ameliorated Ang II-induced $Ca^{2+}$ influx in human VSMC. In conclusion, TRPM2/NCX-induced increase in intracellular levels of calcium may be involved in hypertension-associated vascular dysfunction. Our data also suggests that oxidative stress regulates $Ca^{2+}$ homeostasis through TRPM2-dependent mechanisms.

## 160 CARDIAC DYSFUNCTION IN MICE WITH REDUCED STRIATIN EXPRESSION

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**Purpose** Some protein kinases are regulated in STRIPAK complexes, with striatin (STRN) forming a scaffold. STRN mutations are associated with hypertension in humans and decreased expression of STRN causes arrhythmias and cardiomyopathy in dogs. Because striatin binds $Ca^{2+}$-calmodulin, it was thought to participate in $Ca^{2+}$-dependent signalling, but it is now recognised as regulatory B subunits of protein phosphatase PP2A. Thus, striatin holds the kinase in proximity to PP2A, maintaining it in an inactive state. Global striatin knockout is embryonic lethal in mice, but heterozygotes are viable and fertile. Our hypothesis is that heterozygote deletion of striatin will be detrimental to cardiac function in the context of hypertension.

**Methods** Cardiac function was assessed by echocardiography using a Vevo 2100 system. M-mode images of the short axis view were used for analysis of cardiac dimensions and ventricular function. Pulse-wave analysis of aortic flow was also performed. Following two baseline measurements, Strn ± mice (10–12 weeks) were infused with the pro-hypertensive hormone angiotensin II (AngII; 0.8 mg/kg/day; n=5) via osmotic minipumps for 24 hour. Cardiac function and aortic flow was measured and normalised to the mean of the baseline values.

**Results** Compared to baseline, heart rates were elevated by 18%, whilst ejection fraction was reduced to 61% of baseline. Cardiac output was relatively preserved (87% of baseline). The internal left ventricular (LV) diameter was increased to some extent, but systolic function was severely compromised. Posterior wall thickness during systole was reduced to 79% of baseline measurements and LV internal diameter was increased by 23% giving a calculated increase in systolic volume of 67%.

**Conclusions** STRN plays an important role in maintaining systolic function during hypertension. Thus, the protein kinases that are regulated in striatin complexes must be significant regulators of cardiac contractility.

## 161 BTK INHIBITORS: FRIENDS OR FOES?

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10.1136/heartjnl-2017-311726.160

**Introduction** Bruton's tyrosine kinase (BTK) plays a crucial role in the development and maturation of B-cells. A common side effect of Ibrutinib, a BTK inhibitor approved for the treatment of chronic lymphocytic leukaemia (CLL), is active bleeding in the absence of vascular injury. The mechanisms by which ibrutinib alters haemostasis however are currently unclear. The aim of this study was to investigate the effects of ibrutinib on platelet and endothelial cell function *in vitro*, to determine the mechanisms that underpin ibrutinib-induced bleeding.

**Methods** Platelet rich plasma (PRP) collected from healthy volunteers was treated with increasing concentrations of ibrutinib for 15 min at 37°C, prior to stimulation with collagen (2 µg/ml) or ADP (10 µM). Platelet function and activation were measured by light transmission aggregometry (LTA) and flow cytometry respectively ($CD62P^{+}$, $PAC1^{+}$) and platelet morphology analysed using scanning electron microscopy (SEM). Platelet signalling pathways were analysed by Western blotting and the generation of endothelial microvesicles (EMVs) from HUVECs enumerated by flow cytometry, following 24 hours treatment with ibrutinib (increasing concentrations from 0.1 µM to 10 µM).

**Results** Ibrutinib significantly reduced collagen-mediated platelet aggregation and activation in a dose-dependent manner ($p<0.05$). SEM analysis also demonstrated that collagen-mediated shape change and filopodia formation was defective following ibrutinib treatment. Consistent with these findings, signalling downstream of the collagen GPVI receptor was perturbed, with a marked reduction in PLCÎ³2 phosphorylation. Ibrutinib only exerted mild inhibition of ADP-induced platelet aggregation ($p<0.05$), which was accompanied by reduced $PLA_2$ activation and inhibition of VASP dephosphorylation. Additionally, our results demonstrated that at low concentrations, ibrutinib reduced the generation of pro-thrombotic EMVs, an effect that is reversed at the highest concentrations ($p<0.05$).

**Conclusion** Ibrutinib reduces collagen and ADP-mediated platelet aggregation, and activation by reducing phosphorylation of PLCÎ³2 and $PLA_2$ and inhibiting VASP dephosphorylation. In addition, ibrutinib also appears to alter endothelial cell function by reducing EMV release. Understanding the mechanisms by which ibrutinib alters haemostasis may lead to the identification of novel antithrombotic targets.

## 162 COMBINATORIAL ANALYSIS OF EXOME SEQUENCING DATA AND COPY NUMBER VARIANTS IN CONGENITAL HEART DISEASE PATIENTS

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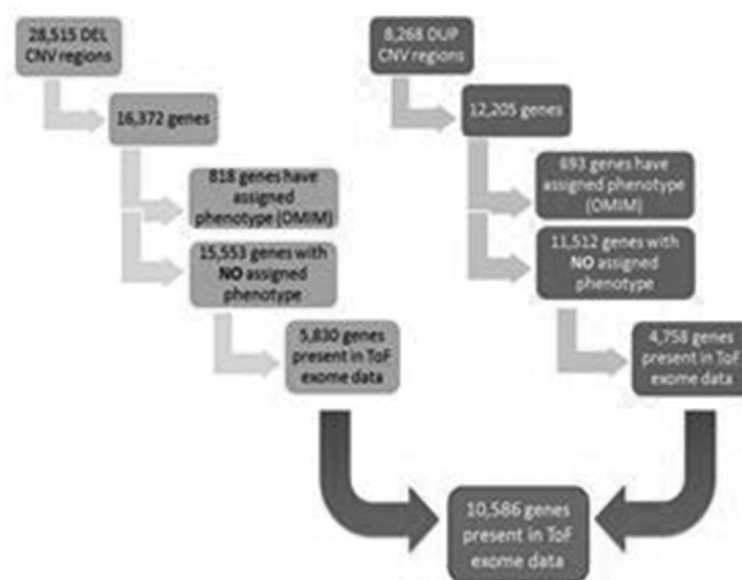
Congenital heart disease (CHD) is the most common type of birth defect in humans. Most cases of CHD are sporadic with the specific interactions between genetic variants and environmental factors involved in their pathogenesis uncharacterised. Various whole exome sequencing studies have identified *de novo* mutations in different genes; however they have only explained a small percentage of CHD cases.Previous work from the group and others has identified chromosomal regions where rare copy number variants (CNVs) were significantly enriched in CHD cases compared to controls. We hypothesise that utilising available CNV data to prioritise candidate regions within which we will interrogate exome sequencing

data in CHD cases will be a productive means of identifying causative genes. In this study we have undertaken various refinement steps to narrow down potentially causative candidate gene/s within deleted (DEL) and duplicated (DUP) CNV regions that have been previously shown to be highly associated with non-syndromic CHD patients. Firstly, we have generated case DEL and DUP CNV lists. This was done by updating a published meta-analysis study (our group have contributed to this study) though utilisation of DECIPHER, ISCA, ECARUCA databases and published studies, using different key terms to identify further non-syndromic CHD patients. We then utilised BedTools to compare these case lists with the corresponding control CNV lists generated by using controls from published literature, DECIPHER, the Database of Genomic Variants and the 1000Genome Phase 3 CNVs. The resulting unique cases CNV regions were annotated and compared against an in-house list of candidate genes (containing novel or rare variants) generated from an exome data analysis of 850 Tetralogy of Fallot (ToF) patients. Genes were further prioritised based on whether they have already an assigned human phenotype, on their ExAC CNV scores, probability of haploinsufficiency (pHI) and loss of function (LoF) intolerance scores. Initially, we have identified 10 586 genes for which 1986 genes are present in both DEL and DUP CNV regions, 2772 genes are unique in DUP and 3842 genes unique in DEL regions (Figure 1). Further analysis of genes in DEL regions and genes present in both types of CNVs revealed that 1,150/3,842 genes and 588/1,986 genes respectively have LoF variants in our ToF exome data. Additional filtering with pHI and pLI scores resulted in 57 genes collectively. This is an on-going work and our plan is to design a next-generation sequencing panel to screen our final candidate gene list in an additional 2000 CHD cases. We will focus on the most promising candidate gene emerging from the discovery experiment to perform functional work. Our experimental strategy will vary depending on what is known about the gene, and whether its involved or not in any well-known signalling pathway during heart development such as the Wnt and VEGF pathways.




**Abstract 162 Figure 1** Flowchart of the initial filtering of CNV regions to genes.

## 163 LOX-1-SPECIFIC AFFIMERS BLOCK OXLDL ACCUMULATION IN VITRO

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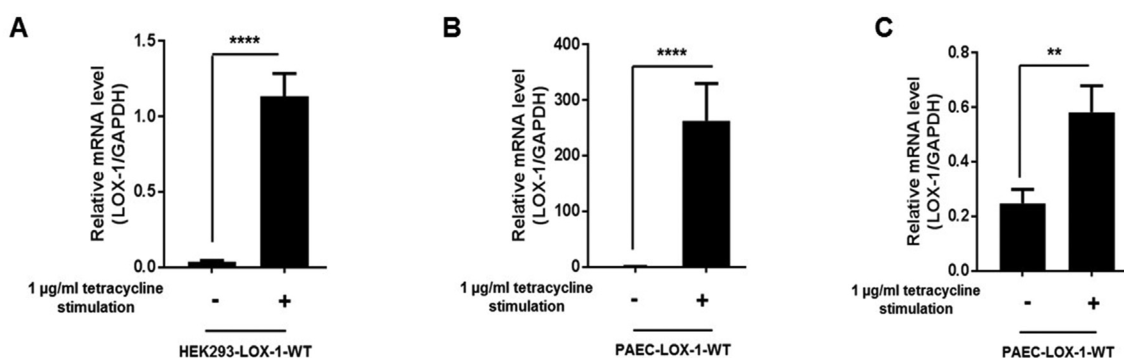
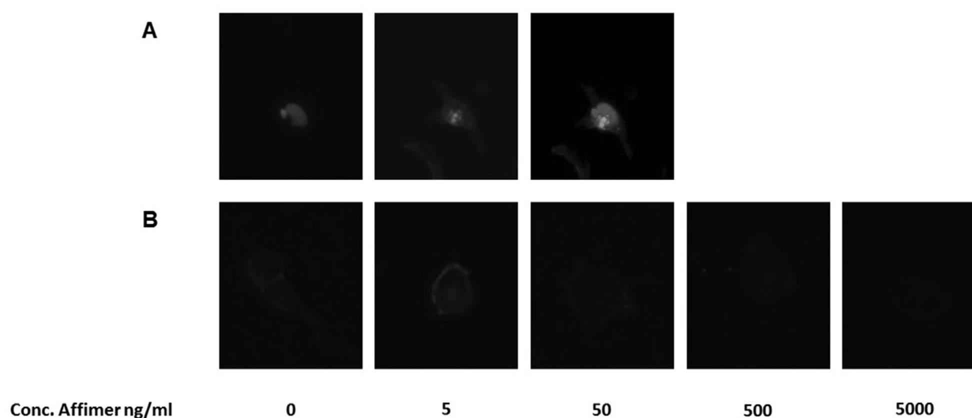
**Introduction** Lectin-like oxidised low density lipoprotein receptor 1 (LOX-1, SREC-1, OLR1) is a class E scavenger which binds oxidised low density lipoproteins (oxLDL). It is expressed on endothelial cells, macrophages and smooth muscle cells, where it has a major role in sensing oxLDL, in the triggering of cellular dysfunction and in contribution to atherosclerosis. LOX-1 is thus an important target for the prevention and/or treatment of atherosclerosis. Affimers are synthetic protein scaffolds containing two hypervariable amino acid loops which enable recognition of a wide range of molecules. We aimed to investigate whether affimers specific for LOX-1 could be used to target oxLDL recognition and uptake using an *in vitro* cellular model.

**Methods** Human embryonic kidney (HEK293T) and porcine aortic endothelial cells (PAEC) with tetracycline-inducible protein expression were engineered to expressed human LOX-1-FLAG construct. Isolated clonal lines for each cell type was checked using RT-PCR, immunoblotting and immunofluorescence microscopy. After overnight incubation with tetracycline (1 microgram/ml), cells were starved and incubated with=1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate




**Abstract 163 Figure 1** Relative mRNA levels of: A) Human LOX-1 in HEKs, B) Human LOX-1 in PAECs and C) Porcine LOX-1 in PAECs




**Abstract 163 Figure 2** Immunofluorescence Microscopy of HEK293T after induction with 1microgram/ml Tetracycline. A) Blue (DAPI) staining of nucleus and green (anti-FLAG) staining of FLAG-tagged LOX-1 B) Affimer effects on oxLDL (red) uptake.

(DiI)-labelled oxLDL (10 microgram/ml) for 30 min. Cells were fixed and imaged to characterise the uptake of OxLDL. Blocking experiments using 5 different affimers, was carried out by adding affimer prior to incubation with DiI-oxLDL. Effects on oxLDL binding and cellular accumulation was evaluated using fluorescence microscopy.

**Results** The levels of LOX-1-FLAG mRNA were significantly raised ($p<0.001$) in both cell types in response to tetracycline. Porcine endothelial cells also had significant levels of endogenous native porcine LOX-1(Figure 1). The presence of human LOX-1-FLAG protein was confirmed by immunofluorescence microscopy (Figure 2a). Incubation with oxLDL demonstrated tetracycline-dependent labelled lipid particle uptake in HEK293T LOX-1-FLAG expressing cells. In contrast, oxLDL uptake was not significantly raised compared to uninduced baseline in PAEC LOX-1-FLAG expressing cells. Incubation with LOX-1 binding affimers led to a decrease in oxLDL uptake in both cell lines after induction of LOX-1-FLAG expression (Figure 2b).

**Conclusion** We have successfully constructed two mammalian cell lines with a stable, inducible LOX-1 expression system. This has allowed us to demonstrate LOX-1-dependent oxLDL uptake. However, such expression is dependent on negligible expression of other scavenger receptors that mediate oxLDL binding and/or uptake. The use of LOX-1-specific affimers show that oxLDL binding and/or uptake is significantly decreased in both cell lines. Future experiments aim to determine the effects of such LOX-1-specific affimers in primary vascular cells and animal models in the context of atherosclerosis and vascular disease.

## 164 ABSTRACT WITHDRAWN

## 165 HUMAN ATHEROSCLEROSIS IS CHARACTERISED BY OXIDATIVE DNA DAMAGE DUE TO DEFECTIVE BASE EXCISION REPAIR

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**Rationale** Human atherosclerotic plaques show extensive oxidative DNA damage in vascular smooth muscle cells (VSMCs) and macrophages, including accumulation of 8-oxo-7,8-dihydroguanine (8-oxoG), the most abundant DNA base lesion on oxidative exposure. 8-oxoG is repaired by base excision repair (BER) mediated by DNA glycosylases, including the specific non-redundant 8-oxoguanine DNA glycosylase-1 (OGG1). OGG1 activity is regulated by acetylation through the p300 acetyltransferase. However, the role of oxidative damage in VSMC function and the regulation of OGG1 in atherosclerosis are unknown.

**Methodology** We stably expressed OGG1 or the $^{K338/K341}$OGG1 acetylation mutant in rat VSMCs *in vitro*, and treated cells with oxidative stress. The functional effects of OGG1 *in vivo* were