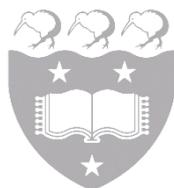


UNIVERSITY OF AUCKLAND

INVESTIGATING ALZHEIMER'S DISEASE IN THE HUMAN BRAIN



THE FREEMASONS FOUNDATION
RESEARCH FELLOW IMPACT REPORT
September 2016



Thank You

We would whole-heartedly like to thank the Freemasons Foundation for your ongoing support towards The Centre for Brain Research and the progression of the Alzheimer's Disease project. Your generosity allows early career researchers, such as post-doctoral research fellows, to undertake cutting-edge scientific research within New Zealand, for New Zealanders. With Alzheimer's Disease becoming a significant nation-wide health issue, this research, utilizing precious tissue samples from our New Zealand Human Brain Bank, is critical for finding new potential therapeutic targets to treat this condition. On behalf of the University of Auckland, and members of the Centre for Brain Research, please accept our sincere gratitude for your highly appreciated support.

Financial Input

In April 2015, the Freemasons Foundation announced an award of \$308,000 over the next four years to fund one of our top postdoctoral research fellows, who is highly qualified in human brain neurochemical studies (Malvinder Singh-Bains) to advance the innovative research on Alzheimer's Disease at the University's Centre for Brain Research (CBR).

The postdoctoral research fellowship was awarded to Dr Malvinder Singh-Bains in the CBR, under the supervision of two highly acclaimed world leaders in Alzheimer's research, Distinguished Professor Richard Faull and Professor Mike Dragunow.

Summary of Outcomes and Impact

Summary of outcomes

Dr Singh-Bains is using a unique method to study human brain tissue from Alzheimer's Disease donor brains, which only requires a 2 millimetre sample! She is able to utilise this method, called a tissue microarray, to create small sample sets of brain tissue embedded into a wax block so she can study them in one experiment. Her first sample set contained 25 Alzheimer's and 25 control brain samples from the cerebellum (a region traditionally considered to be unaffected in Alzheimer's Disease). Strikingly, her assessment of disease indicators, called "biomarkers" in her cerebellum samples have revealed that some of the brain's immune cells, termed

microglia, look different in shape and are more prevalent in the Alzheimer's samples. There are also increases in certain proteins present on brain cells called astrocytes, which are known to be cells that support our brains. She has also found subtle changes in proteins located in and around blood vessels in the Alzheimer's cerebellum samples. These results point to an interesting pattern emerging, suggesting early blood vessel and immune cell changes occur without the overt loss of neurons (main brain cells) in the cerebellum in Alzheimer's Disease. **Could these be the earliest changes occurring in the brain in Alzheimer's Disease?** It is amazing that 2 millimetres of tissue can provide this much information about sensitive protein changes. However, Dr Singh-Bains is now working to validate these changes using larger sections of human cerebellum. Upon the completion of these validation studies, she will be preparing a publication on the usage of tissue microarrays to study the cerebellum in the Alzheimer's Disease human brain.

Dr Singh-Bains's studies have expanded to other areas of the human brain using the tissue microarray method, starting with the middle temporal gyrus, an area more severely affected by Alzheimer's Disease. It is envisaged that her studies will expand to other regions of the cerebral cortex and associated structures which we know are affected in this tragic disease.

Summary of Impact

In many diseases, including Alzheimer's, Parkinson's and Huntington's Disease, administering treatments after brain cells have died would limit the success of the treatment. New therapies to slow down the death of brain cells in Alzheimer's Disease require an understanding of how the disease affects the whole brain. Currently, treatments available for Alzheimer's patients in New Zealand include medications such as Aricept, Exelon and Reminyl, which are only modestly successful in some patients for improving memory and attention skills. However, these medications can only manage the symptoms of dementia by helping the dying brain cells function.

At the CBR, we have the unique opportunity to study human tissue samples generously bequeathed from Alzheimer's Disease patients. Dr Malvinder Singh-Bains is the Freemasons Foundation Postdoctoral Research Fellow who is working to identify the unique features which occur in Alzheimer's brain tissue before the main brain cells (neurons) die. Her continued work to assess the disease patterns throughout various brain regions in Alzheimer's Disease is critical to understanding the mechanisms through which Alzheimer's Disease operates, which will thereby assist in identifying drug targets for treatment.

Research Activity

(Since last report March 2016)

The project involves the usage of a highly specialised tissue microarray machine (a sophisticated machine which systematically arranges small 2 millimetre pieces of brain tissue into a very compact area), which was also generously donated by the Freemasons Foundation. This highly specialised instrument is capable of arranging tissue samples from up to 60 human brains on a single array (an ordered arrangement). This is a highly tissue conserving technique, which allows us to screen for a wide range of proteins or other neurochemicals that could be drug targets for neurodegenerative diseases, including Alzheimer's Disease.

The human **cerebellum**, an area with the highest number of brain cells for its size, is traditionally thought to be spared in Alzheimer's Disease because it has been shown to lack one of the disease-causing proteins specific to Alzheimer's Disease (beta-amyloid), with literature reporting limited cell loss in the disease. However, no studies have assessed other disease indicators of Alzheimer's Disease in this relatively uncharted territory of the brain. Therefore, the first tissue microarray which Malvindar has constructed and focused on analysing utilises human cerebellar tissue from the Neurological Foundation Human Brain Bank. The microarray contains 25 Alzheimer's and 25 control brain samples.

Malvindar's previous report in March detailed the unexpected differences between her control and Alzheimer's cerebellum samples based on her analysis of her first tissue microarray. She summarised these changes in **Table 1 of her previous report (see appendix)**. She had identified that in the Alzheimer's samples, there are changes in the number and appearance of brain cells termed microglia, which are known as the immune cells of the brain; and astrocytes, which are multifunctional cells with roles in support and control of neuronal (main brain cell) communication. She also found changes in the blood vessels within the cerebellum in Alzheimer's Disease. **These results point to an interesting pattern emerging, suggesting early vascular (blood vessel) and non-neuronal cell changes occur without the overt loss of neurons (main brain cells) in the cerebellum in Alzheimer's Disease. Could these be the earliest changes occurring in the brain in Alzheimer's Disease?**

Malvindar's mission has been to follow-up on these interesting results through examining whole human brain sections of the cerebellum. One of the advantages of using the tissue microarray approach for brain research is the fact that it is utilising small tissue quantities per brain (2 millimetres), which maximises tissue usage as it is a precious resource.

However, as the cerebellum is such a large structure in the brain, we need to ensure that the results obtained from the tissue microarray are applicable to the whole cerebellum, and not just the 2 millimetres of tissue biopsied. Malvindar's current work is focusing on addressing this question to ensure that her findings are robust for publication.

Research Output(s)

Currently, Malvindar is carrying out experiments to label whole sections of human cerebellum, from 6 control brains and 6 Alzheimer's brains, with the proteins of interest identified in **Table 1 of her March 2016 report (see appendix)**. These proteins were identified to show quantitative changes in the Alzheimer's samples of her cerebellum tissue microarrays. These proteins include:

Iba1 – a protein that microglia express (immune cells that support neurons in the brain and are involved in inflammation in the brain).

HLA-DR – a protein expressed by a subpopulation of microglia implicated in disease states

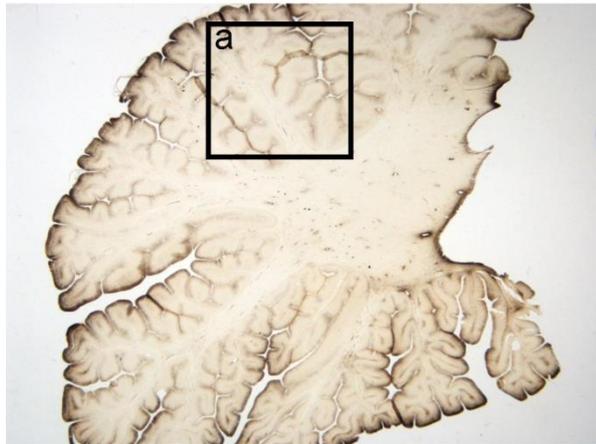
GFAP – a protein expressed by astrocytes (cells which support neurons, and provide support for metabolism in the brain)

CD31 – a marker for blood vessels

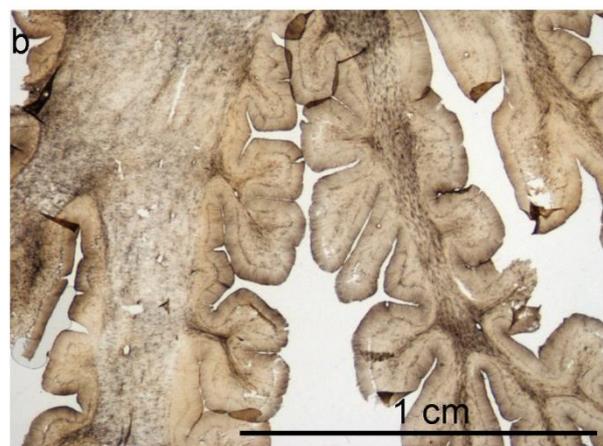
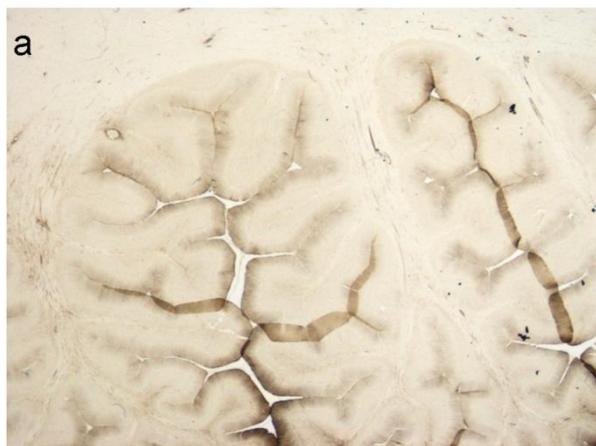
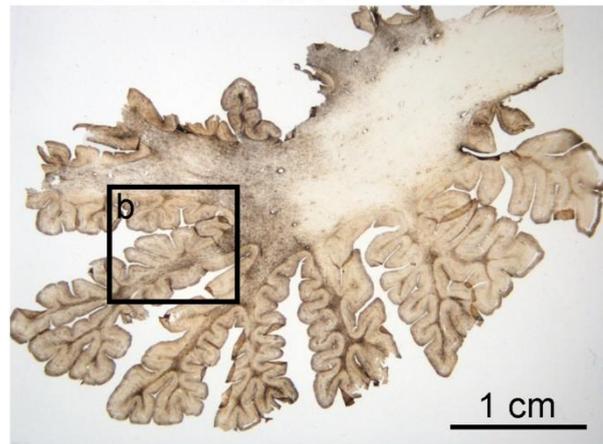
Fibronectin – a marker for a protein located in and around blood vessels that may contribute towards blood vessel disease

These studies are quite strenuous, as 3 sections are being analysed for each marker for each brain, which means a total of 36 sections are being analysed for each marker (i.e. 3 sections per brain, with 12 brains total, gives 36 sections). Currently, Malvindar has successfully conducted the experiment and imaged 36 sections for the marker glial fibrillary acidic protein (GFAP), with representative images presented below:

Control cerebellum GFAP



Alzheimer's cerebellum GFAP



The above figure illustrates the differences in glial fibrillary acidic protein (GFAP) levels in the Alzheimer's human whole cerebellum section compared to a "control" section from a neurologically normal cerebellum. GFAP is a protein expressed in cells in the brain, termed astrocytes, and these cells are thought to express higher levels of this protein, either because there are more of these cells present in the Alzheimer's cerebellum, or because more of this protein is being produced by these cells. An increase in this protein staining (as highlighted by the "darker stained sections") suggests that these cells are responding to the disease (either as a defence mechanism or triggering more damage). Malvindar is currently quantifying how much the intensity of the GFAP staining is increased by in the Alzheimer's sections, which is a work in progress.

For the next few months, Malvindar will be analysing considerable amounts of data obtained from the whole cerebellar sections, in order to verify that her findings in the tissue microarray are applicable to the whole region studied. These validation studies are necessary to ensure that the conclusions Malvindar has drawn about the changes being found in the human cerebellum are robust and true.

Research Outcome(s)

Many parts of the brain are affected in Alzheimer's Disease, and as researchers, we need to find out why certain parts of the brain are more susceptible to the effects of this tragic condition. Some of the areas of the human brain are so severely affected, due to the death of so many cells, that researchers are turning to areas which are more resistant to the disease to study the earliest and more subtle changes. This fellowship is funding a Research Fellow, Dr Malvindar Singh-Bains, to assess disease indicators and potential drug targets for the treatment of Alzheimer's Disease, before all the neurons (main brain cells) have died. She is using a technique, called the tissue microarray, to study several regions of the brain. Her first region, the human cerebellum was traditionally considered an unaffected region in the disease. Malvindar has scanned this region of the brain using the tissue microarray approach and has found some interesting and novel changes occurring in the human cerebellum in Alzheimer's Disease. As a result of the exciting data obtained from the cerebellum Alzheimer's tissue microarray, Malvindar is now following up and validating the interesting changes seen in microglia (immune cells of the brain) and astrocytes (multifunctional cells which support the brain and modulate neuronal communication) and blood vessel marker changes using larger sections of cerebellum tissue. These studies are currently underway and as evidenced by this report, analysing the whole cerebellum is a lot more time-consuming than analysing 2 millimetres worth of tissue! However, the magnitude of the changes observed need to be validated to ensure a robust publication.

In the meantime, Malvindar is also preparing and constructing tissue microarrays of other regions of the brain, including the middle temporal gyrus (an area severely affected in Alzheimer's Disease). In her previous report (March 2016), Malvindar presented some of the data from that tissue microarray, which will serve as a comparison with her cerebellum tissue microarray results. This work is currently ongoing, as Malvindar will be supervising a Master's student in 2017 to carry out expanding on the preliminary findings of the middle temporal gyrus tissue microarray.

The tissue microarray approach allows Malvindar to scan many regions of the human brain with many markers in order to piece together the global effects that Alzheimer's has on the human brain, while assessing the variability across regions at the same time.

(Future) Impact

Currently, treatments available for Alzheimer's patients in New Zealand include medications that increase brain levels of acetylcholine, a neurotransmitter involved in the learning and memory processes. These drugs - Aricept, Exelon and Reminyl - have been modestly successful in some patients for improving memory and attention skills. However, these medications can only manage the symptoms of dementia by helping the dying brain cells function. New therapies to slow down the death of brain cells in Alzheimer's Disease require an understanding of how the disease affects the whole brain. Assessing the disease patterns throughout various brain regions, utilising precious human brain samples, will aid to progress the discovery of novel disease indicators and potential drug targets for the future.

Funding of the Freemasons Foundation Postdoctoral Research Fellow is already making a major impact on our scientific understanding of Alzheimer's Disease in the human brain. In addition, it is already raising the profile of Alzheimer's Disease, and other neurodegenerative diseases, as well as the importance of medical research.

Dr Singh-Bains is a worthy recipient of this funding, not only for her thorough scientific expertise, but also her desire to disseminate and promote her research to the public. **Below is a summary of her public and media engagements in the past few months which have achieved significant community impact.**

12 March 2016 – Spoke in a Sikh temple (Guru Tag Bahadur 24 Dunnotar Rd Papatoetoe) about Brain Health Awareness, Alzheimer's Disease, Parkinson's Disease and Huntington's Disease.

29 March 2016 – Spoke at Hillcrest Baptist Church, Hamilton, as part of the "Continuing education" programme for 60+ ages (160 attendees). Focused on Huntington's, Alzheimer's Disease, and the formation of her charity, the Huntington's Disease Youth Organisation of NZ (HDYO-NZ).

12 April 2016 – Spoke at Bioengineering Institute. "Huntington's Disease, in the lab and in the community" presentation can be found on YouTube: <https://www.youtube.com/watch?v=VzzVXIZ9Ir8>

14 April 2016 – Presentation at Pinehurst School, Albany about Brain Health Awareness, Alzheimer's Disease, Parkinson's Disease and Huntington's Disease.

Radio New Zealand 9-noon with Kathryn Ryan (many slots though out the year).

February 2016

<http://www.radionz.co.nz/national/programmes/ninetoon/audio/201790412/neuroscientist-and-brain-campaigner-malvindar-singh-bains>

June 2016

<http://www.radionz.co.nz/national/programmes/ninetoon/audio/201803688/science-commentator-malvindar-singh-bains>

July 2016

<http://www.radionz.co.nz/national/programmes/ninetoon/audio/201807210/science-commentator-malvindar-singh-baines>

9 June 2016 – Invited to speak at the Brain Health Research Centre Conference about Science communication at the University of Otago.

23 June 2016 – Inducted into the Kiwi Indian Hall of Fame 2016 by the Indian High Commissioner of New Zealand.

<https://www.fmhs.auckland.ac.nz/en/faculty/cbr/news-and-events/news/cbr-news-2015/cbr-neuroscientist-wins-top-kiwi-indian-award.html>

<http://www.halloffame.co.nz/project/hall-of-fame-2016/>

https://issuu.com/indianweekender/docs/iwk1july_final_draft

https://www.youtube.com/watch?v=X_5fhuGvgVg

Aug 24-25 2016 – Attended the Brain and Mind Research in the Asia-Pacific Symposium Conference.

<https://www.fmhs.auckland.ac.nz/en/faculty/cbr/6th-apru-symposium-bmap/cbr-bulletin1111.html>

28 August 2016 – Was a subject of the TV 3 Documentary “Both Worlds” focusing on the topics of Brain Donation and Cultural appropriation, as well as Huntington’s and Parkinson’s Disease.

<http://www.3now.co.nz/shows/both-worlds/season-5-ep-3/77958/129995>

30 August 2016 – Invited guest speaker at KiwiBank Achievers Evening at the Heritage Hotel, Auckland. Spoke about career as a Neuroscientist and what it means to do research.

2 Sept 2016 – Presented Plenary lecture at the Australian and New Zealand Laboratory Animal Association Conference held at SkyCity Convention Centre, Auckland. Spoke about tissue microarrays in the progression of Alzheimer’s Research.

4 Sept 2016 – Profiled in the New Zealand Herald in article titled: Brain Detectives, 10 amazing Kiwi Insights
http://www.nzherald.co.nz/health/news/article.cfm?c_id=204&objectid=11702413

20 Sept 2016 - Presentation at St Kentigern School about Brain Health Awareness, Alzheimer's Disease, Parkinson's Disease and Huntington's Disease

20 and 23 Sept – Was a plenary speaker at the Zeal Tall Poppy Conferences held in Auckland and Wellington. The event focuses on inspiring Youth to achieve their full potential <http://zeal.nz/tallpoppy>

FOR MORE INFORMATION PLEASE CONTACT:

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Appendix:

Table 1 (from Malvindar's March 2016 report): Summarising main changes in the Cerebellum in Alzheimer's Disease using Tissue microarray analysis

Disease indicators	Output (what we have discovered in the Alzheimer's cerebellum)
<p>Microglia (immune cells that support neurons in the brain and are involved in inflammation in the brain)</p>	<ol style="list-style-type: none"> 1. There is an increase in numbers, intensity of protein labelling and ramification (branching out) of a microglial protein called Iba1 in the cerebellum in Alzheimer's Disease. Iba1 is a protein which labels most microglia in the human brain 2. There are less ramified microglia which are labelled with the protein HLA-DR. This is a sign of the presence of "amoeboid" or rounded microglia, which suggests a subpopulation of microglia are present in the Alzheimer's cerebellum which are in an "activated" state to contribute to the defence of and, potentially, damage to the affected cerebellum.
<p>Astrocytes (cells which support neurons, and provide support for neurochemical metabolism in the brain)</p>	<p>There is an increase in the overall amount of glial fibrillary acidic protein (a protein specific for astrocytes) in the Alzheimer's Disease cerebellum. This is likely to be a sign of inflammation occurring in the Alzheimer's cerebellum, as an increase in glial fibrillary acidic protein (GFAP) suggests that the astrocytes are reactive to an insult affecting the cerebellum.</p>
<p>Blood vessels</p>	<ol style="list-style-type: none"> 1. There is an increase in the overall amount of the protein CD31 in the Alzheimer's cerebellum, which labels endothelial cells in the human brain. Endothelial cells line the smallest blood vessels in the human brain, called capillaries. This suggests there are underlying changes in the blood vessels in the Alzheimer's cerebellum 2. There is an increase in the amount of fibronectin in the Alzheimer's cerebellum. Fibronectin is a protein which is deposited around blood vessels and may contribute towards vessel pathology (Disease).