66 ETHNIC DIFFERENCES IN CAROTID INTIMAL MEDIAL THICKNESS AND CAROTID-FEMORAL PULSE WAVE VELOCITY ARE PRESENT IN UK CHILDREN

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[1]P H Whincup, [1]C M Nightingale, [2]A Rapala, [2]D Joysurry, [2]M Prescott, [2]A E Donald, [2]E Ellins, [1]A Donin, [2]S Masi, [1]C G Owen, [1]A R Rudnicka, [1]D G Cook, [2]J E Deanfield. [1]*Division of Population Health Sciences, St George's, University of London, London, UK;* [2]*Vascular Physiology Unit, Institute of Child Health, UCL, London, UK*

Introduction There are marked ethnic differences in cardiovascular disease risks in UK adults; South Asians have high risks of coronary heart disease and stroke while black African-Caribbeans have high risks of stroke and slightly low risks of coronary heart disease when compared with white Europeans. Ethnic differences in cardiovascular risk factors are apparent in childhood, but little is known abut ethnic differences in vascular structure and function during childhood. We set out to measure two vascular markers of cardiovascular risk, common carotid intimal-medial thickness (cIMT) and carotid-femoral pulse wave velocity (PWV) in UK children from different ethnic groups.

Methods We conducted a school-based study examining the cardiovascular risk profiles of 9–10 year-old UK children, including similar numbers of South Asian, black African-Caribbean and white European participants. Following a baseline cardiovascular risk survey with measurements of body build, blood pressure, fasting blood lipids, insulin and HbA1c, 1400 children were invited to have measurements of cIMT (bilateral measurements were made with a Zonare ultrasound scanner). A subgroup of these children (n=900) was also invited for PWV measurements, made with a Vicorder device. All analyses were adjusted for age, gender and allowed for clustering at school level.

Results In all, 939 children (67% response) had measurements of cIMT and 631 children (70% response) had measurements of PWV. Mean cIMT was 0.475 mm (SD 0.035 mm); mean PWV was 5.2 m/s (SD 0.7 m/s). Compared with white European children, black African-Caribbeans had higher cIMT (mean difference 0.014 mm, 95%CI 0.008 to 0.021 mm) and PWV (% difference 3.3, 95%CI 0.4 to 6.2); South Asian children had similar cIMT to white Europeans but slightly higher PWV (% difference 2.7, 95%CI −0.1 to 5.5%). cIMT was positively associated with systolic and diastolic blood pressure but not with other cardiovascular risk markers. In contrast, PWV was positively associated with adiposity, diastolic blood pressure and insulin resistance. Black African-Caribbean children had lower LDL-cholesterol levels and higher insulin and HbA1c levels than white Europeans; South Asian children had higher insulin, HbA1c and triglyceride levels. However, adjustment for these risk factors had little effect on the ethnic differences in cIMT and PWV observed.

Conclusions Ethnic differences in cIMT and PWV, markers of long-term cardiovascular risk, are apparent in childhood. These differences are not fully explained by the ethnic differences in established cardiovascular risk markers observed. The results suggest that there may be important opportunities for prevention of cardiovascular disease before adult life, particularly in high-risk ethnic minority groups.

67 SPONTANEOUS CARDIAC HYPERTROPHY AND ADVERSE LV REMODELLING IN A NOVEL HUMAN RELEVANT MOUSE MODEL OF DIABETES; A MECHANISTIC INSIGHT

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[1]S M Gibbons, [1]Z Hegab, [1]M Zi, [1]S Prehar, [1]T M A Mohammed, [2]R D Cox, [1]E J Cartwright, [1]L Neyses, [1]M A Mamas. [1]*University of Manchester, Manchester, UK;* [2]*MRC mammalian genetics unit, oxford, UK*

Heart failure (HF) is one of the commonest cardiovascular complications of Diabetes Mellitus (DM) with the prevalence of DM reported at around 30% in many pivotal heart failure studies. DM is an independent predictor of mortality in patients with HF, however molecular mechanisms that contribute to HF development in the diabetic population are poorly understood. Using a novel human relevant mouse model of DM (GENA348), identified through the MRC mouse mutagenesis programme with a point mutation in the pancreatic glucokinase (GLK) gene we investigate the molecular mechanisms that contribute to the HF phenotype in DM. GLK is the glucose sensor which regulates insulin secretion and GLK activity is reduced by 90% by the GENA348 point mutation resulting in severe hyperglycaemia. Similar mutations underlie Maturity Onset Diabetes of the Young Type 2 (MODY 2) in humans. Mean random blood glucose was found to be increased in the GENA348 mutant (HO) mice compared to wild type (WT) littermates (WT 6.9±0.3 mmol/l vs HO 20.6±0.8 mmol/l, $p<0.001$). Serial echocardiography was performed, at 3, 6 and 12 months. No significant changes in echocardiographic parameters were observed at 3 months, although by 6 months development of significant cardiac hypertrophy in HO mice was observed. At 12 months of age left ventricular dilatation was evident, characterised by an 8% increase in diastolic diameter (WT 4.08±0.10 vs HO 4.41±0.12, $p<0.05$). Systolic function was preserved although significant diastolic dysfunction was evident at 6 and 12 months with a 31% reduction in the E:A ratio. Histological staining illustrated significant cellular hypertrophy with real time PCR data demonstrating a relative 150% increase in the hypertrophic marker BNP. Hypertrophic pathways were examined through western blot analysis revealing an age dependant increase in Akt phosphorylation (3 months- no increase, 6 months-140%, 12 months-460%). Serum levels of advanced glycation end products (AGE) were also elevated by 86% (WT 21±3.5 ng/ml vs HO 39±8.3 ng/ml, $p<0.05$) as was the protein expression level of the receptor for AGEs (RAGE). In vitro cellular experiments also revealed AGEs directly activate Akt through phosphorylation and increase levels of the receptor RAGE. AGE induced phosphorylation of Akt is inhibited in the presence of wortmannin, suggesting a PI3K dependent signalling mechanism. This was further confirmed in vivo where a bolus injection of wortmannin in 6-month old mutant mice returned Akt phosphorylation levels to those seen in WT mice. In conclusion, using the first human relevant mouse model of diabetes, GENA348 we demonstrate the development of a progressive cardiac phenotype including cardiac hypertrophy, LV dilatation and diastolic dysfunction similar to the clinical manifestations of diabetic cardiomyopathy. We propose that the RAGE/PI3K/Akt pathway contributes to the molecular mechanisms associated with the cardiac phenotype.

68 RARE ALLELES IN GENETIC PREDISPOSITION TO CORONARY ARTERY DISEASE: INSIGHTS FROM THE NOVEL ANALYSIS OF GENE-CENTRIC ARRAY

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[1]P Christofidou, [1]R Debiec, [1]C P Nelson, [1]P S Braund, [1]L D S Bloomer, [2]S G Ball, [2]A J Balmforth, [2]A S Hall, [1]M Tomaszewski, [1]N J Samani. [1]*University of Leicester, Leicester, UK;* [2]*University of Leeds, Leeds, UK*

Background Genome-wide association studies have been successful in identifying association between several common variants and coronary artery disease (CAD). However, collectively these variants explain only a small proportion of CAD heritability. It is becoming increasingly clear that the remainder of the "missing CAD heritability" could be explained by low frequency/rare alleles. Because of the small number of observations for any given rare allele, the power to detect its association with a phenotype is a major limiting factor in genetic analysis. In this study we have undertaken a novel statistical approach that combines information from all low frequency

(MAF<5%) SNPs at one locus in gene-centric analysis of CAD. We hypothesised that patients with CAD will show over-representation of rare alleles compared to controls.

Methods To examine associations between rare alleles and CAD, we have used data from 2119 CAD cases and 2440 healthy controls recruited to the Welcome Trust Case-Control Consortium (WTCCC) Study. DNA from each subject was genotyped for approximately 45 000 SNPs in more than 2000 genes/loci using 50K IBC array (version 1). Association analysis was based on the CCRaVAT (Case-Control Rare Variant Analysis Tool) algorithm that maximises statistical power by combining all rare alleles within defined regions into a single "super locus". Differences in the proportion of cases and controls carrying rare "super loci" were tested by Pearson's or Fisher's exact test. Empirical p values were generated by permuting case-control status a predefined number of times and repeating the analysis for each replicate.

Results 5 candidate regions (MMP23B, VEGFA, DVL1, RIPK1, LPAL2) showed an over-accumulation of rare alleles in patients with CAD when compared to controls (FDR<50%). The number of analysed rare alleles at each of these loci ranged from 4 to 42. The most significant over-representation of rare variants were identified at MMP23B (matrix metallopeptidase 23B gene; $p=1.3/10^4$), a gene previously unsuspected to play a major role in CAD and VEGFA (vascular endothelial growth factor A; $p=2.6\times10^{-4}$). Only one of the identified genes (LPAL2; $p=1.7\times10^{-3}$) lies within the locus that was previously shown to harbour rare variants associated with susceptibility to CAD.

Conclusions Rare alleles are associated with predisposition to CAD and this gene-centric analysis combining information from low-frequency variants of the same locus has a potential to uncover, at least a proportion of, the "missing heritability" of CAD.

69 GENOME WIDE METHYLATION ANALYSIS IN CORONARY ARTERY DISEASE

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K J Dick, C P Nelson, P S Braund, A H Goodall, N J Samani. *University of Leicester, Leicester, UK*

Background Using genome-wide association studies several genes have been identified that affect the risk of CAD. However, these genes only explain part of the heritability. There is increasing evidence of the role of epigenetic regulation in complex diseases that may explain part of the missing heritability. DNA methylation is an important epigenetic change that regulates gene expression. Any role of methylation in CAD is poorly understood. Therefore we undertook an exploratory genome-wide screen to identify genes differentially methylated in CAD cases and controls.

Methods We characterised DNA methylation in 24 CAD patients with a documented history of MI and 24 matched controls from the Cardiogenics case-control cohort. All subjects were male, ranging in age from 40 to 57 years. For each subject, genomic DNA, isolated from whole blood, was bisulphite converted and run on Illumina HumanMethylation27 bead chips. The HumanMethylation27 chips interrogate 27 578 CpG sites spanning 14 495 genes with an average of 2 CpG sites per gene.

Results Global DNA methylation level was significantly higher in cases compared to controls ($p=9.0\times10^{-4}$). Furthermore, 686 individual CpG sites, spanning 633 genes showed statistically significant differences in methylation levels between cases and controls. Significant signals after Bonferroni correction for multiple comparisons included GNAS ($p=7.94\times10^{-5}$), which is involved in receptor-mediated signal transduction, PCMT1 ($p=7.94\times10^{-5}$), ACD ($p=3.48\times10^{-4}$ part of the telosome/shelterin complex), ATXN2 and APOA1 ($p=5.6\times10^{-3}$ and p=0.01). To explore the potential functional importance of differences in methylation level in cases and controls for individual genes, we examined the relationship of methylation level to transcript level in monocytes and macrophages on a gene by gene basis and identified several genes including GNAS and PCMT1 that showed significant correlations between gene expression and methylation. Pathway enrichment analysis of the differentially methylated genes using the DAVID bioinformatics resource identified a number of pathways that showed significant enrichment including the calcium signalling pathway ($p=3.85\times10^{-7}$).

Conclusions This pilot study has shown several significant differences in gene methylation patterns between CAD cases and controls. We also found a correlation between methylation level and gene expression for a number of these genes. Genes differentially methylated in CAD are significantly enriched for a number of pathways including the calcium signalling pathway. While these findings require further validation they suggest that epigenetic changes may play an important role in the pathogenesis of CAD.

70 GENE EXPRESSION AT THE 9p21 LOCUS AND CAD RISK

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[1]C P Nelson, [2]P Lundmark, [1]V Codd, [1]A H Goodall, [2]A C Syvänen, [1]N J Samani. *[1]Department of Cardiovascular Sciences, University of Leicester, Glenfield Hospital, Leicester, UK; [2]Department of Medical Sciences, Molecular Medicine, Science for Life Laboratory, Uppsala University, Uppsala, Sweden*

Background Human chromosome 9p21 harbours a locus that affects risk of coronary artery disease (CAD) through an unknown mechanism. The variants at the locus most strongly associated with CAD lie in non-coding regions suggesting that the affect on CAD risk may be mediated through regulation of gene expression. We investigated the association of single nucleotide polymorphisms (SNPs) across the locus with expression of genes in the locus and compared this with association of the same SNPs with CAD risk.

Methods We quantified transcript levels for CDKN2A, CDKN2B, ARF and MTAP in circulating monocytes from 422 healthy blood donors and 386 CAD cases and obtained genotypes for SNPs in the 9p21 region in the same subjects using genome-wide platforms. We also quantified allelic expression (AE) for these genes and for ANRIL in 186 of the healthy blood donors. We compared expression quantitative trait loci (eQTL) associations for the genes with association findings for the same SNPs for CAD in the Wellcome Trust Case Control Consortium study.

Results In the global gene expression analysis, we found strong cis eQTLs for both CDKN2B ($p=1.3\times10^{-38}$) and MTAP ($p=6.6\times10^{-23}$), explaining 17.0% and 8.0% of the expression of these genes. AE analysis confirmed these findings (CDKN2B, $p=6.0\times10^{-64}$; MTAP, $p=1.4\times10^{-38}$) and also showed a significant cis-eQTL effect on ANRIL expression ($p=3.5\times10^{-28}$). Interestingly, the SNPs associated with CDKN2B and ANRIL expression were the same. However, the SNPs showing e-QTL effects were distinct from SNPs that showed an association with CAD risk ($p=2.2\times10^{-12}$). Even in the region with a physical overlap of variants affecting expression of CDKN2B/ANRIL and CAD risk, the effects of the respective variants were independent of each other. Expression of CDKN2A and ARF was low but did not show any obvious eQTL effect, or differences according to genotype at CAD-associated SNPs.

Conclusions Our findings in monocytes do not support the hypothesis that the chromosome 9p21 locus mediates CAD risk by affecting expression of the genes at the locus. The mechanism by which the chromosome 9p21 locus affects CAD risk requires further elucidation.