Brain intercellular signalling in diabetic neuropathology: integrating single cell 'omics with human iPSC neuro-vascular models to reveal therapeutic opportunities

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Project outline

Background: The neurovasculature is the key physical interface between the brain parenchyma and the rest of the body. Long understood to be pivotal for maintaining brain homeostasis and health via it's barrier and transport functions it is increasingly being recognised that the vasculature is an integral participant in many other brain processes. We have developed an snRNA-seq method to extract and sequence vascular nuclei (endothelial cells, pericytes) from human post-mortem brain with high efficiency whilst simultaneously capturing parenchyma. This is a significant advance over other published methods where either vascular nuclei are tiny fraction (<1%) or parenchymal nuclei are depleted / not captured. This has allowed us to examine cell-cell signalling networks between vascular and parenchymal cells; and we are investigating how such networks are disrupted in Alzheimer's Disease. Preliminary findings highlight that signalling specifically from the vascular compartment is overwhelmingly the most significant in contributing to the disease-evoked and disease-mediating hyper-signalling milieu in this complex disorder. We will shortly also have a snRNAseq dataset from Type II Diabetes post-mortem brain as part of a NN pilot grant.

Hypothesis: Diabetes (Type 1 and 2) is strongly associated with a range of brain disorders including stroke, small vessel disease, cognitive dysfunction, epilepsy and dementia. However the mechanisms by which diabetes increases the risk for these disorders is unknown. The interface between the diabetic state and the brain is the neurovasculature. In diabetes since there is no clear primary insult to the brain itself, we propose that the powerful signalling capacity of the neurovasculature likely comprises a critical mediator of the diabetic state into neurological consequences. Targeting cell-cell signalling pathways has significant advantages for translation as the soluble signal carriers can serve as serum biomarkers and the signalling mechanism (ligand or receptor) can directly be interrupted/modulated for therapeutic benefit.

Aims: Traditionally studies into diabetes examine the impact of the cardinal feature of the disease, namely hyperglycaemia, and one feature of the neurovasculature – the blood brain barrier (BBB). We suggest that isolating studies on just one element of a complex fluctuating biological state upon one function of the vasculature likely fails to adequately model the impact of diabetes on the neurovasculature and brain health. In this project we will:

1. Identify neurovascular to brain parenchymal signalling pathways perturbed in diabetes using existing *in silico* resources
2. Validate key candidate diabetes relevant systemic signalling perturbations on brain capillary endothelial cells using human iPSC models
3. Investigate the consequences of systemic circulation-brain endothelial signalling changes to brain parenchymal cells

Research Methodology

1. We will make use of our recent AD post-mortem brain snRNA-seq data from the IM2PACT consortium and published diabetes brain RNASeq datasets (PMID: 31449493). This will identify critical ligand and receptor changes in each cell type of the neurovasculature and brain parenchyma. We will couple this with an analysis of published longitudinal metabolome and proteome datasets of diabetics (e.g. PMID: 33737653, 32385057). This will reveal candidate systemic signals that may underlie the impacts of diabetes on brain health as well highlight overlapping mechanisms with AD.

2. The Cader lab has established protocols to generate the range of relevant cell types to study brain cell-cell signalling including brain capillary endothelial cells, mural cells (pericytes/smooth muscle), microglia, astrocytes and cortical neurons. We have also established transwell insert models, whereby we can co-culture different combination of cell types, with or without direct contact. Importantly the inclusion of all cell types is requisite for a functional BBB. We will use an adapted form of this system to examine the impact of systemic factors on (i) endothelial barrier function using TEER, dextran-FITC and Rhodamine permeability; (ii) endothelial signalling using transcriptomics and phosphoproteomics of endothelial cells alongside analysis of secreted factors into the cell culture media.

3. We will next use bi-and tri-culture systems so we can assess how induced endothelial signalling might communicate to other cell types and affect their function. We will focus on co-cultured iPSC-pericytes, astrocytes and microglia since they reside proximal to the endothelial cells and are good candidates to mediate neuro-inflammation and BBB breakdown. Specific features to be examined will include pericytic calcium dynamics, and the M1/M2 pro-inflammatory transitions induced in microglia.

Contributions of Oxford and NNRCO supervisors:

Oxford will support the fellow throughout the project and guarantee bench space and access to laboratory resources. This will begin with the omics analysis, which will first require identification and federation of relevant omics data. The Cader lab will support establishing the human iPSC models to investigate the effect of diabetes on brain vascular cell types and signalling to brain parenchymal cells. Where required the fellow will be trained by the Cader group in differentiation protocols and co-culture methods as well performing the relevant assays. The Cader group will also provide the support for interpretation of results.

NNRCO will provide support and training for the in silico analysis, using their extensive experience with single cell datasets and their expertise in molecular phenotypes associated with diabetes. When data from the ongoing pump-priming project become available, Dr. Kitchen and NNRCO researchers will support the fellow in integrating these with in-silico analyses detailed in Aim 1; this

will include a period during which the fellow will work on-site at NNRCO. For the subsequent Aims, NNRCO will support the development of relevant assays and the interpretation of arising results.

Supervisor's recent relevant publications (5 max per supervisor):

1. Pokhilko A, Brezzo G, Handunnetthi L, Heilig R, Lennon R, Smith C, Allan SM, Granata A, Sinha S, Wang T, Markus HS, Naba A, Fischer R, Van Agtmael T, Horsburgh K, **Cader MZ**. Global proteomic analysis of extracellular matrix in mouse and human brain highlights relevance to cerebrovascular disease. J Cereb Blood Flow Metab. 2021 Sep;41(9):2423-2438.
2. Pokhilko A, Handel AE, Curion F, Volpato V, Whiteley ES, Bøstrand S, Newey SE, Akerman CJ, Webber C, Clark MB, Bowden R, **Cader MZ**. Targeted single-cell RNA sequencing of transcription factors enhances the identification of cell types and trajectories. Genome Res. 2021 Jun;31(6):1069-1081.
3. Fang EF, Hou Y, Palikaras K, Adriaanse BA, Kerr JS, Yang B, Lautrup S, Hasan- Olive MM, Caponio D, Dan X, Rocktäschel P, Croteau DL, Akbari M, Greig NH, Fladby T, Nilsen H, **Cader MZ**, Mattson MP, Tavernarakis N, Bohr VA. Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. Nat Neurosci. 2019 Mar;22(3):401-412.
4. Volpato V, Smith J, Sandor C, Ried JS, Baud A, Handel A, Newey SE, Wessely F, Attar M, Whiteley E, Chintawar S, Verheyen A, Barta T, Lako M, Armstrong L, Muschet C, Artati A, Cusulin C, Christensen K, Patsch C, Sharma E, Nicod J, Brownjohn P, Stubbs V, Heywood WE, Gissen P, De Filippis R, Janssen K, Reinhardt P, Adamski J, Royaux I, Peeters PJ, Terstappen GC, Graf M, Livesey FJ, Akerman CJ, Mills K, Bowden R, Nicholson G, Webber C, **Cader MZ**, Lakics V. Reproducibility of Molecular Phenotypes after Long-Term Differentiation to Human iPSC-Derived Neurons: A Multi-Site Omics Study. Stem Cell Reports. 2018 Oct 9;11(4):897-911.
5. Haenseler W, Sansom SN, Buchrieser J, Newey SE, Moore CS, Nicholls FJ, Chintawar S, Schnell C, Antel JP, Allen ND, **Cader MZ**, Wade-Martins R, James WS, Cowley SA. A Highly Efficient Human Pluripotent Stem Cell Microglia Model Displays a Neuronal-Co-culture-Specific Expression Profile and Inflammatory Response. Stem Cell Reports. 2017 Jun 6;8(6):1727-1742.

Additional Information

1. Are there any restrictions on the securing of IP arising from the fellowship project?
   No

2. For projects involving big data, please clearly state below the sources where the data come from or provide details of the other consortia members who contributed the data.
   Omics data from published studies, deposited in public archives such as NIH GEO

3. Any results generated by the fellow will have to be freely shared with Novo Nordisk throughout the fellowship. In addition, any abstract for external presentation and all publications generated by the fellow must be shared with Novo Nordisk 30 days in advance of submission. Can you abide by these rules?
   No

4. For projects jointly supervised between NNRCO and Oxford supervisors, will there be background IP included in the project?

Background IP – snRNAseq data from the IMI IM2PACT project, but Oxford and NN are members of this consortium and free to use this data