Proposed PhD project for BHF CoE clinical fellow (2020-2023)

Title: Genetics of *NOX4/5* DNA variation and cardiovascular function and disease

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Background:

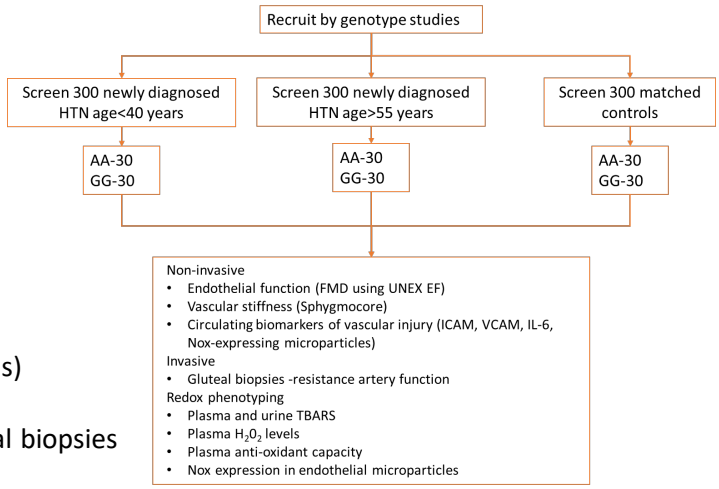
Blood pressure genome wide association studies have so far identified over 500 SNPs associated with blood pressure or hypertension. As with all GWAS, the key challenge next is the functional dissection and clinical application of these discoveries.

One of the interesting signals that has emerged from recent GWAS of blood pressure is the *NOX4* locus. Nox4 is a member of the NADPH oxidase (Nox) family responsible for production of reactive oxygen species (ROS) in vascular cells. While Nox1, Nox2 and Nox5 are associated with vascular injury and cardiovascular disease, Nox4 is cardiovascular protective. A trans-ancestry meta-analysis and a UK Biobank analysis of 192,763 and 140,886 respectively, showed a *NOX4* intronic SNP to be significantly associated with pulse pressure and nominally with systolic blood pressure. Additionally, the minor A allele of a nearby intronic SNP rs2289125 is associated with lower pulse pressure and correlates with increased NOX4 expression in endothelial cells but not in VSMCs. The minor allele of these SNPs are associated with low pulse pressure and increased NOX4 activity which is known to have anti-atherosclerotic and vasoprotective roles. The age-related increase in the prevalence of hypertension is due to an increase in systolic rather than diastolic pressure, reflected in an age-related increase in pulse pressure. Furthermore, A missense SNP in *NOX5* shows nominal association with blood pressure. These findings point to a role of genetic variation in the NOX pathway affecting blood pressure and cardiovascular risk and may explain the role of ROS and oxidative stress in hypertension. The determinants of age-related rise in pulse pressure are unknown and understanding the underlying pathological mechanisms will help develop treatment and preventive strategies for hypertension and cardiovascular risk reduction, especially in the ageing population.

Aim: To unravel the complex relationship of *NOX4* in cardiovascular risk and establishing causality

Experimental Plan: This project comprises a clinical study in genotyped hypertensive patients and a mechanistic study using vascular, molecular and cell biology approaches

1. Recruit by genotype clinical study



The clinical study will select 30 homozygous hypertensive and normotensive individuals who will undergo comprehensive cardiovascular and redox phenotyping.

Non-invasive studies

i). Endothelial function (FMD using UNEX EF)

ii). Vascular stiffness (Sphygmocore)

iii). Circulating biomarkers of vascular injury (ICAM, VCAM, IL-6, Nox-expressing microparticles)

Invasive studies

i). Isolation of subcutaneous arteries from gluteal biopsies for direct assessment of vascular function and structure

ii). Pharmacological interrogation of Nox-derived ROS in isolated arteries

Redox phenotyping

i). Plasma and urine TBARS

ii). Plasma H_20_2 levels

iii). Plasma anti-oxidant capacity

iv). Nox expression in endothelial microparticles

2. **Genetic dissection of SNPs in the *NOX4* and *NOX5* and related loci on blood pressure and cardiovascular in the UK BioBank.**
    a. Phenome-wide analysis of this variant in the UK Biobank (n=480,000) and Generation Scotland(n=20,000) using individual level data. Association testing will be conducted
    b. Arterial stiffness index analysis: In the UK Biobank, 170,000 individuals have available genotype and arterial stiffness index measurement available. .
    c. Mendelian randomization is an analysis technique, which uses genetic information to achieve an unbiased detection of causal effects.
3. **Molecular studies**

    To explore the putative role of the Nox4 and Nox5 SNPs in endothelial (EC) and vascular smooth muscle cell (VSMC) function, studies will be performed in primary culture human ECs and VSMCs harboring the SNPs. In particular cells will be exposed to pro-hypertensive stimuli (Ang II) and effects on Nox activity, ROS production, redox signaling and cell function (contraction, migration, inflammation, proliferation) will be assessed. These processes play an important role in vascular dysfunction arterial remodeling and target organ damage associated with hypertension.

Significance

Using genotyping-phenotyping approaches, big data and molecular strategies, these novel studies will elucidate a clear relationship between Nox4 activity, vascular function and blood pressure and will demonstrate that Nox4 loss of function and Nox5 gain of function promote oxidative stress and associated vascular injury in hypertension.

This is a multidisciplinary study involving big data analysis, clinical phenotyping, vascular physiology and molecular, cellular and redox biology. Hence the fellow will have opportunities to study molecular mechanisms in clinically relevant conditions.