Project Details	
Project Code	MRCNMH26Ex Ryu
Title	Functional analysis of novel rare coding variants for schizophrenia using zebrafish
Research Theme	NMH
Project Type	Wet lab
Summary	Schizophrenia is a severe psychiatric disorder with debilitating effects on a patient's quality of life. Because current medications do not work well, we need to study its molecular cause to develop new therapeutics. Recent research identified a number of genetic mutations in Schizophrenia patients called “rare coding variants.” These mutations change the proteins they produce, but for many of them, we do not yet know the functional consequences. In this project we will study the functions of the rare coding variants using zebrafish and compare the results with mouse models of equivalent mutations and human post-mortem brain tissue.
Description	Schizophrenia is a severe psychiatric disorder with debilitating effects on a patient's quality of life and reduction of life expectancy. Despite the significant lifetime risk of disease at 0.7%, existing therapies do not adequately target its varied symptoms. Also, responses to current medications are highly variable with 30% of patients classified as treatment resistant. Improved development in therapy requires better understanding of the molecular aetiology of Schizophrenia. It is crucial to understand how molecular alterations contribute to disease-relevant phenotypes. In this project we will carry out functional analysis of so-called “rare coding variants” (RCV) of schizophrenia which are large-effect risk variants which appear at much lower frequencies in the population. These mutations are often found within gene coding regions altering protein functions and/or gene expression. They are therefore more tractable for functional analysis in animal models as protein-coding regions and protein functions are much more likely to be conserved across species compared to non-coding regions. Identification of RCV in schizophrenia is an active and emerging area of current schizophrenia research. Mouse models are available for some of the published RCV and are beginning to be analysed, but for most other unpublished rare coding variants, animal models do not exist yet. Therefore the first goal of this study is to develop new animal models to study those RCVs that have not been functionally characterised before. We propose to take advantage of the zebrafish model, which offers low cost genetic manipulation combined with ease of whole brain activity analysis. We will generate mutants that harbor mutations representing human RCV and analyse their function in the context of the whole animal. In parallel, we will generate zebrafish mutants for two RCVs for which mouse models are already available in the Clifton lab to compare and synergise the functional analyses of those RCVs using two species. Further, we will combine zebrafish mutants with one of the strongest risk factors for developing schizophrenia, namely early life stress. We will then assess the effects of combining genetic and environmental risk factors in a large number of functional assays already established in the Ryu lab. Finally, we will perform RNA sequencing analysis of select brain

	regions in the zebrafish mutants to identify molecular alterations caused by RCVs and compare them to a wide range of molecular data available from human post-mortem cortex with and without a schizophrenia diagnosis. Specific Aims: 1. Generate zebrafish KO of 5 rare coding variants of Schizophrenia and perform behavioural and whole-brain activity analysis 1.1 Using CRISPR-Cas9 technology, we will generate zebrafish mutants. We will choose 2 variants for which mouse models are already available in the Clifton lab. In addition, we will generate mutants for 3 additional unpublished variants guided by the human data obtained by Dr Elliott Rees 1.2 A battery of behavioural assays will be used to assess the phenotype in larval, juvenile and adult fish including – social behaviour, pre-pulse inhibition, stimuli responsiveness, and anxiety-related assays 1.3 Whole brain activity analysis of the mutants will be carried out using histological methods to detect immediate early gene expression and compared with anatomical atlas. 2. Determine how early life stress exposure affects the mutant phenotype The Ryu lab has established an early life stress zebrafish model where the level of stress hormone, cortisol, can be elevated during development. This model has been well-characterized and shows behavioural and transcriptomic alteration in adulthood. We will combine RCV mutants with this early life stress model and carry out phenotypic analysis as above. 3. Perform RNA sequencing of affected regions from the mutant to identify altered genes- To identify molecular consequences of the RCV mutation, we will carry out RNA seq using dissected tissues representing hippocampus from mutant zebrafish 4. Determine whether any of the identified genes are also altered in mouse models and human post mortem tissue multi-omic data. The RNA seq data from zebrafish will be compared with equivalent data sets in mouse and human brain. The student can take ownership and steer the project throughout the project. In particular phenotypic analyses proposed in specific aims 1 and 2 will require evaluation of the results obtained and planning and choosing the appropriate next assays. This will require the student to steer the project based on the results that he/she has obtained. Also, molecular analyses proposed in specific aims 3 and 4 require the student to take ownership in deciding which molecular alterations to focus and characterise further.
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