ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Research letter

# *COL18A1* genotypic associations with endostatin levels and clinical features in pulmonary arterial hypertension: a quantitative trait association study

Catherine E. Simpson, Megan Griffiths, Jun Yang, Melanie K. Nies, R. Dhananjay Vaidya, Stephanie Brandal, Lisa J. Martin, Michael W. Pauciulo, Katie A. Lutz, Anna W. Coleman, Eric D. Austin, D. Dunbar Ivy, William C. Nichols, Allen D. Everett, Paul M. Hassoun, Rachel L. Damico

Please cite this article as: Simpson CE, Griffiths M, Yang J, *et al*. *COL18A1* genotypic associations with endostatin levels and clinical features in pulmonary arterial hypertension: a quantitative trait association study. *ERJ Open Res* 2022; in press (https://doi.org/10.1183/23120541.00725-2021).

# *COL18A1* genotypic associations with endostatin levels and clinical features in pulmonary arterial hypertension: a quantitative trait association study

Catherine E. Simpson, MD MHS[1], Megan Griffiths, MD[2], Jun Yang, PhD[2], Melanie K. Nies, MD[2], R. Dhananjay Vaidya, PhD[3], Stephanie Brandal, MS[2], Lisa J. Martin, PhD[4], Michael W. Pauciulo, MBA[4], Katie A. Lutz, MS[4], Anna W. Coleman, MS[4], Eric D. Austin, MD MSCI[5], D. Dunbar Ivy, MD[6], William C. Nichols, PhD[4], Allen D. Everett, MD[2], Paul M. Hassoun, MD[1], and Rachel L. Damico, MD PhD[1]

[1]Johns Hopkins University Department of Medicine, Division of Pulmonary and Critical Care Medicine
[2]Johns Hopkins University Department of Pediatrics, Division of Pediatric Cardiology
[3]Johns Hopkins University Department of Medicine, Division of General Internal Medicine
[4]Cincinnati Children's Hospital Medical Center and University of Cincinnati College of Medicine, Department of Pediatrics, Division of Human Genetics
[5]Vanderbilt University Department of Pediatrics, Division of Allergy, Immunology, and Pulmonary Medicine
[6]Children's Hospital Colorado, Department of Pediatric Cardiology

**Corresponding authors:**
Rachel L. Damico and Paul M. Hassoun
1830 E. Monument St., Fifth Floor
Baltimore, MD 21205
Tel 410-614-6311, Fax 410-614-7451
rdamico1@jhmi.edu, phassou1@jhmi.edu

**Authors' contributions:**
C.E.S., R.L.D., P.M.H., and A.D.E. designed the study; S.B. and J.Y. performed the experiments and interpreted the results; C.E.S., M.G., J.Y., M.K.N., M.W.P., E.D.A., D.D. I., W.C.N. performed data collection, maintenance, and analysis; C.E.S., L.J.M., and R.D.V. performed statistical analyses; C.E.S. drafted the manuscript; all authors critically revised the manuscript for important intellectual content and approved the final version; P.M.H. and R.L.D. had access to all the data in the study and take full responsibility for the integrity and accuracy of the work.

**Funding information:**
This study was supported by National Institutes of Health/National Heart, Lung, and Blood Institute award R01HL135114 and R01 HL150070 (A.D.E., M.N., J.Y., R.L.D., D.V., W.C.N., D.D.I and E.D.A.), R01HL132153 (R.L.D., P.M.H.), R24 HL105333 (W.C.N., M.W.P.), K12-HD000850 (M.G.) and K23HL153781 (C.E.S.).

**To the Editor:**

Endostatin (ES) is a circulating peptide derived from collagen XVIII, alpha 1 (COL18A1) known to inhibit angiogenesis (1, 2). Decreased angiogenesis is a feature of pulmonary arterial hypertension (PAH) in animal models (3) and human subjects (4). Our group has reported strong associations between circulating ES levels and hemodynamics and survival in PAH (5-7). We have also reported that a missense variant in *COL18A1,* which encodes ES, confers lower ES and longer survival, suggesting variation within the gene contributes to circulating levels (5). With this study, we assessed *COL18A1* variant associations with clinical phenotypes and outcomes, including *COL18A1* associations with circulating ES levels, in a large, multicenter PAH cohort in which we previously investigated ES as a prognostic biomarker (6).

This study was approved by the Johns Hopkins University Institutional Review Board. Serum samples contributed to the National Heart, Lung, and Blood Institute-sponsored PAH Biobank underwent single nucleotide polymorphism (SNP) genotyping using the Omni5-4 BeadChip (Illumina) and whole exome sequencing (WES) through the Regeneron Genetics Center (8). An electrochemiluminescence assay was developed to quantitate ES. Sample collection and processing methods have been previously published (6, 9, 10).

ES measurements were regressed on genotypes of *COL18A1* variants to perform a multivariable protein quantitative trait loci (pQTL) analysis. Linear regression models adjusted for age and sex were restricted to subjects of European (EA) or African ancestry (AA). To determine whether ES-associated SNPs also impacted regulation of *COL18A1* gene expression, pQTLs were queried in a publicly available expression quantitative trait loci (eQTL) database of whole-blood RNA samples (11). Associations with clinical phenotypes and survival were

modeled using multivariable linear and Cox regressions. Minor allele frequencies (MAF) for *COL18A1* variants were compared with the Genome Aggregation Database (gnomad.broadinstitute.org). Linkage disequilibrium (LD) across the *COL18A1* region was assessed using D' (12). A *p* value of <0.05 was considered nominally significant. An LD-adjusted correction for multiple testing was applied for QTL analyses equaling 0.0016 in EA and 0.0013 in AA subjects. Statistical tests were performed using Stata version 15.1 (StataCorp., College Station, TX, USA), SAS version 9.4 (SAS Institute, Cary NC), and PLINK (13) version 1.9 (http://pngu.mgh.harvard.edu/purcell/plink/).

The cohort consisted of 2,017 subjects with median age 53 years, of whom 80% were female and 82% were of European ancestry (EA). Full clinical characteristics of this cohort have been published previously (6). Briefly, subjects had prevalent disease (median duration at sample collection 48 months, interquartile range 14-92 months) and moderately severe PAH at enrollment, with mean pulmonary artery pressure (mean ± standard deviation) 50 ± 15 mmHg, pulmonary vascular resistance 10 ± 6 Wood units, and 45% with New York Heart Association Functional Class III or IV symptoms. Most subjects had IPAH (n=870) or CTD-PAH (n=623). From the Omni5 SNP array, 100 *COL18A1* variants in 1400 EA subjects and 126 *COL18A1* variants in 209 subjects of African ancestry (AA) passed quality control (HWE >0.001, MAF >0.05 and genotype missing rate <5%), with 91 variants present in both EA and AA subjects. In multivariable pQTL analysis, 26 cis-acting SNPs were associated with ES levels in EA individuals, and eight were associated with ES levels in AA individuals. There were no pQTLs in common for EA and AA subjects. Twenty-three of 26 pQTLs in EA and five of eight pQTLs in AA individuals were associated with differences in *COL18A1* gene expression. In EA subjects, two Omni5 SNPs

demonstrated associations with cardiac index (CI): the T allele was associated with a 0.11 L/m$^2$ lower CI for rs7281138 (*p* 0.043), and a 0.12 L/m$^2$ lower CI for rs2838917 (*p* 0.028). QTL data and genotype-phenotype associations are shown in the Table.

Of 102 *COL18A1* WES variants, 22 had a frequency of 5% or greater; none deviated from HWE. Six SNPs overlapped between exonic SNPs and Omni5 SNPs. Three of the 16 unique exonic variants in EA and one in AA subjects were associated with differences in serum ES. All four exonic pQTLs identified were also associated with significant differences in *COL18A1* gene expression in eQTL analysis. In EA subjects, two exome variants demonstrated associations with survival: the A allele was associated with 23% lower mortality for rs7499 (HR 0.77, 95% CI 0.62-0.96, *p* 0.018), and the A allele was associated with 24% lower mortality for rs1050351 (HR 0.76, 95% CI 0.61-0.95, *p* 0.015). Six exonic variants with chromosomal positions in close proximity were associated with longer 6MWD, and an additional three exonic variants, also in close proximity, were associated with higher CI (Table). There were no observed differences in MAFs of *COL18A1* variants compared to available controls.

Our QTL results suggest circulating ES levels are partially genetically influenced by variants in and around *COL18A1*. The eQTL results suggest some variation in ES abundance may be due to variations in mRNA expression. Most known QTLs are associated with changes in mRNA expression, with downstream effects on ribosome occupancy and protein abundance (14). Thus, eQTLs often have smaller effect sizes on protein levels than on gene expression (14, 15), consistent with our results. We found some signal for genetically influenced phenotypic variation, though none of the phenotypically-associated variants were ES-associated pQTLs, and all but one (rs7499 in the 3' untranslated region) were synonymous variants. Interestingly,

rs7499 has been associated with significantly reduced risk of hepatocellular carcinomas in patients with hepatitis B infection (16), suggesting some biologic significance of this variant in humans.

In contrast to our 2015 report (5), we did not find an association between rs12483377 and ES levels or outcomes. This discrepancy may be due to the smaller sample size in the first study. Genotype at rs12483377 was not associated with survival in two large PAH genome-wide association studies (GWAS) later published (17), though these GWAS excluded patients with connective tissue disease and may have investigated genetically different cohorts.

This study has several limitations. We are limited by the cohort size available for a rare disease; consequently, some of our results are of nominal significance, with a higher likelihood of observation due to chance alone. The QTL results are based on associations in whole blood, as mRNA or protein expression data from human tissues most relevant to disease are not available. The genetic and clinical associations with ES are based on a single time point for each subject. Further, OMNI5 genotyping and WES leaves many genetic variants uncharacterized. Therefore, the identified genotype-phenotype associations may not be causal themselves, but rather in LD with true, unidentified QTLs.

Aside from reports on BMPR2, our study is one of only a few (17, 18) that offer insights into genetic influences on disease severity and heterogeneity in PAH, a strength of our work. Heritable modifiers of phenotype have not been well-established in PAH, and most genetic studies have focused on identifying loci contributing to disease susceptibility, rather than disease severity or prognosis.

In conclusion, these results suggest PAH disease heterogeneity is influenced in part by genetic variation around the COL18A1 gene. ES levels have been linked to variation in PAH severity and outcomes, and our results suggest ES levels may be genetically influenced. Understanding influences on transcription and translation of genes implicated in disease can clarify therapeutic targeting strategies. Future work on ES/COL18A1 is needed to better understand genetic and cellular mechanisms underlying PAH pathobiology.

**Acknowledgements**

Exome sequencing and genotyping data were generated by Regeneron Genetics Center, Regeneron Pharmaceuticals, Tarrytown, NY.

**Disclosure Statement**

C.E. Simpson reports grants from the NIH/NHLBI during the conduct of the study. M. Griffiths reports grants from the NIH during the conduct of the study. J. Yang has nothing to disclose. M.K. Nies has nothing to disclose. R.D. Vaidya reports grants from the NIH during the conduct of the study. S. Brandal has nothing to disclose. L.J. Martin reports grants from the NIH during the conduct of the study. M.W. Pauciulo has nothing to disclose. K.A. Lutz has nothing to disclose. A.W. Coleman has nothing to disclose. E.D. Austin reports grants from the CMREF and NIH/NHLBI during the conduct of the study. The University of Colorado has contracts with Actelion, Altavant, Bayer, and Gossamer Bio for Dr Ivy to be a consultant. W.C. Nichols reports grants from the NIH/NHLBI during the conduct of the study. A.D. Everett reports grants from the NIH/NHLBI during the conduct of the study. P.M. Hassoun served on a scientific advisory board for Merck & Co and reports grants from the NIH/NHLBI during the conduct of the study. R.L. Damico reports grants from the NIH/NHLBI during the conduct of the study.

**References**


1. Taddei L, Chiarugi P, Brogelli L, Cirri P, Magnelli L, Raugei G, Ziche M, Granger HJ, Chiarugi V, Ramponi G. Inhibitory effect of full-length human endostatin on in vitro angiogenesis. *Biochem Biophys Res Commun* 1999; 263: 340-345.
2. O'Reilly MS, Boehm T, Shing Y, Fukai N, Vasios G, Lane WS, Flynn E, Birkhead JR, Olsen BR, Folkman J. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997; 88: 277-285.
3. Sutendra G, Dromparis P, Paulin R, Zervopoulos S, Haromy A, Nagendran J, Michelakis ED. A metabolic remodeling in right ventricular hypertrophy is associated with decreased angiogenesis and a transition from a compensated to a decompensated state in pulmonary hypertension. *J Mol Med (Berl)* 2013; 91: 1315-1327.
4. Vogel-Claussen J, Shehata ML, Lossnitzer D, Skrok J, Singh S, Boyce D, Lechtzin N, Girgis RE, Mathai SC, Lima JA, Bluemke DA, Hassoun PM. Increased right ventricular Septomarginal trabeculation mass is a novel marker for pulmonary hypertension: comparison with ventricular mass index and right ventricular mass. *Invest Radiol* 2011; 46: 567-575.
5. Damico R, Kolb TM, Valera L, Wang L, Housten T, Tedford RJ, Kass DA, Rafaels N, Gao L, Barnes KC, Benza RL, Rand JL, Hamid R, Loyd JE, Robbins IM, Hemnes AR, Chung WK, Austin ED, Drummond MB, Mathai SC, Hassoun PM. Serum endostatin is a genetically determined predictor of survival in pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2015; 191: 208-218.
6. Simpson CE, Griffiths M, Yang J, Nies MK, Vaidya RD, Brandal S, Martin LJ, Pauciulo MW, Lutz KA, Coleman AW, Austin ED, Ivy DD, Nichols WC, Everett AD, Hassoun PM, Damico RL. The angiostatic peptide endostatin enhances mortality risk prediction in pulmonary arterial hypertension. *ERJ Open Res* 2021; 7.
7. Daly CM, Griffiths M, Simpson CE, Yang J, Damico RL, Vaidya RD, Williams M, Brandal S, Jone PN, Polsen C, Ivy DD, Austin ED, Nichols WC, Pauciulo MW, Lutz K, Nies MK, Rosenzweig EB, Hirsch R, Yung D, Everett AD. Angiostatic Peptide, Endostatin, Predicts Severity in Pediatric Congenital Heart Disease-Associated Pulmonary Hypertension. *J Am Heart Assoc* 2021: e021409.
8. Zhu N, Pauciulo MW, Welch CL, Lutz KA, Coleman AW, Gonzaga-Jauregui C, Wang J, Grimes JM, Martin LJ, He H, Investigators PAHBEC, Shen Y, Chung WK, Nichols WC. Novel risk genes and mechanisms implicated by exome sequencing of 2572 individuals with pulmonary arterial hypertension. *Genome Med* 2019; 11: 69.
9. Simpson CE, Chen JY, Damico RL, Hassoun PM, Martin LJ, Yang J, Nies M, Griffiths M, Vaidya RD, Brandal S, Pauciulo MW, Lutz KA, Coleman AW, Austin ED, Ivy DD, Nichols WC, Everett AD. Cellular sources of interleukin-6 and associations with clinical phenotypes and outcomes in pulmonary arterial hypertension. *Eur Respir J* 2020; 55.
10. Simpson CE, Damico RL, Hassoun PM, Martin LJ, Yang J, Nies MK, Vaidya RD, Brandal S, Pauciulo MW, Austin ED, Ivy DD, Nichols WC, Everett AD. Noninvasive prognostic biomarkers for left heart failure as predictors of survival in pulmonary arterial hypertension. *Chest* 2020.
11. Jansen R, Hottenga JJ, Nivard MG, Abdellaoui A, Laport B, de Geus EJ, Wright FA, Penninx B, Boomsma DI. Conditional eQTL analysis reveals allelic heterogeneity of gene expression. *Hum Mol Genet* 2017; 26: 1444-1451.
12. Slatkin M. Linkage disequilibrium--understanding the evolutionary past and mapping the medical future. *Nat Rev Genet* 2008; 9: 477-485.
13. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559-575.

14. Battle A, Khan Z, Wang SH, Mitrano A, Ford MJ, Pritchard JK, Gilad Y. Genomic variation. Impact of regulatory variation from RNA to protein. *Science* 2015; 347: 664-667.
15. Li JJ, Biggin MD. Gene expression. Statistics requantitates the central dogma. *Science* 2015; 347: 1066-1067.
16. Wu X, Wu J, Xin Z, Wang H, Zhu X, Pan L, Li Z, Li H, Liu Y. A 3' UTR SNP in COL18A1 is associated with susceptibility to HBV related hepatocellular carcinoma in Chinese: three independent case-control studies. *PLoS One* 2012; 7: e33855.
17. Rhodes CJ, Batai K, Bleda M, Haimel M, Southgate L, Germain M, Pauciulo MW, Hadinnapola C, Aman J, Girerd B, Arora A, Knight J, Hanscombe KB, Karnes JH, Kaakinen M, Gall H, Ulrich A, Harbaum L, Cebola I, Ferrer J, Lutz K, Swietlik EM, Ahmad F, Amouyel P, Archer SL, Argula R, Austin ED, Badesch D, Bakshi S, Barnett C, Benza R, Bhatt N, Bogaard HJ, Burger CD, Chakinala M, Church C, Coghlan JG, Condliffe R, Corris PA, Danesino C, Debette S, Elliott CG, Elwing J, Eyries M, Fortin T, Franke A, Frantz RP, Frost A, Garcia JGN, Ghio S, Ghofrani HA, Gibbs JSR, Harley J, He H, Hill NS, Hirsch R, Houweling AC, Howard LS, Ivy D, Kiely DG, Klinger J, Kovacs G, Lahm T, Laudes M, Machado RD, MacKenzie Ross RV, Marsolo K, Martin LJ, Moledina S, Montani D, Nathan SD, Newnham M, Olschewski A, Olschewski H, Oudiz RJ, Ouwehand WH, Peacock AJ, Pepke-Zaba J, Rehman Z, Robbins I, Roden DM, Rosenzweig EB, Saydain G, Scelsi L, Schilz R, Seeger W, Shaffer CM, Simms RW, Simon M, Sitbon O, Suntharalingam J, Tang H, Tchourbanov AY, Thenappan T, Torres F, Toshner MR, Treacy CM, Vonk Noordegraaf A, Waisfisz Q, Walsworth AK, Walter RE, Wharton J, White RJ, Wilt J, Wort SJ, Yung D, Lawrie A, Humbert M, Soubrier F, Tregouet DA, Prokopenko I, Kittles R, Graf S, Nichols WC, Trembath RC, Desai AA, Morrell NW, Wilkins MR, Consortium UNBRD, Consortium UPCS, Consortium UPB. Genetic determinants of risk in pulmonary arterial hypertension: international genome-wide association studies and meta-analysis. *Lancet Respir Med* 2019; 7: 227-238.
18. Rhodes CJ, Otero-Nunez P, Wharton J, Swietlik EM, Kariotis S, Harbaum L, Dunning MJ, Elinoff JM, Errington N, Thompson AAR, Iremonger J, Coghlan JG, Corris PA, Howard LS, Kiely DG, Church C, Pepke-Zaba J, Toshner M, Wort SJ, Desai AA, Humbert M, Nichols WC, Southgate L, Tregouet DA, Trembath RC, Prokopenko I, Graf S, Morrell NW, Wang D, Lawrie A, Wilkins MR. Whole-Blood RNA Profiles Associated with Pulmonary Arterial Hypertension and Clinical Outcome. *Am J Respir Crit Care Med* 2020; 202: 586-594.

**Table.** *COL18A1* variants associated with serum endostatin levels (pQTLs), gene expression (eQTLs), and clinical measures

| QTL Data | | | | | |
|---|---|---|---|---|---|
| | **ES β** | **ES *p* value** | **eQTL β** | **eQTL *p* value** | **FDR** |
| ***EA Subjects*** | | | | | |
| ***OMNI5 Array*** | | | | | |
| **rs9976834** | -3510 | 0.0001983 | -0.66 | 2.22E-197 | <1.34e-05 |
| **rs9976784** | -3205 | 0.0003702 | -0.666 | 2.27E-200 | <1.34e-05 |
| **rs2838932** | -3137 | 0.0004473 | -0.636 | 1.17E-184 | <1.34e-05 |
| **rs2236461** | -3089 | 0.0004758 | -0.643 | 4.78E-191 | <1.34e-05 |
| **rs9977482** | -2903 | 0.0006399 | -0.705 | 9.81E-254 | <1.34e-05 |
| **rs2236470** | -2864 | 0.0007104 | -0.707 | 2.57E-255 | <1.34e-05 |
| **rs8133622** | -2988 | 0.0007197 | -0.632 | 1.27E-182 | <1.34e-05 |
| **rs9980531** | -2947 | 0.0008603 | -0.625 | 5.94E-177 | <1.34e-05 |
| **rs11702782** | -3139 | 0.001041 | -0.584 | 6.75E-37 | <1.34e-05 |
| **rs12482088*** | -2758 | 0.001153 | -0.695 | 1.02E-248 | <1.34e-05 |
| **rs201577993** | -2552 | 0.001393 | NA | NA | NA |
| **rs2330180** | -2898 | 0.001514 | -0.632 | 5.80E-180 | <1.34e-05 |
| **rs2236459** | -2475 | 0.001694 | -0.513 | 1.35E-143 | <1.34e-05 |
| **rs2236464** | -2677 | 0.002145 | -0.654 | 1.06E-196 | <1.34e-05 |
| **rs11702494** | -2567 | 0.00237 | -0.674 | 1.76E-231 | <1.34e-05 |
| **rs2236451*** | -2289 | 0.00332 | -0.55 | 1.20E-176 | <1.34e-05 |
| **rs55684533** | -2479 | 0.004203 | NA | NA | NA |
| **rs10854470** | -1915 | 0.01018 | -0.445 | 1.32E-122 | <1.34e-05 |
| **rs2236454** | -1902 | 0.01065 | -0.465 | 9.84E-134 | <1.34e-05 |
| **rs4819124** | -1802 | 0.01436 | -0.367 | 1.74E-86 | <1.34e-05 |
| **rs61633029** | -1864 | 0.02498 | -0.473 | 1.06E-115 | <1.34e-05 |
| **rs17338076** | -2409 | 0.02935 | -0.674 | 1.38E-34 | <1.34e-05 |
| **rs2236479** | -1641 | 0.03106 | -0.455 | 5.77E-127 | <1.34e-05 |
| **rs2150443** | -1544 | 0.03625 | -0.367 | 2.40E-86 | <1.34e-05 |
| **rs7409857** | -1531 | 0.03885 | -0.32 | 6.64E-65 | <1.34e-05 |
| **rs7281138** | 1752 | 0.04298 | NA | NA | NA |

| | | | | | |
|---|---|---|---|---|---|
| ***WES*** | | | | | |
| **rs9979845** | -2675 | 0.0013 | -0.705 | 9.81E-254 | <1.34e-05 |
| **rs11702425** | -2107 | 0.0058 | -0.532 | 4.67E-157 | <1.34e-05 |
| **rs749627** | -1682 | 0.022 | -0.304 | 6.79E-58 | <1.34e-05 |
| ***AA Subjects*** | | | | | |
| ***OMNI5 Array*** | | | | | |
| **rs4819124** | -5598 | 0.01829 | -0.367 | 1.74E-86 | <1.34e-05 |
| **rs2150443** | -5415 | 0.0219 | -0.367 | 2.40E-86 | <1.34e-05 |
| **rs73370840** | 6899 | 0.02508 | 0.31 | 1.30E-23 | <1.34e-05 |
| **rs2838917** | -4931 | 0.02828 | 0.153 | 7.35E-13 | <1.34e-05 |
| **rs114255716** | 10260 | 0.0306 | NA | NA | NA |
| **rs78620106** | 10810 | 0.0308 | NA | NA | NA |
| **rs61633029** | 5548 | 0.03098 | -0.473 | 1.06E-115 | <1.34e-05 |
| **rs56327327** | -4398 | 0.04565 | NA | NA | NA |
| ***WES*** | | | | | |
| **rs749627** | 5172 | 0.025 | -0.304 | 6.79E-58 | <1.34e-05 |

| **Phenotypic Data** | | | |
|---|---|---|---|
| | **Clinical Measure** | **Effect Estimate** | ***p* value** |
| ***EA Subjects*** | | | |
| **rs7499** | Survival | 0.77 (0.62-0.96) | 0.018 |
| **rs1050351** | Survival | 0.76 (0.61-0.95) | 0.015 |
| **rs1131100** | 6MWD (m) | 30.61 (2.09-59.13) | 0.035 |
| **rs1131101** | 6MWD (m) | 30.61 (2.09-59.13) | 0.035 |
| **rs2236467** | 6MWD (m) | 30.16 (1.80-58.53) | 0.037 |
| **rs1131102** | 6MWD (m) | 30.11 (1.72-58.49) | 0.038 |
| **rs2236466** | 6MWD (m) | 28.49 (-0.02-57.01) | 0.050 |
| **rs7281138**** | Cardiac Index (L/m2) | -0.11 (-0.23- -0.004) | 0.043 |
| **rs2838917**** | Cardiac Index (L/m2) | -0.12 (-0.23- -0.01) | 0.028 |

| | | | |
|---|---|---|---|
| **rs2230688** | Cardiac Index (L/m2) | 0.19 (0.04-0.33) | 0.010 |
| **rs2230687** | Cardiac Index (L/m2) | 0.19 (0.05-0.33) | 0.009 |
| **rs2236456** | Cardiac Index (L/m2) | 0.14 (0.002-0.28) | 0.047 |

*Definition of abbreviations. ES: endostatin; β: beta coefficient; eQTL: expression quantitative trait loci; FDR: false discovery rate; EA: European ancestry; NA: no association between variant and gene expression; AA: African ancestry.*
*QTL data: ES beta coefficients reflect differences in ES in pg/mL for each copy of the minor allele. eQTL beta coefficients reflect differences in robust multi-array analysis (RMA), a measure of intensity derived from Affymetrix gene expression data.*
*Linear regressions on ES levels are adjusted for age and sex. Methods for eQTL models have been previously published (11). Phenotypic data: Effect estimates are hazard ratios for associations with survival and beta coefficients for associations with all other clinical measures. Coefficients reflect differences in clinical measures for subjects with the presence versus the absence of the minor allele. Associations with cardiac index are adjusted for age at enrollment, sex, PAH subtype, and PAH therapies. Associations with survival are additionally adjusted for difference in time from PAH diagnosis to cohort enrollment. Associations with 6MWD are adjusted for body mass index and the following comorbid conditions: hypertension, diabetes, obstructive lung disease, cardiomyopathy, and chronic kidney disease. Phenotypic data are reported for EA only.*
** denotes SNPs that appears on both OMNI5 and WES arrays*
*** denotes SNPs from Omni5 array; all others are WES SNPs.*