Final Report

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Title of Project: Does a familial gene variant drive neurodegeneration in Multiple Sclerosis?

Summary:

Multiple sclerosis (MS) is an autoimmune and neurodegenerative disease with no clear cause and no known cure. It is estimated that genetics account for 15-72% of MS risk depending on the population studied. Genome-wide association studies (GWAS) have identified >200 gene variants that account for <20% MS heritability. Only a small number of these 'risk genes' have a known biological function in MS and by design, GWAS can only identify common genetic variants, even in larger sample populations. This means they miss the significant proportion of MS heritability that must be attributed to rare, family-specific, genetic variants. Our team has studied families with an unusually high incidence of MS (e.g. 4 out of 8 siblings have MS) and have identified 6 rare, but highly penetrant genetic variants that are only carried by the family members that have MS. In this project, **we aimed to determine the functional effects of one of these MS-family specific gene variants, in different brain cell types**. We focused on a variant identified in the NLRX1 gene, a known regulator of anti-inflammatory signalling. Currently, our results are not fully finalised, but we have made substantial progress towards this goal.

Notably, this funding allowed me to establish my research team at UTAS. It enabled the recruitment of an Honours student, Ms Elizabeth Read, who completed some of this work and graduated with First-Class Honours (H1) in December 2022, and PhD students, Ms Surbhi Agarwal and Ms Negar Sadeghi Hassanabadi, who commenced work on studying the MS-associated gene variants in late 2022. Work from this project was also presented at the Asia-Pacific Neural Tissue Repair Symposium in October 2022 and the Australasian Neuroscience Society Annual Meeting in December 2022. The data generated from this grant will be included as background data for pending NHMRC Ideas Grant and MS Australia funding applications in 2023.

Hypothesis vs Findings

Our overall hypothesis is that dysregulated NLRX1 signalling, leads to MS-associated neurodegeneration. NLRX1 is a protein expressed in the mitochondria of all cells, including brain cells. Its primary known function is to downregulate or 'dampen' inflammatory signals that occur after tissue damage or viral infections. It

does this by binding to a protein called MAVS (Mitochondrial AntiViral Signalling). The gene variant we identified is predicted to cause a change in the region of the protein that binds MAVS, and so we hypothesised that the MS-associated NLRX1 variant would not be able to bind to MAVS effectively. We were able to successfully overexpress both the normal (wildtype) and variant forms of NLRX1 in a cell culture system, and 'pull down' NLRX1 from the mitochondria of cultured cells. However, we encountered technical difficulties in determining if MAVS bound to the wildtype version of NLRX1, that is not associated with MS. Without this control, we are unable to make strong conclusions about whether the variant form of NLRX1 is able or unable to bind to MAVS. We are currently trouble shooting this problem using other commercially available antibodies and believe that we will be able to make a strong conclusion soon.

In the second part of this project, we aimed to examine the function of the NLRX1 variant in brain cell types derived from the family members and unrelated people with MS, as well as people without MS (controls) using induced pluripotent stem cell (iPSC) technology. We have successfully generated microglia, astrocytes and oligodendrocyte progenitor cells (OPCs) and verified their cellular identity using immunocytochemistry and bulk RNA-sequencing (Fig.1) to ensure that they are the correct cell type. It is important to note that generation of astrocytes and OPCs takes approximately 3 months, and due to staff changes, some of our progress in this area slowed. However, we have successfully generated each of these brain cell types and verified their cellular identity and are proceeding with our analysis of the functional consequences of the MS-associated NLRX1 variant in each of these brain cell types. This is the focus of our newly recruited PhD students.

Unanswered Questions

Although, the final outcomes of this project are ongoing, one of the key research questions that remains unanswered is whether it is the cumulative genetic background of the MS family members that leads them to develop MS? As MS is a complex, polygenic disease, it is unlikely that the NLRX1 variant by itself, is sufficient to initiate MS development, but it could be a significant factor contributing to the progression or severity of MS that affected family members experience. To get at these questions we have recently sent some of our iPSCs from this family to have NLRX1 or GRIK4 (a second gene, containing a variant identified in this family to be associated with MS) 'corrected.' This means that the iPSCs will have the same genetic background as the family members, except for each of these genes, which will be 'corrected' back to the most common version of NLRX1 or GRIK4 not associated with MS. This will allow us to determine if the functional effects we see, are due to either of these gene variants individually, or if it is due to some additional genetic burden carried by the MS family members.

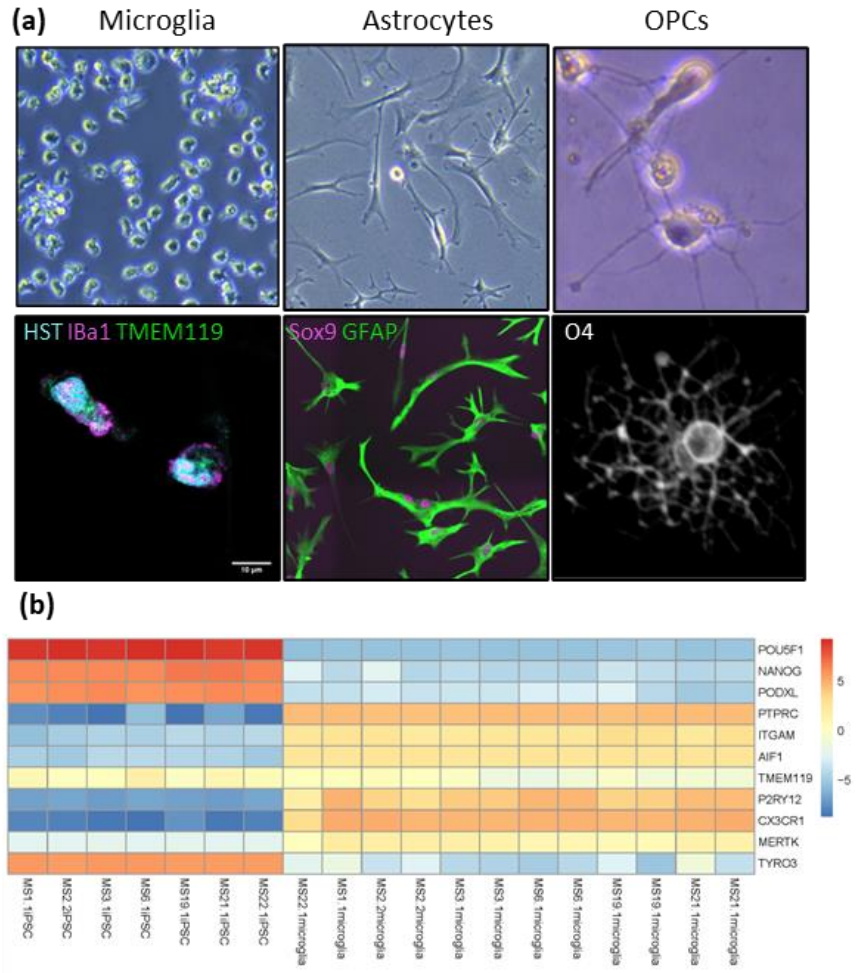

Fig. 1: (a) *Representative images of brain cells, including microglia, astrocytes and OPCs, derived from iPSCs from family members with MS. Bright field images (top), cells positive for cell identity markers (bottom).* ***(b)*** *Example heatmap of bulk-RNA sequencing to confirm cellular identity of iPSC-derived microglia against the original iPSC line. Warm colours (red, orange) indicate high levels of gene expression, cool colours (blue) indicate low levels of gene expression. Genes PTPRC, ITGAM, AIF1, TMEM119, P2RY12, CX3CR1, and MERTK are typically highly expressed by microglia. POU5F1, NANOG and PODXL are markers of pluripotency, meaning the cell is still able to become any cell type.*

What these research outcomes mean

These research outcomes are early and incomplete. However, they are the first step into developing a new ‘humanised’ model of MS. This very important for both our ability to design effective brain repair therapies and for our ability to understand how MS develops and progresses. One of the key limitations of current preclinical models is that none of them faithfully recapitulate either the cause of MS or the full range of disease processes that occur in people with MS. By determining if these gene variants that are strongly associated with MS development in families have biological effects linked to neurodegeneration, we will be able to establish a novel cellular model of MS. This will be a highly significant outcome for MS brain research, generating a novel platform of therapeutic testing for new brain repair treatments.