pulmonary arterial hypertension (PAH) cases and approximately 25% of idiopathic PAH cases. PAH may arise due to endothelial dysfunction as mice with BMPR-II deficiency exhibit increased pulmonary vascular permeability.

BMP9 is an endothelial quiescence factor and is thought to maintain the integrity of the endothelium. We previously reported that BMPR-II and ALK1 are the key receptors through which BMP9 inhibits the proliferation of human pulmonary artery endothelial cells (hPAECs). We hypothesised that BMPR-II deficiency impacts on endothelial cell connectivity and may contribute to endothelial dysfunction in PAH.

Methods Human pulmonary artery endothelial cells were obtained from Lonza and blood outgrowth endothelial cells (BOECs) were isolated from peripheral blood of unaffected controls or PAH patients with identified BMPR-II mutations. Cells were transfected with siRNAs targeting BMPR-II followed by stimulation with BMP9. RNA was extracted and the expression of candidate genes determined by quantitative PCR. Further siRNA studies were performed for ALK1 and endoglin siRNAs. The promotion of gap junction assembly by BMP9 and BMP10 were assessed by immunofluorescence, Western blotting and functionally using parachute assays.

Results Screening of candidate BMP9-induced junctional and structural proteins highlighted a subset of endothelial connexins that are BMP9 and BMP10-responsive and dependent on BMPR-II and ALK1. BMP9 and BMP10 increased the expression of the connexins, assessed by Western blotting and immunostaining. In addition, BMP9 and BMP10 significantly increased the transfer of calcein from labelled donor cells to unlabelled acceptor cells, indicating a promotion of endothelial cell connectivity.

Conclusion In addition to their roles promoting endothelial quiescence, BMP9 and BMP10 directly promote endothelial cell connectivity by increasing connexin expression and assembly. The central contributions of BMPR-II and ALK1 to this process may implicate impaired endothelial connectivity as a pathological component of PAH and HHT.

S36 FERROPORTIN IS EXPRESSED IN HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS: IMPLICATIONS FOR PULMONARY ARTERIAL HYPERTENSION

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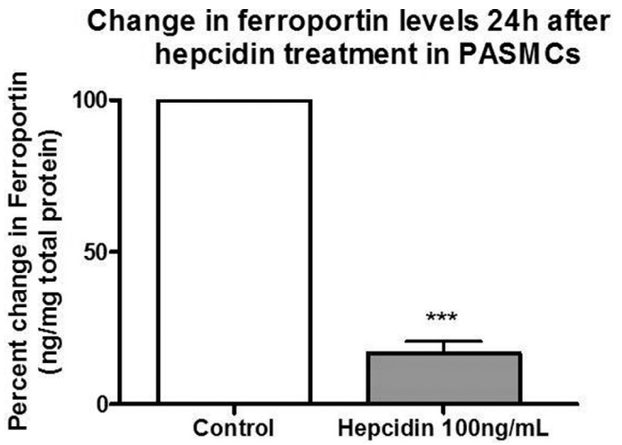
Background Pulmonary Arterial Hypertension (PAH) is a rare but fatal condition manifested by pulmonary vascular remodelling, increased pulmonary vascular resistance and right-heart failure. Disruption in iron handling and anaemia, caused by elevated iron-regulatory hormone hepcidin, is observed in PAH. Ferroportin the only known cellular iron-export protein is downregulated by hepcidin. As such, iron supplementation as a therapy is currently under clinical trial. However, it is also known that iron is both pro-oxidant and pro-proliferative. Latest evidence also points to sub-clinical haemolysis and the presence of free haemoglobin in PAH patients. We hypothesised that ferroportin would be expressed; be responsive to hepcidin challenge and have implications for the proliferation of human pulmonary artery smooth cells (hPASMCs).

Methods The mRNA levels of ferroportin was measured by RT--PCR, the protein expression was detected by western-blot analysis and quantified by ELISA. The sub-cellular distribution of ferroportin was visualised by immunocytochemistry (ICC). hPASMCs were pre-incubated with or without free haemoglobin and further challenged with increasing doses of hepcidin and the proliferative responses assessed by cyquant and/or BrdU incorporation assays. Some cells were also pre-incubated with LY2928057 (monoclonal antibody against ferroportin that stabilises cellular expression, Eli-Lily) in proliferation assays.



Abstract S36 Figure 1 Confluent PASMCs (passage 4–8) were treated with 100 ng/ml hepcidin for 22–24 h and total protein extracted using cell-lysis buffer. Ferroportin expression was quantitated using an ELISA kit and normalised to total protein estimated by bradford reagent. n = 4, *** = $p < 0.001$

Results Basal ferroportin mRNA was detected in hPASMCs, but the mRNA levels were largely unaltered with hepcidin exposure (n = 3). A ~50KDa protein band representing ferroportin was detected under resting conditions while hepcidin challenge caused decrease in ferroportin protein levels (Figure 1). Basal ferroportin was uniformly distributed in the cells; however hepcidin treatment led to intense punctate/vesicular staining (n = 3). Finally, exposure to free haemoglobin alone or along with hepcidin increased proliferation of hPASMCs by 13.6% and 12.4% ($p < 0.05$, n = 3) respectively. Interestingly, pre-incubation of the cells with LY2928057 partly reversed this effect.

Conclusion This is the first report of ferroportin expression and regulation in hPASMCs. We suggest that targeting and manipulating the hepcidin-ferroportin axis using LY2928057 might prove a novel therapeutic approach for PAH.

S37 VASCULAR ENDOTHELIAL CELL GROWTH FACTOR-A (VEGF-A) SIGNALLING AND NEOVASCULARISATION OF PULMONARY ENDARTERECTOMY MATERIAL IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION (CTEPH)

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Background Despite recent advances in the medical treatment of patients with CTEPH, relatively little is understood surrounding the underlying pathological mechanisms. Many patients have a historical documented venous thromboembolic event (VTE) and consequently, failed resolution of an acute VTE has been proposed as a key initiating factor in the subsequent development of CTEPH. Here we investigated VEGF-A levels, a key regulator of angiogenesis, in CTEPH patients prior to and following