An integrative genetic and experimental study of mitochondrial oxidative stress in hypertrophic heart disease

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Project outline

Background: Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease with a prevalence of 1:250-1:500 and is a leading cause of sudden death in young adults and athletes particularly[1]. Genetic studies indicated that HCM-causing mutations are commonly found in sarcomere genes (e.g., MYH7, MYBPC3, and ACTC1)[2]. However, despite significant progress in identifying disease-causing gene mutations and clinical interventions for symptoms and risk mitigation of sudden death[3], there is still a lack of effective and specific treatments for mitigating the disease progression of HCM[4]. This may reflect the challenge of treating genetic mutations and the resulting complicated pathogenesis of the disease. Abnormalities in mitochondria (MT) have been reported in HCM patients[5]. On the other hand, work by us and others over the past decade has laid the groundwork for identifying the key roles of p21-activated kinase 1 (PAK1), a group of serine/threonine protein kinases, in cardiac physiology and their therapeutic potential in cardiac hypertrophic disease and heart failure (HF)[6-10]. In line with these experimental findings, stimulating PAK1 signalling genetically or pharmacologically showed a beneficial effect in hypertrophic and HF mouse hearts.[9-11]. Intriguingly, alternation of *PAK1* activity has been observed in HCM mouse hearts and our initial treatment of HCM mice with a PAK1 modulator shows a promising effect on disease progression (unpublished observation). Therefore, PAKs are likely potential new druggable targets for defending against the development of cardiac hypertrophy, HF and HCM.

Hypothesis: Modulating MT oxidative stress signalling provides a novel therapeutic intervention for the development of HCM and other forms of cardiac hypertrophy. Integrating multi-omics data (e.g., genomics, proteomics, metabolomics) in both human and animal models may elucidate new targets for designing such therapeutic interventions that target mitochondrial abnormalities
Aims: (1) Delineate MT metabolomic and redox signalling molecules and genes that are involved in the (causal) pathways of development and disease progression of HCM and HF (2) Determine the role of modulating PAKs on MT oxidative stress as a therapeutic option for the management of HCM.

Description of the work to be undertaken:
Study #1: Delineate the role of *PAK* genes and other MT genes (both nuclear- and MT-encoded) that are involved in the (causal) pathways of development and disease progression of HCM and HF by using large-scale human omics data (GWAS, eQTL & pQTLs), as well as cardiac MRI imaging (UK Biobank).

We will also use publicly available data on metabolites and proteins to identify circulating biomarkers for those conditions. To achieve these goals, we will initially identify genetic associations shared between cardiac hypertrophy and heart failure risk loci and quantitative traits which highlight disease related intermediate phenotypes, using genome-wide association studies (GWAS). We will use in-house tools/pipelines, such as fine mapping/colocalization, Mendelian randomisation and rare variant analysis in order to determine if *PAK* genes are the underlying causal genes for cardiac hypertrophy, contractility and heart failure risk. Finally, we aim to determine both the directionality for therapeutic modulation and potential adverse effects. Collectively, these approaches help to prioritise genetic support for PAK genes in relation to HCM and HF. Moreover, there is also the potential to validate and further characterise any causal variants of interest in recall-by-genotype studies in some of the cohorts Novo Nordisk has access to.

Study #2: We will establish the efficacy of PAK modulators on HCM and mechanisms of action. We have already established well-validated HCM mouse models carrying human HCM mutation of cardiac α-actin (ACTC E99K). Once the target tissue threapeutic concentration has been established, chronic treatment of the lead compound will be conducted in mouse models carrying human HCM mutation of ACTC E99K. The therapeutic efficacy of the compound and clinical endpoints will be assessed by echocardiography, MRI, histology and biomarker assessment. We will also examine the metabolites and proteins to identify circulating biomarkers for HCM in this model. Plasma will be taken for compound concentration, histology on LV tissue sections will quantify the hypertrophic remodeling at the cardiomyocyte level and fibrosis measured by histology (e.g. Picrosirius Red staining) and real-time RT-PCR for expression of hypertrophic genes. We will determine the signalling mechanisms of activation of PAK1 on mitochondrial oxidative stress including key signalling molecules in regulating mitochondria function.

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