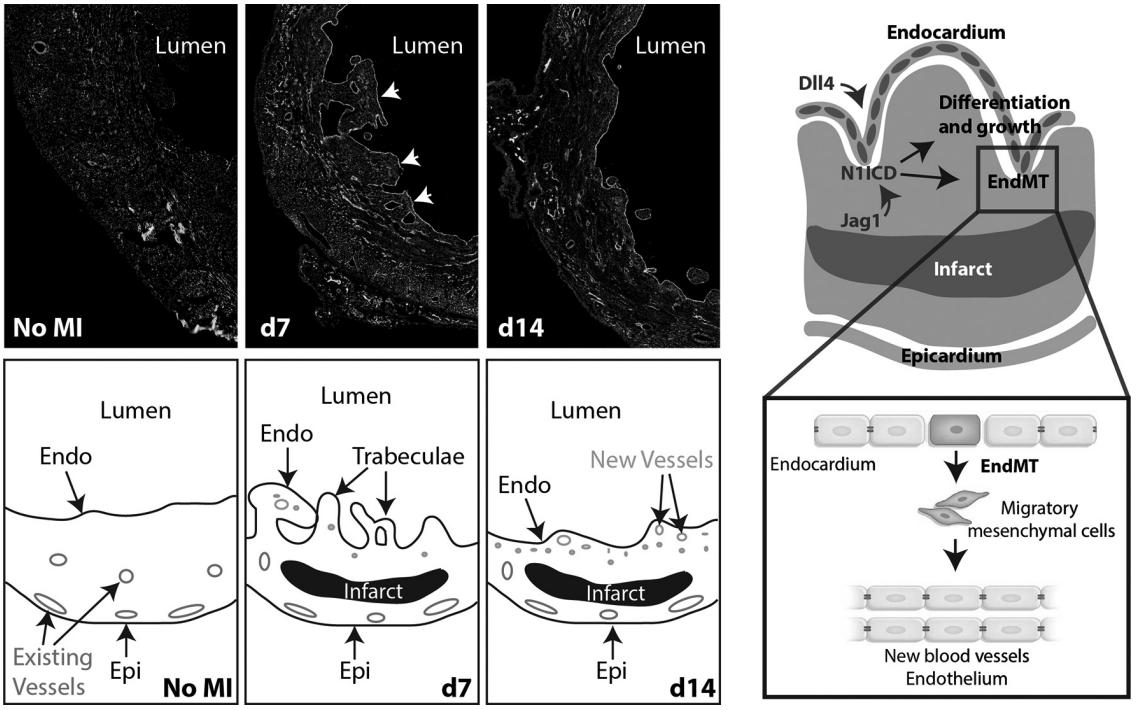

Abstract BS1 Figure 1

Results We demonstrated that the adult heart reverts to a hypertrabeculated state between 1 and 5 days post-MI and repeats the process of endocardial compaction from 7–14 days. This process appears to facilitate endocardium-derived neovascularization, leading to formation of mature sub-endocardial vessels after infarction. We observed reactivation of the Notch pathway in the endocardium following MI, in keeping with its role as a key regulator of these processes in development. Using a Notch1 loss-of-function mouse model targeted by an endothelial-specific Cre, we observed impaired trabeculation with reduced sub-endocardial vessel count after disruption of Notch activity. (Figure 1)

Conclusion We established that de novo vessel formation constitutes a significant component of the neovascular response and revealed that the endocardium is a major contributory source. Notch1 was identified as a key driver in this remodelling process and, moreover, our data suggest a role for Notch in driving endothelial-mesenchymal transition (EndMT) to provide smooth muscle support to newly formed vessels. Ongoing work seeks to determine whether targeting trabeculation in the adult heart will contribute to improved neovascularisation post-MI.

Conflict of interest N/A

BS2 STX18-AS1 IS A LONG NONCODING RNA GOVERNING IN VITRO CARDIOMYOCYTE DIFFERENTIATION AND PREDISPOSING TO ATRIAL SEPTAL DEFECT VIA DOWNREGULATION OF NKX2-5

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Objectives A number of risk variants have been identified from Genome-wide association studies (GWAS) of Congenital Heart Disease (CHD). However, none of them has been functionally confirmed as yet. We sought to identify the gene and mechanism responsible for the GWAS signal (OR=1.46; p=2.61×10–10) which we previously identified at chromosome 4p16 for atrial septal defect (ASD).

Methods and results Exploration of the linkage disequilibrium pattern between SNPs in the region indicated association was restricted to the interval spanning the long noncoding RNA STX18-AS1. Since no homolog of STX18-AS1 is found beyond primates, all experiments were conducted in human tissues and cell lines. With RNA from 108 Right Atrial Appendages and corresponding blood DNA, we confirmed the risk SNPs (rs870142 and rs16835979) were eQTLs for STX18-AS1 in human cardiac tissues. In RNA expression analyses using qPCR on embryonic heart samples, the transcription of STX18-AS1 was detected to be the highest at CS14-CS18, the critical time for atrial septation during human heart development. With in situ hybridization on whole embryonic hearts of CS17-CS19, the expression of STX18-AS1 was also determined in the myocardium of Atrial Septum.

We next identified STX18-AS1 as a regulator of the key cardiac transcriptional factor NKX2-5 using CRISPR/Cas9 knockdown of STX18-AS1 in HepG2 cells. Mutations in NKX2-5 cause septal defects in humans. Reduced STX18-AS1 transcription inhibited the expression of NKX2-5 accompanied by decreased H3K4me3 at its promoter region. We demonstrated the interaction between STX18-AS1 RNA and WDR5 protein, which supported the epigenetic regulation effects of STX18-AS1. Using in vitro cardiomyocyte differentiation from human embryonic stem cells, we demonstrated that the knockdown of STX18-AS1 depleted the potential of human embryonic stem cells in differentiating into beating cardiomyocytes without changes in cell viability and pluripotency.

Conclusion STX18-AS1 is the first long noncoding RNA influencing CHD risk identified from GWAS. The mechanism involves downregulation of the NKX2-5 gene through epigenetic mechanisms.
Conflict of interest No

BS3 NOTCH LIGAND JAG1 PROMOTES ENDOTHELIAL-TO-MESENCHYMAL TRANSITION AND ATHEROSCLEROSIS AT REGIONS OF DISTURBED FLOW

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Introduction Atherosclerosis is a chronic inflammatory disease marked by hardening and thickening of the arteries. The disease develops predominantly at arterial branches and bends. These atheroprone areas are subjected to disturbed blood flow, which generates low and oscillatory wall shear stress (LOSS), a frictional force exerted on endothelial cells (EC). LOSS increases EC inflammatory activation and drives endothelial-to-mesenchymal transition (EndoMT), which promotes atherosclerosis. The molecular basis of EC responses to shear stress is not fully elucidated.

Notch signaling is a major regulator of vascular development and homeostasis. It plays a critical role in communication between EC, initiated by the interaction of a receptor (i.e. Notch1 or 4) with a ligand (Dll4 or Jag1) present on a neighbouring cell. A recent study revealed that Notch1 is a mechanosensor protecting adult arteries from developing atherosclerosis (Mack et al., 2017, Nat Commun.;8(1):1620). However the role of other Notch actors in this disease is unknown. Here we investigated the role of the Notch ligand Jag1 in EC pathophysiology and atherosclerosis.
Methods To study the effect of shear stress on Notch actors, Human Coronary Aortic EC (HCAEC) were exposed to LOSS (4dynes/cm^2 ±1Hz) versus high shear stress (12dynes/cm^2) using a parallel plate system (Ibidi). After 2 to 4 days of culture under flow, expression levels of transcripts and proteins were assessed by qRT-PCR and western blotting. The function of Jag1 in HCAEC exposed to LOSS was studied using neutralizing antibodies. The function of Jag1 in EC was studied in vivo by tamoxifen inducible conditional deletion in mice (CDH5Cre/Ert2; Jag1fl/fl). The EC phenotype was analysed by immunostaining, qRT-PCR and western blotting.
Results qRT-PCR and en face staining revealed that Jag1 is enriched at LOSS sites in both the pig ($p<0.05$) and mouse aorta ($p<0.05$). We concluded that LOSS is responsible for focal Jag1 expression at atheroprone areas because it induced Jag1 in HCAEC ($p<0.05$). We then investigated whether Jag1 influences EC dysfunction in response to LOSS. Blocking Jag1 activity reduced expression of inflammatory markers (e.g. VCAM-1, E-Selectin and MCP-1) as well as mesenchymal markers (e.g. Snail, N-Cadherin and SMA) in HCAEC exposed to LOSS. It also significantly increased proliferation. Thus LOSS activation of Jag1 drives EC activation and EndoMT and reduces proliferative potential. Co-staining of Jag1 with markers of inflammation and EndoMT revealed a strong colocalization in the mouse aorta. Jag1 was also detected in EC above atherosclerotic plaques. To investigate whether endothelial Jag1 plays a role in atherosclerosis, we carried out an inducible knockout of Jag1 in EC, which resulted in a decrease of plaque formation.
Conclusion Our data reveal a critical role for the Notch ligand Jag1 as a pro-atherogenic factor, controlling inflammation, EC fate and proliferation at atheroprone sites.
Conflict of interest none

BS4 IRON DEFICIENCY IN PULMONARY ARTERIAL SMOOTH MUSCLE CELLS INDUCES PULMONARY ARTERIAL HYPERTENSION THROUGH ENDOTHELIN-1

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Introduction Iron deficiency augments hypoxic pulmonary arterial pressure in healthy individuals and exacerbates pulmonary arterial hypertension (PAH) in patients, even in the absence of anaemia. In supplementation has been shown to be beneficial in both settings. The mechanisms underlying the detrimental effects of iron deficiency and the beneficial effects of iron supplementation are not known, owing to a lack of understanding of how specific cells of the pulmonary vasculature respond to changes in iron levels. The iron export protein ferroportin (FPN) and its antagonist peptide hepcidin control systemic iron levels by regulating its release from the gut, spleen and liver, the sites of iron absorption, recycling and storage, respectively. We found FPN to be present in pulmonary arterial smooth muscle cells (PASMCs) and to be regulated by hepcidin. Therefore, we set out to interrogate the physiological function of the hepcidin/FPN axis in PASMCs.
Methods We generated a murine model with a smooth muscle-specific knock-in of fpn C326Y, which encodes a FPN with intact iron export function but impaired hepcidin binding. We then studied pulmonary hemodynamics and cardiac function over time.
Results While retaining normal systemic iron and haemoglobin levels, this model developed PAH and right heart failure as a consequence of intracellular iron deficiency and increased expression of the vasoconstrictor endothelin-1 (ET-1) specifically within PASMCs. PAH was prevented and reversed by intravenous iron treatment and by the ET receptor antagonist BQ-123. The regulation of ET-1 by iron was further demonstrated in healthy humans exposed to hypoxia and in PASMCs from PAH patients.
Conclusion This study presents the first evidence that intracellular iron deficiency localised specifically within PASMCs is sufficient to impair pulmonary vascular function, even in the absence of anaemia (fig 1). It offers a mechanistic underpinning for the known effects of iron availability on the pulmonary vasculature in the human setting.
Conflict of interest None