Project Details	
Project Code	MRC23NMHCa Harwood
Title	A combined in vitro and in silico molecular analysis of CHD8 gene-mediated risk for ASD
Research Theme	Neuroscience and Mental Health
Summary	This project investigates how risk for autism may arise due to changes in high-level, 3-dimensional structure of DNA in cell nucleus. It focuses on the gene CHD8, mutants of which are strongly associated with risk for autism. It will use a combination of Next-Generation Sequencing, human iPSC biology and computational analysis to establish how CHD8 may increase risk for autism via altered gene expression due to changes in genome architecture.
Description	Aim: To understand the molecular mechanisms by which mutation of the CHD8 gene increases risk of Autism Spectrum Disorder (ASD). Background: CHD8 (chromodomain helicase DNA-binding protein 8) is a chromatin-remodelling factor that has been implicated in neuropsychiatric and neurodevelopmental disorders, including ASD and schizophrenia (SCZ) [1]. Mutations of CHD8 alter expression of ASD or SCZ risk genes [2], however the sequence positions of CHD8 binding to DNA poorly correlate to the genes that they regulate, hampering in silico prediction of target genes and suggests an indirect interaction between CHD8 and control of gene transcription. As CHD8 functions to position nucleosomes on DNA and hence overall chromatin topology, we propose that CHD8 exerts its effects on gene expression via long range chromatin interactions that are mediated by changes in 3D genome architecture. Objectives: 1. The student will differentiate control (IBJ4) and isogenic CHD8 mutant human induced stem cell (iPSC) lines to neural progenitor cells (NPC), a key stage in neurodevelopment when neuronal gene transcription begins. CHD8 mutants for this project are already created in the IBJ4 cell background by CRISPR, and preliminary analysis identified a set of mis-regulated genes. We have also created data for pluripotent iPSC cells using promotor-capture Hi-C sequencing (PC-HiC), a technique established via a WT ISSF collaborative project. This maps long-range chromatin interactions between regulatory sequences and gene promoters and provides baseline data to identify those genes with disrupted or enhanced chromatin interaction due to loss of CHD8. In this project new PC-HiC data will be generated for NPC allowing dynamic changes in the 3D genome to be mapped as cells enter this critical stage of neurodevelopment. Each sample will be accompanied by a transcriptional profile (RNA-seq) data set to confirm downstream targets 2. In silico analysis of our existing pluripotent and the new NPC stage data will map chromatin interactions sensitive to CHD8 regulation at both high scale across the whole genome and onto individual genes and their long-range, trans-regulation sequences. These changes will be integrated with RNA-seq data to identify the core set of CHD8-sensitve genes regulated during neurodevelopment. The resulting gene set will be investigated for their risk association with ASD and SCZ by integration with previous GWAS studies. At the population level, data from the 100,000 genomes project will be analysed for sequence variants that map to the CHD8-mediated interaction sites. These analyses should predict the CHD8-mediated neurodevelopmental pathways directly that

	underlie psychiatric symptoms. 3. In a final wet lab project, quantitative PCR-based methods will be used to validate our gene set for changes in gene transcription and promoter sequence interaction across an extended neurodevelopmental time course, including early and mature neurons. In a "stretch challenge", the student will design and employ a chromatin-locus visualisation method [3] to observe chromatin interactions in single cells, including different neuronal and glial cell types are sensitive to CHD8 hypofunction. This project is a standalone project that will be developed and managed by the student under guidance of the supervisory team. As an overall package, the student will develop a unique set of inter-disciplinary expertise, spanning both in vitro human stem methods and in silico computational and informatic skills, thus training the next generation of researchers in an area of important need. It will build the personal skills in the student needed to pursue a route to independence. References: [1] McCarthy et al (2014) Mol Psych 19:652–658; [2] Cotney et al (2015) Nat Commun 6:6406; Wilkinson et al (2015)Trans Psych 5:e568; Wang et al (2017) Mol Autism 6:55; [3] Bradnao et al (2021) Curr Opin Cell Biology 70:18-26
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