Project Details	
Project Code	CMD27Ca Fowler
Title	Linking structure, function and genomics at single cell resolution: an integrative approach to understanding inherited heart disease
Research Theme	Cardiometabolic Disease
Project Type	Wet lab
Summary	This project aims to address a research priority for patients with the inherited heart condition, hypertrophic cardiomyopathy (HCM), by investigating how the same mutation can result in widely different phenotypes. Increased variability can manifest between different individuals, regions of the heart, and from one cell to the next. To address this, you will develop a unique process for capturing structural (confocal microscopy), functional (electrophysiology and Ca2+ imaging), and transcriptomic data all from the same cell. Integrating function and genomics will provide unprecedented power to identify possible causal factors responsible for pathology, and opportunities for intervention.
Description	Hypertrophic cardiomyopathy (HCM) is the most common genetic cause of heart disease (affecting ~1 in 500 people) and is the leading cause of sudden death in young adults and currently there is no cure. A better understanding of the factors driving the disease could identify new opportunities for intervention and ultimately improve patient outcomes. HCM-causing variants in myofilament proteins (essential for muscle contraction) are usually autosomal dominant, meaning that one copy of a faulty gene is sufficient to cause disease, however there is substantial variability in disease penetrance, meaning that some develop severe early onset symptoms whereas others with the same mutation may be lifelong symptom-free. HCM can unpredictably affect both atria or ventricles and their regions , the extent of hypertrophy and dysfunction of physiological processes in cardiac myocytes, and the organization of contractile proteins within cardiac myocytes themselves. Structural and functional heterogeneity may have a detrimental impact on outcomes, but the cause(s) are poorly understood. RNA sequencing (RNA-Seq) provides insight into gene expression profile in bulk tissue homogenates but does not account for variation between/within regions. Single cell RNA-Seq provides a more detailed view of the heterogeneity in transcriptome between cells and has been combined with FACS to link single parameters, such as cell size, with differentially expressed (DE) hypertrophic gene profiles. However, these methods do not reveal how DE genes impact the functional properties of cells, nor do they capture more detailed subcellular abnormalities. Patch-Seq is technique that combines the resolution of single-cell RNA sequencing with detailed physiological assessment achieved using patch-clamp electrophysiology and Ca2+ imaging, yielding fully integrated multidimensional data on each cell's electrophysiology, morphology, and individual transcriptome. We have previously used Patch-Seq to investigate causes of action potential failure in neural stem cells and neurons. Transcriptomic data generated using Patch-Seq successfully predicted experimental electrophysiological properties of neurons, demonstrating the mechanistic value of understanding transcriptome at the cellular level. Thus Patch-Seq has the potential to be a transformative tool for understanding the molecular causes of cellular

	dysfunction, however no studies have used it to investigate cardiovascular disease. The key research question this project will address is whether DE genes underlie the pathological phenotype and functional heterogeneity present in HCM. The student will establish, for the first time, Patch-Seq in cardiac cells and use it to identify the molecular pathways associated with functional disruption in HCM. Specifically, the student will use cardiac electrophysiology and Patch-Seq on mouse cardiomyocytes and on human induced pluripotent stem cell-derived cardiac myocyte (hiPSC-CM). This is an excellent opportunity for the student to take ownership by developing unique skills that will open new fields of further study in cardiometabolic diseases. The project will address the following aims: 1) Establish Patch-Seq method for RNA extraction from atrial and ventricular cardiac myocytes and validate with complementary methods (qPCR). 2) Identify DE transcripts associated with the severity of cellular structural and functional abnormalities in a mouse model of HCM using confocal microscopy. 3) Compare and contrast identified DE transcripts with a hiPSC-CM model of HCM Training will be provided in the following areas to achieve these aims: In Patch-Seq, the structural and functional properties of individual cells are first recorded using patch clamp electrophysiology and fluorescence imaging, then the cell is aspirated into the electrode for single-cell cDNA library preparation. We will first optimise the procedure for mRNA extraction from single atrial and ventricular cardiac myocytes from wildtype (WT) mice, following functional characterisation using confocal imaging and patch clamp electrophysiology in the same cell. These cell types have distinct structural, physiological, and transcriptional profiles, and therefore will be ideal positive controls to validate the methodology by correlating known functional characteristics with expression levels of relevant genes. These experiments will demonstrate that Patch-Seq can distinguish between types of cardiac myocytes based on known DE genes, and to correlate gene expression levels with known functional properties. The second aim will be addressed using transgenic mice with a severe HCM phenotype, including atrial and ventricular hypertrophy, susceptibility to tachyarrhythmias and aberrant heart functions. Using this mouse model we have identified greater variability in heart weight, electrical repolarization and myofilament organisation, however the molecular causes of these differences are not known. We will apply the Patch-Seq methodology to test whether DE genes explain the structural and functional variability within and between HCM and WT cells. Finally, we will extend the methodology to hiPSC-CM, that are widely used for academic and commercial drug screening research, to validate DE genes associated with structural and functional abnormalities using a human model system. This improves the translational potential of the project to human medicine.
Can be completed part time?	No
Supervisory Team	

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