

for patients with an IL-6 level of <1.6 pg/ml, $P=0.0013$, figure 1A). IL-6 appears to outperform NT-proBNP in this respect (cumulative survival for patients with an NT-proBNP level of >130 ng/ml at 3 years was 76% compared to 84% for patients with an NT-proBNP level of ≤ 130 ng/ml, $P=0.37$, figure 1B). The converse was true in PAH without mutation (cumulative survival for patients with an IL-6 level of >1.3 pg/ml at 3 years is 81% compared to 92% for patients with an IL-6 level of ≤ 1.3 pg/ml, $P=0.048$, figure 1C). Cumulative survival for patients with a NT-proBNP level of >239 ng/ml at 3 years was 80% compared to 96% for patients with NT-proBNP level of <239 pg/ml, ($P=0.01$, figure 1D).

Conclusions BMPR2-mutation positive patients have a different inflammatory profile compared to PAH patients without mutations. The selection of biomarkers of inflammation to predict clinical outcomes may therefore differ between these groups.

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HEPCIDIN AND INTERLEUKIN-6 DOWNREGULATE BMPR2 AND DYSREGULATE BMPR2 DOWNSTREAM PATHWAYS; IMPLICATIONS FOR PULMONARY ARTERY HYPERTENSION

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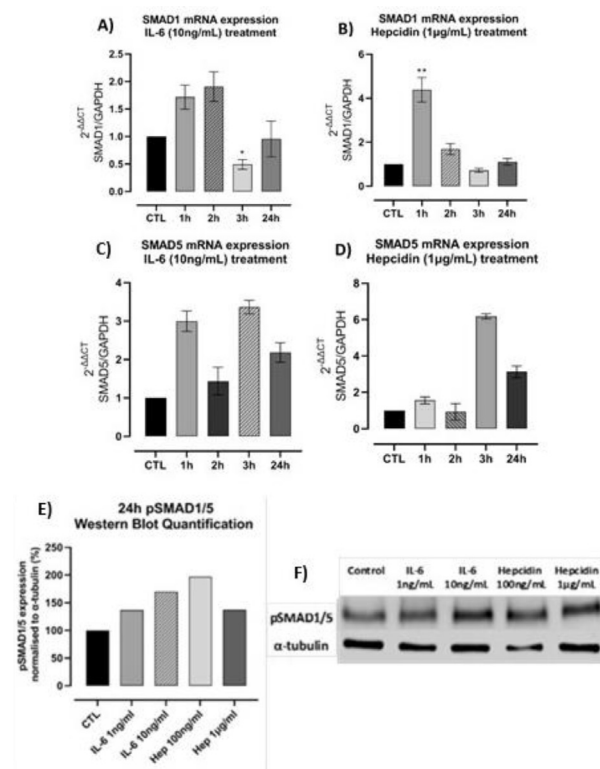
Background Pulmonary Arterial Hypertension (PAH) is a condition resulting from vascular remodelling and elevated pulmonary arterial pressure. Bone Morphogenetic Protein Receptor II (BMPR2) mutations have been strongly associated with heritable forms of PAH. Previous studies have demonstrated that the hepcidin/ferroportin axis acts to downregulate BMPR2 and promote proliferation in pulmonary artery smooth muscle. However, the role of hepcidin on pulmonary artery endothelial cells (PAEC) and BMPR2 downstream pathways is yet to be elucidated.

Aims To investigate the influence of Hepcidin/IL-6 on PAEC proliferation, mitochondrial dysfunction, as well as BMPR2 and downstream pathway perturbation.

Methods PAECs were treated with Hepcidin-25 (100 ng/mL or 1 μ g/mL) or Interleukin-6 (IL-6) (1 ng/mL or 10 ng/mL). Proliferation was determined using CyQuant assay, gene transcription was analysed using real-time PCR and protein expression by Western Blot (WB) and Enzyme-linked Immunosorbent Assay (ELISA).

Results Hepcidin and IL-6 after 24h treatment cause PAECs to proliferate ($n=4$; $p<0.05$). Hepcidin and IL-6 treatments both upregulate hepcidin mRNA and protein expression ($n=4$ and $n=17$; $p<0.05$). BMPR2 mRNA and protein expression, as measured by rt-PCR ($n=4$; $p<0.05$) and WB analysis ($n=1$) is downregulated in PAECs by hepcidin and IL-6. However, WB and mRNA analysis also show that hepcidin treatment increases expression and phosphorylation of SMAD1/5 at different time-points (figure 1). Furthermore, ID mRNA expression was dysregulated at several time-points compared to control. Hepcidin upregulates PINK1 mRNA expression 6-fold compared to control ($n=4$; $p<0.0001$).

Conclusions These study findings uncover the complexity of the relationship between hepcidin and BMP-signalling in PAECs. Disruption in iron homeostasis and elevation in hepcidin levels have been reported in PAH populations, thus a role for a dysregulated hepcidin/ferroportin axis and downstream



Abstract S92 Figure 1 qPCR of SMAD protein expression A) SMAD1 mRNA expression by IL-6 (10 ng/mL) at different time points ($n=4$) B) SMAD1 mRNA expression by Hepcidin (1 μ g/mL) treatment at different time points ($n=4$) C) SMAD5 mRNA expression by IL-6 (10 ng/mL) at different time points ($n=2$) D) SMAD5 mRNA expression by Hepcidin (1 μ g/mL) treatment at different time points ($n=2$). Western Blot analysis of phosphorylated SMAD1/5 expression by hPAECs E) Western Blot quantification of pSMAD1/5 expression after 24h treatment with IL-6 (1 ng/mL and 10 ng/mL) and Hepcidin (100 ng/mL and 1 μ g/mL), $n=1$. Protein expression normalized to α -tubulin F) Image of Western Blot qPCR expression data is normalized to GAPDH. t-tests were performed against each respective control. Data presented as mean \pm SEM

pathway disruption presents a potential mechanism for these observations. Nonetheless, further research is pivotal to fully elucidate the role of hepcidin in disrupting PAEC iron homeostasis and BMP-signalling.

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REPAIR: LONG-TERM EFFECTS OF MACITENTAN ON THE RIGHT VENTRICLE (RV) IN PULMONARY ARTERIAL HYPERTENSION (PAH)

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Introduction and Objectives In a study of patients with PAH (REPAIR), macitentan improved RV stroke volume (RVSV)