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Letter to the Editor, in response to: “Iron Homeostasis and Pulmonary Hypertension: Iron Deficiency Leads to Pulmonary Vascular Remodeling in the Rat”

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We read with great interest the article by Cotroneo et al, and the accompanying editorial, which provides evidence that iron deficiency is associated with pulmonary vascular remodeling in an animal model.^{1,2} We provide additional support for their results in humans and suggest that the observed relationship between iron homeostasis and pulmonary vascular remodeling is driven in part by mechanisms shared between pulmonary arterial hypertension (PAH) and disorders of iron metabolism.

We analyzed data from BioVU, Vanderbilt's integrated de-identified electronic medical record system (EMR) and DNA biorepository, to identify clinical diagnoses associated with single nucleotide polymorphism (SNP) variation in and around genes previously associated with PAH by a genome-wide association study (GWAS)³. We used 29,349 unrelated BioVU subjects of European ancestry who were genotyped on the Illumina Human Exome BeadChip, which has approximately 33,000 common SNPs with a minor allele frequency (MAF)>1%. We used 1,309 curated collections of diagnostically-related ICD-9 diagnosis codes that have been extensively validated for use in genetic studies.⁴ Cases are subjects with 2 or more instances of a grouped ICD-9 code appearing in their medical record. Age- and sex- frequency matched controls were selected for each phenotype. We next identified a set of 133 genes that contained SNPs that replicated in the same direction between discovery and replication cohorts (p value < .008) from a large GWAS of PAH patients.³ Sixty-five of the 133 genes overlapped with the Exome Chip, and we evaluated all SNPs located within 25,000 base pairs of these genes (n=311 SNPs). We used a genetic generalized linear mixed models approach to test for an association between each phenotype and the joint variation by these 311 SNPs.⁵

We found that SNP variation around our 65 genes was significantly associated with only one phenotype, “Disorders of Iron Metabolism” (n=111 cases, $p=4.8\times10^{-9}$), after adjusting for multiple testing. This phenotype is a composite of conditions related to iron regulation. While our list of candidate genes did not include the Hemochromatosis (*HFE*) gene, it did include genes within the same region including *SLC17A3*, *SLC17A2* and *TRIM38* on chromosomal region 6p. Of note, SNPs in this region have been associated with variation in red blood cell indices including mean hemoglobin content (*MHC*) suggesting that SNP

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variation in this region has a role in modulating iron regulation.⁶ The findings suggest a possible genetic correlation between PAH and conditions related to iron homeostasis, offering some evidence that genetic variants related to iron maintenance, including iron deficiency, associate with PAH.

As elucidated in the article by Cotroneo et al, and its accompanying editorial, emerging data suggest that the pulmonary vascular disease phenotype of PAH occurs in the context of broad irregularities in the cellular metabolic milieu.^{1, 2} Metabolic alterations characteristic of PAH at the molecular level indicate a pathologic shift toward aerobic glycolysis and disruption of normal mitochondrial metabolic activity to promote the emergence of an apoptosis-resistant cellular phenotype associated with disorganized and dysregulated cellular growth and proliferation.^{2, 7} Because of its direct relevance to crucial cellular processes, deficiency of iron may contribute to PAH in a number of ways, including enhancing expression and activity of the hypoxia-inducible factor family of transcription factors (HIFs). Among its repercussions, exuberant HIF activity directly perturbs mitochondrial function.^{2, 8} Intriguingly, systemic iron deficiency is more prevalent in PAH patients, is associated with increased morbidity and mortality, and under study as a therapeutic target via iron supplementation (NCT01447628).⁹⁻¹¹

As the precise relationship between iron and PAH becomes clearer, abnormalities of gene or protein networks related to iron may reveal important information about inherent risk factors for PAH pathogenesis and progression. We plan to determine which genes, either in concert or isolation, are driving the association between PAH and disorders of iron metabolism. This paper by Cotroneo et al provides additional biological plausibility for our exploratory genetic findings, and advances the concept that exogenous and endogenous factors relevant to iron storage and metabolism are an appealing target for therapeutic, and perhaps preventative, approaches.

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