Uncovering the mechanism of action for the coronary artery disease GWAS gene JCAD

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Project outline

Background: Genome-wide association studies (GWAS) and large-scale gene-centric genotyping have identified (and robustly replicated) many common genetic variations that contribute to increased coronary artery disease risk. Given the focus of current treatments on lipid lowering, genes which do not associate with lipid QTLs but rather implicate other effects on plaque biology have the potential to discover novel mechanisms. One such gene, JCAD was identified in previous coronary artery disease GWAS. Using *in vitro* studies in primary human endothelial cells, *in vivo* murine models of atherosclerosis and *in vivo* and *in vitro* functional human data we showed a key role for endothelial cell JCAD in atherosclerosis and flow-induced vascular remodelling via modulation of the shear stress mechanotransduction pathway. However, the consequences of JCAD overexpression on atherosclerosis progression, and how overexpression alters physiological vascular remodelling are yet to be determined. A complete understanding of JCAD biology is key if JCAD is to become a rational therapeutic target for vascular disease. This research has the potential to open up important new avenues of research in atherosclerosis and other vascular diseases.

Hypothesis: Overexpression of JCAD accelerates atherosclerosis and impacts on flow induced vascular remodelling.

Aims: We now aim to use both human cells, biobank data and targeted murine models to investigate the role of overexpression of JCAD on pathological and physiological vascular remodelling.

Description of Work: We will use an unbiased genomic (RNA-seq) and proteomic based approach to investigate the impact of overexpression of JCAD in primary endothelial cell function. Expression plasmids will be used to overexpress JCAD, and cells will then be exposed to pro-atherogenic stimuli such as oxidised low density lipoprotein, pathological flow or physiological stimuli e.g VEGF. Pathway analysis will be used to establish which cellular processes are modified by overexpression of JCAD. Targeted *in vitro* assays in primary cells e.g. angiogenesis (proliferation, tube formation) or metabolism (mitochondria function) will then be used to interrogate the cellular pathways implicated in the genomic/proteomic analysis. Once the cellular role of JCAD has been established, mutant PCSK9 and high fat feeding in Jcad over expressing mice will be used to test atherosclerosis progression and the impact of overexpression of Jcad on metabolic homeostasis. The role of JCAD in physiological flow induced vascular remodelling will be interrogated using our voluntary running system. As carried out in our previous work, we will confirm the relevance of these findings by examining data from Oxford based biobanks. The overall approach will be to establish the

mechanism of action using in vitro assays in human primary cells, establish causation using mouse models of atherosclerosis and physiological vascular remodelling, and confirm relevance using functional human data. In addition to the valuable information gained regarding JCAD the genomic and proteomic data from control cells will also be interrogated for changes in other novel coronary artery disease GWAS hits. This data set will help inform us potential targets to take forward for further analysis.

Supervisor's recent relevant publications:

1. **Douglas G**, Mehat V, Al Haj Zen A, Akoumianak I, Goel A, Rashvrook VS, Trelfa L, Donovan L, Drydale E, Chuaiphichai S, Antoniades C, Watkins H, Kyriakou T, Tzima E, **Channon KM**. A key role for the novel coronary artery disease gene *JCAD* in atherosclerosis via shear stress mechanotransduction. Cardiovascular Research, *Epub*. 2019. PMID: 31584065

2. Chuaiphichai S, Rashbrook VS, Trelfa L, Hale AB, McNeill E, Lygate, CA, Channon KM, **Douglas G**. Endothelial Cell Tetrahydrobiopterin Modulates Sensitivity to Angiotensin II-induced Resistance Vessel Remodelling, Blood pressure and Susceptibility to Aortic Abdominal Aneurysm. Hypertension. 2018: 72 (1) 128-138. PMID: 29844152

3. Jones PD, Kaiser MA, Ghaderi Najafabadi M, Koplev S, Zhao Y, **Douglas G**, Kyriakou T, Andrews S, Rajmohan R, Watkins H, **Channon KM**, Ye S, Yang X, Björkegren JLM, Samani NJ and Webb TR. JCAD Gene at the 10p11 Coronary Artery Disease Locus Regulates Hippo Signaling in Endothelial Cells. ATVB. 2018: 38 1711-1722. PMID: 29794114

4. **Douglas G**, Hale, AB, Patel J, Chuaiphichai S, Haj Zen A, Rashbrook VS, Trelfa L, Crabtree MJ, McNeill E, **Channon KM**. Roles for endothelial cell and macrophage Gch1 and tetrahydrobiopterin in atherosclerosis progression. Cardiovascular Research. 2018: 114 (10) 1385-1399. PMID: 29596571

5. Chuaiphichai S. Crabtree MJ, McNeill E, Hale AB, Trelfa L, **Channon KM**, **Douglas G**. A key role for tetrahydrobiopterin-dependent endothelial NOS regulation in resistance arteries: studies in endothelial cell tetrahydrobiopterin-deficient mice. British Journal of Pharmacology. 2017: 174 (8): 657-671. PMID: 28128438