general population of up to 2%. There has been growing evidence supporting its familial predisposition with an autosomal dominant pattern of inheritance. It is often associated with ascending aortic dilatation and dissection, occurring at a younger age than in patients with idiopathic aortic aneurysms. BAV disease carries a 6% lifetime risk of aortic dissection, 9 times higher than that of the general population. Thus, the presence of BAV and dilatation of the ascending aorta requires regular monitoring with a view to timely pre-emptive surgery. Current ACC/AHA guidelines state that echocardiographic screening of first degree relatives of patients with BAV is recommended. This, however, to our knowledge, is not routinely done within the UK.

Methodology and Results We set out to explore the practicalities of running a routine echocardiographic screening programme for first degree relatives of patients with BAV disease. We identified a total of 47 patients who had undergone aortic valve surgery performed by the same Consultant Cardiothoracic Surgeon in the context of BAV disease in the period May 2007–September 2009. Screening of first degree relatives was offered to these patients. 24 patients (51%) gave us information regarding family members who would like to attend for an echocardiogram. A total of 75 first degree relatives were referred—an approximate average of 3 per patient. Out of these, 52 relatives (70%) actually attended for an appointment. The remainder did not undergo testing with us as they either lived in a different geographic region or expressed a personal preference not to be scanned at this time. The incidence of newly diagnosed bicuspid aortic valve disease in our cohort of first degree relatives was 8% (4 out of 52 relatives). One of these asymptomatic individuals had a significant ascending aortic aneurysm, which required prompt surgery. Among the relatives of the 24 index patients, there were a total of 8 cases (3: 1 ratio) of bicuspid aortic valve disease—either known or newly diagnosed via screening.

Conclusions There is a relatively high prevalence and incidence of bicuspid aortic valve disease among first degree relatives of patients with this common congenital cardiac abnormality. Routine echocardiographic screening should be offered to these families. Implementing such a programme is limited by adequate motivation to attend for a screening test if well, and by varying clinical practice in different geographic regions. Patients with bicuspid aortic valve disease should be made aware of its familial pattern of inheritance and screening of their first degree relatives should be actively pursued in order to reduce the potential morbidity and mortality associated with this condition and its related aortopathy.

137 A CITED2->VEGFA PATHWAY COUPLES MYOCARDIAL AND CORONARY VASCULAR GROWTH IN THE DEVELOPING MOUSE HEART

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Introduction Myocardial development is dependent on the concomitant growth of cardiomyocytes and a supporting vascular network. The coupling of myocardial and coronary vascular development is mediated in part by VEGFA signalling. *Cited2* is a transcriptional co-factor that can inhibit hypoxia-activated transcription and also acts as a co-activator for transcription factors such as TFAP2. Genetic evidence indicates that *Cited2* is essential for cardiac left-right patterning via regulation of the *Nodal-Pitx2c* left-right patterning pathway. Zygotic and epiblastic deletion of *Cited2* results in atrio-ventricular septation, outflow tract and aortic arch abnormalities, as well as left-right patterning defects such as right-isomerism. *Cited2* is also essential for adrenal, neural crest, liver, lung, lens and placental development. However, the early requirement of *Cited2* in

left-right patterning and placental development makes it difficult to identify a later specific role for *Cited2* in myocardial development. To overcome this problem we therefore investigated the role of *Cited2* in the myocardium by conditional deletion in cardiomyocyte precursors.

Methods *Cited2* was selectively deleted from cardiomyocytes by intercrossing mice transgenic for *Cited2* and *Nkx2-5Cre*. Embryos were collected and processed for analysis by histology, MRI, X-Gal staining, quantitative reverse transcriptase PCR (Q-RT-PCR), chromatin immunoprecipitation and transient transfection assays.

Results The cardiomyocyte specific knockout of *Cited2* results in abnormal myocardial compact zone growth and ventricular septal defects. This is associated with a decreased ratio in the number of small vessels to large vessels, and a reduction in *Vegfa* expression. We also show that CITED2 is present at the *Vegfa* promoter in mouse embryonic hearts, and that it stimulates human *VEGFA* promoter activity in cooperation with TFAP2 transcription factors in transient transfection assays. However, we observed no change in the myocardial expression of the left-right patterning gene *Pitx2c*, a known target of *Cited2*.

Conclusions The myocardial and capillary defects observed in myocardial loss of *Cited2* are not associated with *Pitx2c* deficiency and suggests that *Cited2* can cause myocardial and vascular defects via a mechanism that is distinct from its effect on the left-right patterning pathway. Our results delineate a novel mechanism of *Vegfa* regulation by CITED2 and TFAP2 transcription factors, and indicate that coupling of myocardial and coronary vascular growth in the developing mouse heart occurs, at least in part, through a *Cited2*->*Vegfa* pathway. This pathway may be targeted for the treatment of heart failure resulting from ischaemic heart disease.

138 CELL-SPECIFIC ROLE OF NOX2 NADPH OXIDASE IN DEVELOPMENT OF ANGIOTENSIN II-INDUCED CARDIAC FIBROSIS IN VIVO

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Introduction Mice globally deficient in Nox2 are protected against cardiac fibrosis in response to chronic AngII infusion even though the degree of hypertrophy was unaltered. The selective effect of Nox2 on fibrosis may reflect its activation in a non-cardiomyocyte cell type. We hypothesised that Nox2, which is expressed in endothelial cells and inflammatory cells, may be important for cardiac fibrosis in these cell types.

Methods To investigate the role of Nox2 in inflammatory cells, we generated chimeric mice by irradiation (10Gy, 15 min) to deplete resident bone marrow cells, followed by bone marrow (BM) transplantation, using the following permutations: wild-type (WT) recipient with either KO or WT BM, and KO recipients with WT BM. To assess the role of endothelial Nox2, we used transgenic mice with endothelial-specific overexpression of Nox2 (TG) utilising the tie2 promoter construct.

Result AngII (1.1 mg/kg/day, 14-day) infusion caused similar increase in systolic hypertension and cardiac hypertrophy in all 3 chimeric groups. However, cardiac fibrosis assessed by Sirius red staining was significantly lower in KO mice receiving WT BM (0.5±0.1%) compared to the WT:WT group (2.7±0.7%) or in WT receiving KO BM (2.3±0.6%). These data suggested that resident Nox2-expressing cells are responsible for the protective effect observed in global Nox2 KO mice. TG mice developed the same level of systolic hypertension and hypertrophy as WT littermates after AngII infusion. However, the extent of cardiac fibrosis was significantly greater in TG than WT by ~2-fold (p<0.05). This was associated with a greater degree of infiltration by CD45+

inflammatory cells (~ 1.8 -fold, $p < 0.05$) and 30% ($p < 0.05$) more VCAM-1 positive blood vessels in AngII treated TG hearts. Furthermore, isolated TG endothelial cells recruited ~ 2 -fold ($p < 0.05$) more leukocytes than WT upon AngII treatment.

Conclusion These results indicate there is a cell-specific role of endothelial Nox2 in the development of fibrosis. Endothelial Nox2 enhances AngII-induced cardiac fibrosis, possibly by enhancing inflammatory cell recruitment and influx via VCAM-1 expression. Although inflammatory cells may be important for the development of fibrosis, our results indicate that Nox2 in these cells is not essential for any pro-fibrotic effect.

139 ENDOTHELIAL SPECIFIC INSULIN RESISTANCE PROMOTES THE DEVELOPMENT OF ATHEROSCLEROSIS

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Background Global insulin resistance and endothelial dysfunction have been identified as predisposing factors for atherosclerosis. However, it is unclear whether selective insulin resistance in endothelial cells alone, is sufficient to promote atherosclerosis. Here we addressed this question by crossing Endothelial Specific Mutant Insulin Receptor Over-expressing (ESMIRO) mice with ApoE null mice. ESMIRO mice over-express a human insulin receptor with Ala-Thr1134 mutation in the tyrosine kinase domain (which disrupts insulin signalling) selectively in endothelial cells under the control of the tie-2 promoter/enhancer.

Methods Male ApoE^{-/-}ESMIRO mice were compared with sex-matched littermate ApoE^{-/-} mice (both on a C57Bl6 background) after feeding a Western-style diet for 12 weeks.

Results ApoE^{-/-}ESMIRO mice were morphologically indistinguishable from ApoE^{-/-} control littermates, with normal development and no difference between groups in body mass. Heart rate, systolic blood pressure, glucose tolerance, insulin sensitivity and fasting glucose levels were similar in ApoE^{-/-}ESMIRO and ApoE^{-/-} mice. Aortic lipid deposition, assessed by en-face oil red O staining, was similar in ApoE^{-/-}ESMIRO and ApoE^{-/-} mice ($6.4\% \pm 0.5\%$ vs $5.8\% \pm 0.5\%$; $p = 0.39$). However, atherosclerotic lesion area in cross sections of aortic sinus was significantly increased in ApoE^{-/-}ESMIRO mice compared to ApoE^{-/-} controls ($24.8\% \pm 2.4\%$ vs $16.6\% \pm 2.4\%$; $p = 0.02$). Absolute plaque size was also significantly increased in ApoE^{-/-}ESMIRO mice compared to ApoE controls ($226\,448.9 \pm 16\,154\ \mu\text{m}^2$ vs $149\,424.41 \pm 24\,221\ \mu\text{m}^2$; $p = 0.01$).

Conclusions Endothelial specific insulin resistance is sufficient to promote atherosclerosis and increase lesion area in ApoE null mice. This suggests that enhancing endothelial insulin sensitivity may be an appropriate target to prevent atherosclerosis in insulin-resistant conditions.

140 IN VIVO DEPLETION OF ENDOGLIN RESULTS IN SIGNIFICANT LEFT VENTRICULAR REMODELLING

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Endoglin, a TGF β co-receptor, is essential for cardiovascular development. However, endoglin also has an important role in fibrosis in adult life. Endoglin heterozygous mice have been shown to have reduced fibrosis in response to renal injury. They also have significantly

reduced cardiac function following myocardial infarction. In rat cardiac fibroblasts, endoglin expression is up regulated following stimulation with angiotensin II and TGF β , resulting in reduced expression of MMP1 and increased expression of collagen I. These effects are inhibited by an endoglin specific antibody. Using our conditional endoglin knockout mice we sought to investigate the role of endoglin in cardiac healing following myocardial infarction. Adult Eng fl/fl Rosa26-Cre^{ERT2} or control (Eng fl/fl) mice were treated with intraperitoneal injection of tamoxifen for 5 days to activate Cre^{ERT2} and deplete endoglin by Cre-lox recombination. Mice then underwent surgical coronary artery ligation or sham operation. Cine cardiac MRI was performed 28 days after injury. Measurement of left ventricular (LV) volumes and myocardial mass were made using ImageJ, and parameters of cardiac function were calculated. We found that LV volumes and mass were significantly increased ($p < 0.001$) and ejection fraction significantly reduced ($p = 0.005$) in endoglin deficient mice compared to controls. However, we also noticed LV volume and mass were increased in sham operated endoglin deficient mice. This led us to investigate the effect of endoglin knockdown on normal heart structure and function in adult mice. Cine cardiac MRI was therefore performed on mice without any surgical procedure after endoglin knockdown. We found that in the endoglin deficient mice, LV volume and mass were again significantly increased ($p < 0.03$). However, ejection fraction did not differ significantly from controls. These results demonstrate that depletion of endoglin results in significant left ventricular remodelling and suggest that endoglin plays an essential role in the maintenance of normal cardiac structure. The fact that cardiac function was preserved indicates that this is not a cardiomyopathic process and we hypothesise that the increased left ventricular volume in the endoglin-deficient mice may be the result of alterations in the extracellular matrix. We are currently investigating this potential molecular mechanism for left ventricular remodelling in the absence of endoglin.

141 TISSUE FACTOR PATHWAY INHIBITOR REGULATES VASCULAR DEVELOPMENT IN ZEBRAFISH

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Introduction Angiogenesis requires the coordinate regulation of multiple biological pathways, including haemostasis. Tissue factor pathway inhibitor (TFPI) is a potent anticoagulant molecule, inhibiting tissue factor-led coagulation. However, the role of TFPI in vascular development is unknown. Zebrafish (*Danio rerio*) provide a unique model system to study vascular development in vertebrates. Despite the divergence of jawed fish (teleosts) over 430 million years ago, there is notable conservation of the constituent molecules of the clotting cascade. Multiple features make this vertebrate model unique, including its genetic accessibility, the ability to titrate the degree of genetic knock-down, external embryonic development, and the transparent nature of the embryos.

Methods Using in-situ hybridisation techniques, we demonstrate TFPI expression during early vertebrate development. We then utilise transgenic fish with labelled endothelium (Eli1GFP) and erythrocytes (GATA1dsRed), to study in real time, concomitant fluorescent imaging of both structural development and dynamic blood flow observation, in living zebrafish embryos.

Results TFPI expression was identified at 24 h post fertilisation (hpf) in the pronephros (Abstract 141 figure 1—dark blue staining denotes TFPI expression; none seen in control embryos). Subsequently, TFPI mRNA became more abundant, localising to