71 A GENOME-WIDE ASSOCIATION STUDY IN INDIAN ASIANS IDENTIFIES FOUR SUSCEPTIBILITY LOCI FOR TYPE-2 DIABETES

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Background Type-2 diabetes (T2D) is a major risk factor for cardiovascular disease, and a leading causing of mortality worldwide. T2D is 2–4 fold more common among Indian Asians than Europeans, and contributes to higher cardiovascular disease mortality in Asians. Little is known of the genetic basis of T2D in Indian Asians.
Methods We carried out a genome-wide association (GWA) study of T2D in 5561 Indian Asian cases and 14 458 controls from LOLIPOP, PROMIS and SINDI cohorts. Whole genome scans were performed using the Illumina 317 k or 610 k arrays. Further testing of suggestive SNPs was carried out in independent cohorts of Indian Asian (12 K T2D cases and 25 K controls) and European ancestry (DIAGRAM+, 8 K T2D cases and 39 K controls).
Results There were two novel loci associated with T2D at $p<10^{-6}$, and an additional 57 loci associated with T2D at $p<10^{-4}$ in the GWA study. We used results from DIAGRAM+ to prioritise 19 loci for further testing in Indian Asians. In combined analysis of results from GWA and further testing, four loci now reached genome-wide significance ($p<5\times10^{-8}$) among Indian Asians. Coding variant and eQTL studies at these loci identify genes closely involved in insulin secretion and signalling.
Conclusion We identify four novel genetic loci associated with T2D in Indian Asians. Our observations provide new insights into the biological mechanisms underlying T2D, a major risk factor for cardiovascular disease.

72 ANGIOGENESIS IN RESPONSE TO UPREGULATED HYPOXIC SIGNALLING IS DEPENDENT ON HAEMODYNAMIC FLOW

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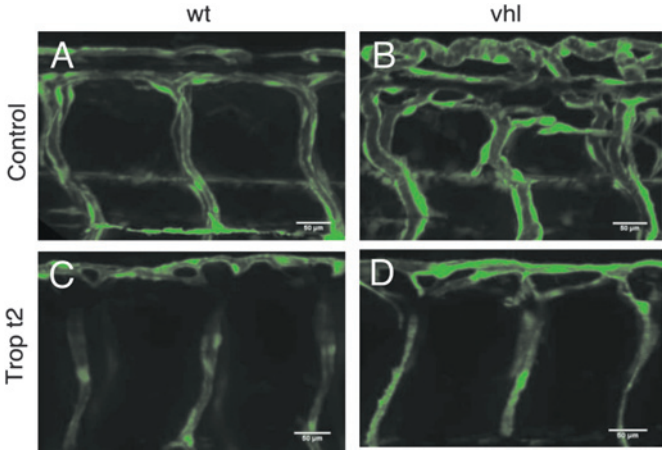
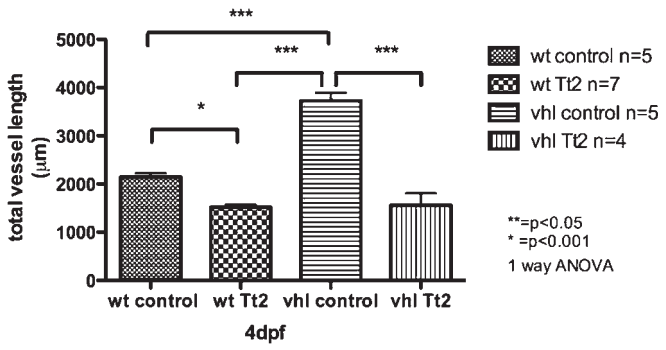
Introduction Hypoxia drives angiogenesis in a range of pathologies. Mutations in von hippel lindau protein (vhl) lead to excessive angiogenesis via upregulation of hypoxic signalling, due to impaired HIF-1α degradation. Physical forces exerted by blood flow have been shown to contribute to vascular remodelling. We therefore used vhl mutant zebrafish to observe the interplay between hypoxic signalling, haemodynamic flow and vascular development. Since NO has been shown to be both pro-angiogenic and released in response to haemodynamic force, we assessed whether NO contributed to angiogenesis in this model.
Methods Vhl mutant zebrafish were crossed with a fli1; GFP transgenic that expresses Green Fluorescent Protein (GFP) in the endothelium. Embryonic vascular development was observed in mutants and wild type siblings by confocal microscopy. To determine the role of blood flow in the angiogenic response, cardiac troponin t_2 was knocked down by morpholino antisense injection. To assess the contribution of nitric oxide, embryos were treated with either L-NAME (nitric oxide synthase inhibitor) (1mM) or sodium nitroprusside (NO donor) (100μM) from 24-h post fertilisation (hpf) until imaging at 4dpf.
Results Imaging of the developing trunk vasculature revealed that vhl mutant embryos display excessive and aberrant angiogenesis from 3dpf (Abstract 72 figure 1A, B). Cardiac troponin T_2 knockdown prevented any cardiac contraction, but embryos develop normally due to passive oxygen diffusion. Loss of blood flow did not alter normal intersegmental vessel patterning in either controls (Abstract 72 figure 1C) or vhl mutants (Abstract 72 figure 1D). However, loss of blood flow completely prevented excessive angiogenesis in vhl mutants (Abstract 72 figures 1D and 2), implying that both blood flow and hypoxic signalling are required for "pathological" angiogenesis but not developmental angiogenesis (vasculogenesis). NO synthase inhibition with L-NAME had no effect, suggesting that the contribution of flow to excessive angiogenesis in response to upregulated hypoxic signalling is NO independent.



Abstract 72 Figure 1



Abstract 72 Figure 2 Effect of troponin T knockdown on total vessel length.

Conclusion Angiogenesis in response to hypoxic signalling is critically dependent upon haemodynamic force, compared with developmental vasculogenesis that can proceed in the absence of any blood flow. This indicates a different mechanism of development for hypoxia driven angiogenesis and vasculogenesis which may have important therapeutic implications.

73 HERITABILITY OF CORONARY FLOW RESERVE

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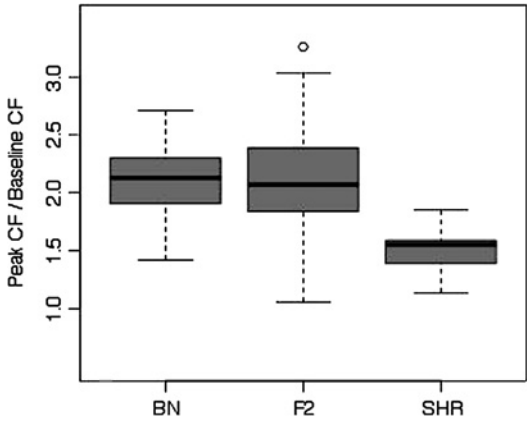
Introduction Coronary flow reserve (CRF) is the ratio of peak coronary flow during maximal coronary artery dilatation to basal

coronary flow and is an important predictor of coronary microvascular function. A variety of environmental stimuli have been shown to affect CFR but little is known about the genetic component of CFR. To characterise the genetics of CFR we initially measured in vivo blood pressure (BP) and ex vivo cardiac phenotypes including CFR in two inbred rat strains, Brown Norway (BN) and Spontaneously Hypertensive Rat (SHR) which is a genetic model for hypertension and microvascular dysfunction. We then studied BP and coronary flow (CF) phenotypes in F_1 and F_2 crosses derived from BN and SHR to estimate the heritability of CFR and its relationship with BP.

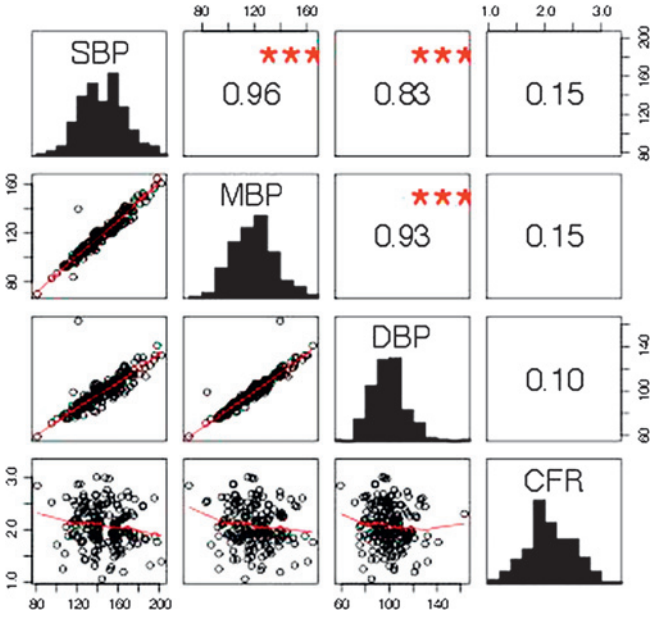
Methods Animals were anaesthetized using a mixture of Oxygen and Isoflurane. BP was measured invasively by cannulation of carotid artery. Following BP measurement hearts were excised and rapidly transferred to the ex vivo perfusion apparatus where retrograde perfusion was established using the Langendorff technique. Hearts were perfused with Carbogen buffered Kreb's solution and paced constantly at 360 bpm. A fluid filled balloon was placed in the left ventricular (LV) cavity to measure the pressure indices. CF, LV developed pressure, myocardial contractility (LV dP/dt_{max}) and myocardial relaxation (LV dP/dt_{min}) were recorded at baseline, during peak hyperaemia, regional ischaemia (induced by ligation of the proximal left anterior descending artery) and reperfusion.

Results 1) CFR differs significantly between the two inbred parental rat strains. (BN=2.1 ± 0.32, SHR=1.5 ± 0.18, $p=2.6\times10^{-7}$, n=16 each). 2) Heritability of CFR: Broad sense heritability (the proportion of total phenotypic variance attributable to total genetic variance) for CFR is 62% indicating a large and previously unrecognised genetic component of CFR. 3) Relationship between CFR and BP: We did not find statistically significant correlation between CFR and BP in the F_2 intercross (r=0.11, p=0.11, n=176). 4) Relationship between CF and myocardial relaxation (LV dP/dt_{min}): LV dP/dt_{min} correlated strongly with CF during all stages of the experiment (baseline CF, r=−0.36, p<0.0001, reperfusion CF, r=−0.40, p<0.0001).

Conclusions Our results demonstrate that CFR has a significant genetic component and is largely independent of BP effects. Furthermore we demonstrate a very significant relationship between CF and LV dP/dt_{min} indicating a link between LV diastolic dysfunction and impaired CF. Using 768 SNP genotyping assay for linkage mapping and gene expression analysis with Affymetrix rat gene chip, we will determine the quantitative trait loci and transcripts associated with CFR to improve our understanding of the genomic architecture of CFR.



Abstract 73 Figure 1 Coronary flow reserve.



Abstract 73 Figure 2 Correlation between BP and CFR.

74 MECHANISTIC STUDY FOR THE ROLE OF ADVANCED GLYCATION END PRODUCTS IN THE DEVELOPMENT OF DIABETIC HEART FAILURE

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Advanced glycation end products (AGEs) are thought to play a crucial role in the development of diabetic complications including heart failure, a leading cause of morbidity and mortality in diabetic patients. However, the molecular mechanisms that underlie the pathophysiological contribution of AGEs to heart failure development are not yet fully understood. We therefore investigated the effects and mechanisms of action of AGEs on isolated neonatal rat cardiomyocytes (NRCM). Standard molecular techniques were applied. Western blot showed that RAGE receptor is expressed in NRCM and adult mouse cardiomyocytes. Incubation of NRCM for 24 h with AGEs showed a dose dependant reduction of calcium transient amplitude with a maximum of 52% at 1 g/l ($p<0.01$) accompanied with 32% reduction in SR calcium content with no significant changes in the protein expression of calcium handling proteins. We demonstrated a 24% increase ($p<0.01$) in the production of reactive oxygen species ROS in AGE treated cardiomyocytes mediated through increased NADPH oxidase activity ($p<0.05$). Subsequent translocation of NF-KB, a transcriptional factor from the cytoplasm to the nucleus together with increased NF-KB activity resulted in a 56% increase in iNOS gene protein expression ($p<0.01$), a downstream target of NF-KB. The latter was associated with 10% increase in NO production ($p<0.05$) with subsequent nitrosylation of the Ryanodine receptor shown through immunofluoresence. Changes in calcium transient were completely inhibited when we incubated the cardiomyocytes with inhibitors of NADPH oxidase, NOS or NF-KB prior to their incubation with AGEs. In conclusion, AGEs directly decline cardiomyocytes function through binding to their RAGE receptor leading to calcium handling impairment through increased ROS production inducing activation and translocation of NF-KB to the nucleus. The latter increased transcription of iNOS with increased NO production. Coexistence