Identifying Alzheimer's Disease Causal Gene Networks in Brain Vascular and Immune Cells

Supervision
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Genome-wide association studies (GWAS) have illuminated the importance of pathways involving amyloid and tau biology, lipid processing, and immunity in Alzheimer's Disease (AD). However, existing single-cell/nuclei RNA-sequencing datasets primarily lack adequate representation of brain vascular cells, limiting our ability to fully understand the contributions of these cells to AD.
To address this gap, we developed a novel protocol for simultaneous isolation of vascular and parenchymal cells from post-mortem prefrontal cortex samples as part of the IMI IM2PACT project. This approach has produced a high-purity dataset of both parenchymal and vascular cell types, including pericytes, smooth muscle cells, and perivascular fibroblasts (AD n=20, healthy controls, HCs n=20). Our preliminary analysis highlights specific enrichment of AD GWAS genes in pericytes, perivascular fibroblasts, and activated microglia, with gene expression profiles implicating processes such as amyloid clearance, immune cell activation (including T cell recruitment), and cytoskeletal organization. This is the first time vascular cells have been robustly shown to carry AD genetic risk where all cellular constituent of the prefrontal cortex are considered and it represent an attractive and novel therapeutic opportunity. This will however require validation and better understanding of the relevant biology.

Challenges and Hypotheses
- **Challenges**: The main challenge lies in analyzing this large, multimodal dataset to accurately model cellular interactions and identify disease-driving mechanisms by validating causal variants and the genes they regulate.
- **Hypothesis**: Specific vascular and parenchymal cell types collaborate in amyloid-related and immune processes, which may contribute to the early pathogenic events in AD.

Aims and Objectives
The overall aim is to leverage advanced computational methods and available transcriptomic and epigenetic dataset to explore the cellular mechanisms linking vascular and parenchymal cells to AD pathogenesis. Chromatin accessibility data for neurovascular cells that we have identified as carrying genetic risk will be obtained as these are presently not available. The project will also leverage rich dataset from a recently started multi-omic AD GSK-IMCM project including combined snRNA-seq and ATAC analysis in the amygdala of 332 post-mortem brains (AD= 245, HC n=87).

Objectives:
1. Perform genomic chromatin footprinting and analyse chromatin contact maps in AD implicated cell types: microglia, pericytes and fibroblasts
2. Integrate AD GWAS data with epigenetic data to validate causal variants and causal genes. Perform eQTL, pQTL and Mendelian randomisation as post-GWAS analysis to develop further support
3. Validate the role of neurovascular cells and microglia using the AD GSK-IMCM project multi-omics dataset

Potential Applications and Benefits
Insights into the neurovascular unit's contribution to AD pathogenesis, paving the way for targeted therapeutic strategies. Improved understanding of vascular and immune contributions to AD may inform biomarker discovery and drug development.

Data sharing and Ethics
The data we have already generated will be published and will be open access. We will also look to share new data from this project and data from ongoing AD studies through the existing Oxford-GSK agreement. No additional ethical approvals are necessary.

Methodology, Milestones, and Training Opportunities

1. ATAC-seq and analysis: Analyze chromatin accessibility data (ATAC-seq) generated from post-mortem tissue and induced pluripotent stem cell (iPSC)-derived models for key cell types (microglia, pericytes, and fibroblasts) to identify open chromatin regions and infer transcription factor binding. Integrate AD GWAS results with ATAC-seq data to identify causal variants and their target genes.
2. Post-GWAS Analyses: Conduct post-GWAS analyses, including expression quantitative trait loci (eQTL), protein quantitative trait loci (pQTL), and Mendelian randomization, to validate causal genes and pathways. Undertake chromatin capture (e.g. Micro-CC) to validate.
3. Validation Using Multi-Omic Data: Leverage the AD GSK-IMCM project multi-omics dataset to validate findings - Analyze transcriptomic and proteomic profiles to elucidate disease mechanisms. Construct protein-protein interaction networks and perform clustering to identify functional gene modules. Use multi-cell-type interaction models to understand collaborative roles in AD-related processes such as amyloid clearance and immune regulation.

Timeline and Milestones

- Year 1: Generate and initial analysis of chromatin accessibility data for relevant cell types from post-mortem tissue and iPSC-derived models.
- Year 2: Complete eQTL and pQTL analyses and initiate Mendelian randomization studies to validate causal genes. Conduct chromatin contact mapping on prioritised regions and integrate epigenetic data with GWAS results.
- Year 3: Validate findings using the AD GSK-IMCM project multi-omics dataset and develop a network of causal gene modules

Training Opportunities: The fellow will develop expertise in the following areas:

1. Advanced bioinformatics workflows for RNA-seq, ATAC-seq, and chromatin contact map data.
2. Statistical methods for post-GWAS analysis, including eQTL, pQTL, and Mendelian randomization.
3. Integrative multi-omics analysis to uncover gene networks.
4. Hands-on experience in generating and analyzing chromatin accessibility data using ATAC-seq.
5. Use of iPSC models to study cell-type-specific regulatory mechanisms.
6. Opportunities to present research at internal and external meetings, enhancing communication skills.
7. Access to workshops on transferable skills, including project management, grant writing, and science communication.

Research Environment

The Cader Lab has extensive expertise in single-cell RNA-seq analysis and integration of epigenetic data, making it an ideal environment for the proposed work. Additionally the Cader lab has the required iPSC models, wet-lab assays and techniques including chromatin capture. The Parkkinen lab has leading expertise in AD neuropathology and is leading on the multi-omics study of AD with GSK-IMCM.

Institutional Support

- Wet-lab facilities and expertise and assay platforms
- Access to high-performance computing facilities for large-scale data analysis.
- Support for transferrable skills development
- Opportunities for internal and external research meetings to develop communication skills and networks

Clinical Commitments: The fellow will have an honorary clinical contract if required, to support ongoing training but the main focus will be on research activities.