPFC Neuron cfDNA within their plasma (P-value=0.035) validating the presence of brain-derived cfDNA within the blood plasma of individuals.
3. To extend cfDNA to the detection of brain-region specific cfDNA we were able to identify locations in the genome with DNA methylation specific to Cerebellum, DLPFC, Hippocampus and Ventral White Matter. We designed and validated targeted epigenetic sequencing assays for these 4 brain regions which extends from our DLPFC neuron assays. Furthermore, we generated sequencing results from the cfDNA from the 99 patient samples (above) and are currently performing bioinformatic data analysis to determine the presence of brain-region specific cfDNA within these patient samples.

*Hypothesis vs Findings*

We hypothesised that DNA (cfDNA) from the brain would be detectable within the blood (plasma) of patients suffering neurodegeneration (Frontotemporal Dementia). We identified the presence of brain-derived cfDNA in both FTD/MND patients and, unexpectedly, in healthy controls. However, the presence of brain-derived cfDNA was associated with brain imaging results. This indicates that brain-derived cfDNA is a sensitive measure of neurodegeneration.

Unanswered Questions

A key unanswered question is how many locations in the genome have DNA methylation that can distinguish brain-cell types and brain-cells from brain-regions? We hope to gain insight into this question when we analyse the single-cell DNA methylation data from distinct brain-regions in 2020. Another key unanswered question is can epigenetic assays with brain-region specificity detect brain-derived cfDNA from brain regions affected during the disease course i.e. motor cortex in MND? We hope to gain insight into this question when we compare structural imaging (MRI) results to our brain-region specific epigenetic assays applied to cfDNA within the FTD/MND and HC cohort.

What these research outcomes mean

The research outcome for this project is the identification of a new class of brain-derived molecule (brain-derived cfDNA) within the blood plasma of patients that have identifiable neurodegeneration by MRI. Future studies will expand on the utility of these biomolecules to diagnose and monitor disease in FTD, MND and virtually any neurodegenerative disease.