

Project Details	
Project Code	MRC23IIARCa Peters
Title	Epigenetic regulation of microglial gene expression in Alzheimer's disease
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Neuroinflammation is a prominent event in Alzheimer's disease (AD) pathogenesis driven by activation of microglia, the brains resident immune cells. To define how microglial gene expression is regulated in AD, this project utilises a state-of-the-art human induced pluripotent stem cell models of AD coupled with epigenomic profiling and bioinformatic analysis. The role of prioritised genes will then be described through functional assays of key microglial processes.
Description	<p>Neuroinflammation is emerging as a key pathogenetic event in risk and progression of Alzheimer's disease (AD). GWAS have identified single nucleotide polymorphisms (SNPs) at genetic loci associated with microglia function enriched in AD cohorts, however how these alter gene expression within loci is often unclear. Profiling transcriptional regulation of AD associated gene by epigenome wide association studies of post-mortem human cortex has identified enriched patterns of DNA methylation in microglial genes of AD patients. Whilst further illuminating the contribution of microglia in AD pathogenesis, a caveat of these experiments is the tissue utilised; post-mortem samples of mixed cell types derived from patients dying with late-stage AD with profound neurodegeneration and inflammation. Such tissues may not capture more subtle, early changes in epigenomic regulation that underpin functional changes in cells in prodromal patients. To better understand these events, we will profile the epigenome of human microglia in in vitro co-culture models of AD, hypothesising that essential microglial functions are disrupted through altered epigenetic regulation of microglia specific genes in AD. Aim 1: To define epigenetic regulation of microglia genes associated with extracellular amyloid 1a. CU: To generate an AD relevant population of microglia for epigenomic profiling, a hiPSC model of amyloid accumulation will be utilised. APP mutant and isogenic control KOLF2 hiPSCs will be differentiated to establish the following microglia/cortical neuron co-cultures: 1) wild type neurons, wild type microglia (WT co-culture); 2) APP mutant expressing neurons, wild microglia (AD co-culture). Microglia will next be recovered from the co-cultures using CD11 magnetic beads (MACS) for cell separation and genomic DNA and RNA isolation. 1b. UEx: To define the genome wide patterns of DNA methylation and gene regulation, microglia DNA and RNA collected from WT or AD co-cultures will undergo quantitative genome wide profiling for methylation by microarray profiling (Infinium MethylationEPIC BeadChip platform, 850k CpG sites) and bulk RNASeq transcriptome profiling. Epigenomic and transcriptomic profiles will be bioinformatically analysed using established analysis and bioinformatic approaches. Differentially regulated targets will be triaged for further analysis, based on i) statistical significance, ii) association with the expression of proximal genes, iii) contribution to microglia specific function and iv) potential for therapeutic intervention. Aim 2: Contribution of epigenetically regulated microglia genes in AD-relevant phenotypes. 2a. CU/UEx:</p>

	<p>Methylation associated regulation of genes in prioritised loci will be further validated by bisulphite sequencing, qPCR and/or antibody staining, assessing microglia in either WT or APP co-cultures. We will triage 3 genes where methylation is associated with robust changes in expression for further analysis. We will next mis-express three lead genes in hiPSC microglia, where appropriate utilising targeted methylation by Cas9-DNMT, and/or CRISPRi for gene disruption (Cas9-KREB), CRISPRa (dCas9-VP64) for activation (transgenic siRNA/cDNA as contingency). Changes in gene expression will be confirmed by qPCR, western blotting and immunostaining. 2b. CU: The consequences of gene manipulation in 2a will be examined for AD-relevant microglia phenotypes. Assays will include inflammatory responses of microglia stimulated with INFY/LPS, quantified by cytokine release via a flow cytometry-based immunoassay (BD cytokine bead array); changes in morphology and motility of INFY/LPS stimulated microglia in neuronal WT and AD co-cultures; and phagocytosis of pHrodo labelled E. coli or fluorescent amyloid oligomers assessed by live imaging. Our approach will define AD relevant epigenetic regulation of microglia and explore how altered expression of these genes contributes to neuroinflammatory function.</p>
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