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RESEARCH ARTICLE

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# Insulin-like growth factor binding Protein-4: A novel indicator of pulmonary arterial hypertension severity and survival

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## Abstract

Proteomic analysis of patients with pulmonary arterial hypertension (PAH) has demonstrated significant abnormalities in the insulin-like growth factor axis (IGF). This study proposed to establish associations between a specific binding protein, insulin-like growth factor binding protein 4 (IGFBP4), and PAH severity as well as survival across varying study cohorts. In all cohorts studied, serum IGFBP4 levels were significantly elevated in PAH compared to controls ($p < 0.0001$). IGFBP4 concentration was also highest in the connective tissue-associated PAH (CTD-PAH) and idiopathic PAH subtypes (876 and 784 ng/mL, median, respectively). After adjustment for age and sex, IGFBP4 was significantly associated with worse PAH severity as defined by a decreased

**Abbreviations:** 6MWD, 6-min walk distance; AUC, area under the curve; CHD, congenital heart disease; CTD, connective tissue disease; ELISA, enzyme-linked immunosorbent assay; IGFBP4, insulin like growth factor binding protein 4; IQR, interquartile range; JHPH, Johns Hopkins Pulmonary Hypertension Cohort; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA-FC, New York Heart Association—Functional Classification; PAEC, pulmonary artery Endothelial cells; PAH, pulmonary artery hypertension; PAHB, PAH Biobank cohort; PAP, pulmonary arterial pressure; PASMC, pulmonary artery smooth muscle cells; PVR, pulmonary vascular resistance; RAP, right atrial pressure; WHO, World Health Organization.

6-min walk distance (6MWD), New York heart association functional class (NYHA-FC), REVEAL 2.0 score and higher right atrial pressures. In longitudinal analysis provided by one of the study cohorts, IGFBP4 was prospectively significantly associated with a shorter 6MWD, worse NYHA-FC classification, and decreased survival. Cox multivariable analysis demonstrated higher serum IGFBP4 as an independent predictor of survival in the overall PAHB cohort. Therefore, this study established that higher circulating IGFBP4 levels were significantly associated with worse PAH severity, decreased survival and disease progression. Dysregulation of IGF metabolism/growth axis may play a significant role in PAH cardio-pulmonary pathobiology.

**KEYWORDS**
biomarker, outcomes, prognosis, proteomics

Pulmonary arterial hypertension (PAH) is a progressive disease with high morbidity and mortality, defined by a resting mean pulmonary arterial pressure (mPAP) greater than or equal to 25 mmHg, pulmonary vascular resistance (PVR) greater than 3 Wood units, a pulmonary capillary wedge pressure ≤15 mmHg, and the absence of significant heart, lung, or thromboembolic disease.[1,2] Although there are multiple mechanisms causing progressive PVR and associated right heart failure, PAH therapies primarily target pulmonary vasodilation. Thus, the 5-year mortality rate for PAH remains over 40%.[3,4]

Detection of PAH in its early stages remains a challenge, particularly as emergence of symptoms often appear later on in disease. Circulating N-terminal pro-brain natriuretic peptide (NTproBNP), a marker of cardiac function and stretch not related to PAH etiology is commonly used clinically. However, NTproBNP is confounded by other factors such as left heart disease and renal function[5,6] and is ultimately a marker of heart failure, a late finding in PAH. Thus, a more specific, precise and causally related biomarker which could improve noninvasive diagnosis and monitoring of disease progression is urgently needed.

Reprogrammed energy metabolism pathways, and their associated role in facilitating cell growth and proliferation, have recently been studied in their relationship with PAH. One of these pathways is the insulin-like growth factor (IGF) axis. The IGF axis consists of two hormones (IGF1 and 2), two types of receptors (IGFR1 and 2), and 7 binding proteins (IGFBP1-7) with differing levels of binding affinity to IGFs.[7,8] Complexes between IGF and IGFBPs not only provide an IGF hormone reservoir (with its associated regulation of bioavailability) but also directly affect cell function via IGF-independent mechanisms.[9–14]

Our group has previously shown an association between increased IGFBP-2 expression in the PAH lung and decreased survival along with increased mortality.[15] In a recent mass spectrometry proteomics discovery study, IGFBP4 was also increased in PAH and verified with a small PAH cohort by enzyme-linked immunosorbent assay (ELISA).[16] In the current study, we measured circulating IGFBP4 in multiple PAH and healthy control cohorts to evaluate the value of these proteins as diagnostic/prognostic biomarkers for PAH. We also evaluated the relationships of these protein biomarkers with PAH progression and severity.

## STUDY DESIGN AND METHODS

### Study cohorts

***The Johns Hopkins pulmonary hypertension (hereafter JHPH)*** cohort includes adult patients enrolled through the JHPH Program ($n = 127$).[17] Inclusion, exclusion criteria and clinical assessments and therapy have been previously published.[15] Briefly, adults aged 18 years or older with a diagnosis of PAH established by right heart catheterization were included. Classification groups were defined by the Fifth World Symposium on Pulmonary Hypertension guidelines. Patients were followed prospectively until the data were censored for analysis in 2018. No patients were lost to follow-up. Survival was determined by review of the electronic record and search of the Social Security Death Index.

Median follow-up was 4.2 years with a range of 0.2–10 years. This was designated as our test cohort given available information and overall number of participants. Demographic and clinical data are summarized in Table 1.

***National biological sample and data repository for pulmonary arterial hypertension, or PAH Biobank (hereafter PAHB)*** is a NHLBI funded resource of World Health Organization Group 1 PAH patient biological samples, genetic data, and clinical data from 38 US Centers (www.pahbiobank.org). Subjects were included if they were at least 18 years old at the time of diagnosis and the time of enrollment, with the initial dataset including 2904 subjects. The vast majority of subjects had a prior diagnosis of PH at the time of enrollment (median time to enrollment 3.36 years, interquartile range [IQR]: 0.97–7.29 years). There was a small subset of subjects ($N = 9$) who were enrolled at the moment of diagnosis. Hemodynamic assessment for most enrollees via right heart catheterization (RHC) was at diagnosis. Only a small number ($N = 495$) had a diagnostic RHC within 6 months before enrollment, 349 within 3 months, 216 within 1 month, 4 after enrollment (3 of 4 within a couple of days), 39 with no date of diagnostic RHC. A total of 46 subjects were excluded from the dataset due to the absence of clinical data (i.e., enrollment or demographic). Median follow-up was 40.1 months (IQR: 23.5–49.5). This was designated as

**TABLE 1** Demographics and clinical characteristics of study participants.

| | JHPH cohort | PAHB cohort | VLPH cohort | Control cohort |
|---|---|---|---|---|
| Demographics | | | | |
| Subjects, *n* | 127 | 2579 | 127 | 90 |
| Age, years | 62 (50–69) | 52 (39–63) | 55 (44–65) | 37 (28–45) |
| Follow-up, years | 4.2 (0.2–10) | 3.3 (1.9–4.1) | 5 | |
| Sex, *n* female (%) | 105 (85%) | 2020 (79%) | 103 (81%) | 69 (78%) |
| Race—EA, AA, other *n* (%) | 95/20/12 (75%/16%/9%) | 2243/249/87 (86%/10%/4%) | 104/14/9 (82%/11%/7%) | 69/9/12 (77%/10%/13%) |
| NYHA FC, *n* I/II/III/IV (%III/IV) | 13/53/60/0 (47%) | 117/537/953/148 (63%) | 6/29/34/4 (52%) | |
| IPAH/APAH/FPAH, *n* (%) | 46/81/0 (36%/64%/0%) | 1097/1226/101 (43%/48%/4%) | 62/65/0 (49%/51%/0%) | |
| 6MWD (m) | 377 (298–455) | 343 (254–422) | 381 (309–432) | |
| REVEAL 2.0 Class (I/II/III) | 124/2 | 1326/13/0 | | |
| Deaths, *n* (%) | 52 (28%) | 392 (15%) | 44 (35%) | |
| Hemodynamics | | | | |
| RAP, mmHg | 7 (4–9) | 8 (5–12) | 7.5 (5–10) | |
| mPAP, mmHg | 39 (29–50) | 49 (39–58) | 45 (39–56) | |
| PAWP, mmHg | 10 (7–12) | 10 (7–13) | 11 (8–14) | |
| PVR, Wood units | 6.4 (3.4–10.3) | 8.7 (5.6–12.8) | 6.2 (4.0–9.0) | |
| Cardiac output, L/min | 4.4 (3.7–5.5) | 4.3 (3.4–5.4) | 4.6 (3.9–6.0) | |
| Cardiac index, L/min/$m^2$ | 2.6 (2.1–3.1) | 2.5 (2.0–3.1) | 2.6 (2.2–3.3) | |
| Therapies, *n* (%) | | | | |
| PDE5 monotherapy | 38 (31) | 747 (30) | 67 (53) | |
| PDE5/ERA | 53 (43) | 950 (37) | 48 (38) | |
| PDE5/ERA/IV/PCA | 32 (26) | 844 (33) | 35 (27) | |

*Note*: All data presented as median and IQR, unless otherwise specified.

Abbreviations: 6MWD, 6-min walk distance; AA, African American; CCB, calcium channel blocker; EA, European American; ERA, endothelin receptor antagonist; JHPH, Johns Hopkins pulmonary hypertension; mPAP, mean pulmonary arterial pressure; NYHA FC, New York Heart Association Functional Class; PAHB, pulmonary arterial hypertension Biobank; PAWP, pulmonary artery wedge pressure; PCA, prostacycline; PDE5, phosphodiesterase-5; PVR, pulmonary vascular resistance; RAP, right atrial pressure.

our validation cohort given available clinical and survival information as well as overall number of participants. Demographic and clinical characteristics are summarized in Table 1.

***Vanderbilt longitudinal (hereafter VLPH) cohort*** is a NHLBI funded resource consisting of PAH patients' biological samples, as well as genetic, clinical, and invasive hemodynamic data. Enrolled participants ($N = 127$) were followed longitudinally for 60 months with outcome data and IGFBP4 levels determined at three longitudinal time points.

The control serum from healthy adult volunteers were collected at the Johns Hopkins Pulmonary Center and Vanderbilt University Medical Center. A total of 90 subjects served as control ($N = 30$ and 60 from JHPH and Vanderbilt, respectively) with demographic information summarized in Table 1.

## IGFBP4 measurements in serum

IGFBP4 serum levels were measured using a commercially-available ELISA (Meso Scale Discovery). All procedures and data analysis were performed in the same lab and followed the same manufacturer instructions. The inter-plate percent coefficient of variation was 7.8% across 49 plates. All assays were performed as a continuous single batch over 3 months.

## Primary cells and cell culture conditions

Using primary pulmonary artery smooth muscle (PASMC) and endothelial cells (PAEC) from PAH or nontransplanted donors in a method similar to previously published,[15] conditioned media was harvested and centrifuged at 3000 rpm for 5 min at 4°C. Supernatants were aliquoted and stored at −80°C initially, and subsequently used for ELISA to measure IGFBP4 level. The IGFBP4 protein level in media was normalized by the protein concentration of cultured cells.

## RNA extraction and RNAseq analysis

Reagents and methodology were as described previously.[15] In summary, total RNA was extracted from PASMC and PAECs. Transcript abundance for each sample was estimated with StringTie,[18] and the FPKM[19] value for gene and transcript level were calculated with the R package Ballgown.[20–22] IGFBP4 gene expression levels (FPKM values) were extracted from the data analysis results.

## Statistical analysis

IGFBP4 levels, demographic and functional test data are presented as median and IQR, or number and percentage, as appropriate. Serum IGFBP4 levels were logarithmically transformed to achieve normality for regressions. Associations were established with various clinical measures using unadjusted Spearman's rank correlation test for continuous variables and Kruskal Wallis test for categorical variables. Age- and sex-adjusted linear regression analysis was used for continuous variables, with logistic regression used for dichotomous variables. IGFBP4 was also dichotomized at the median for survival analysis using Kaplan–Meier analysis and Cox proportional hazard regression analysis adjusted for age and sex. To assess IGFBP4 as a marker of clinical improvement, hemodynamics were compared between first and last visits in the VLPH cohort. Subjects with a 10% or greater improvement in the majority of hemodynamic variables (10% decrease in mPAP, PVR, and stable or improved cardiac output) were listed as improved. IGFBP4 levels in improved subjects were compared between initial and final visits to determine change in IGFBP4 with improved hemodynamics. A $p$ value less than 0.05 was considered statistically significant. Statistical analysis was performed using STATA (Version 15, StataCorp LLC) and MedCalc (2019 version; MedCalc Software).

# RESULTS

As shown in Table 1, test (JHPH cases), verification (PAHB) and VLPH cohorts had similar median (IQR) ages of 62 (50–69), 52 (39–63), and 55 (44–65), respectively. Sex was predominately female, at 85%, 79%, and 81%, respectively. The PAH subtypes were predominately IPAH and APAH (36% and 64%, 43% and 48%, 49% and 51%, for each of the respective cohorts). In the JHPH cohort, all APAH cases were secondary to CTD-PAH, whereas in the PAHB and VLPH cohorts it represented about a third of each cohort (31% and 37%, respectively). The CHD-PAH subtype was the least common in the PAHB and VLPH cohorts (7% and 15%, respectively). Functionally, the cohorts had similar 6-min walk distances (6MWDs) (median, IQR) of 377 (298–455), 343 (254–422), and 381 (309–432) meters, respectively. Severity of PAH as assessed by hemodynamic data was comparable between the cohorts,

though the PAHB cohort had the highest mPAP with median (IQR) of 49 (39–58) mmHg and PVR with median (IQR) of 8.7 (5.6–12.8) WU. Last, therapeutic modalities for treatment of PAH were multimodal in all cohorts. Use of PDE5 inhibitors was predominant with use in 85% of participants in JHPH, 72% in PAHB, and 53% in VLPH cohort.

As shown in Figure 1 and Table 2, the circulating IGFBP4 concentration was significantly increased in the JHPH test cohort ($N = 187$) compared with healthy control subjects ($N = 90$; median = 475 ng/mL vs. 329 ng/mL, $p < 0.001$), respectively. A serum IGFBP4 cut-off value of 352.2 ng/mL was established to distinguish PAH from controls by Youden analysis in the JHPH cohort. This cut-off value had a sensitivity and specificity for PAH of 75.4% and 58%, respectively.

The initial (baseline) sample in the VLPH cohort ($N = 127$) also had higher IGFBP4 levels compared to controls (median 593 vs. 329 ng/mL, respectively). Logistic regression analysis of longitudinal samples noted a small but significant overall increase in IGFBP4 concentrations over time (coefficient = 0.013, $p < 0.001$).

The larger multicenter PAHB cohort ($N = 2579$) was used as a validation cohort. Circulating IGFBP4 was also significantly increased compared with healthy control subjects (median = 788 ng/mL vs. 329 ng/mL, $p < 0.001$, respectively).

## IGFBP4 correlates with PAH severity

The relationship between serum IGFBP4 and invasive resting hemodynamics, exercise tolerance assessed by 6MWD, functional class, and REVEAL was also evaluated. A significant negative correlation between IGFBP4 and 6MWD was observed for all cohorts ($r = -0.28$, $p = 0.003$ for JHPH; $r = -0.31$, $p < 0.001$ for PAHB; $r = -0.26$, $p = 0.005$, Table S1). After adjustment for age and sex, linear regression revealed associations between each log-unit increase of IGFBP4 and a shorter 6MWD in the test JHPH (coefficient = −34.43, $p = 0.1$) and validation PAHB (coefficient = −31.41, $p < 0.001$) cohorts (Table 3). In the JHPH cohort, each log-unit increase in IGFBP4 was also associated with an approximate 6-mmHg higher mPAP (coefficient = 6.21, $p = 0.01$), higher PVR (coefficient = 2.76, $p = 0.005$) and a 0.87 L/min decrease in cardiac output (coefficient = −0.87, $p = 0.005$). These associations remained consistent after adjustment for PAH subtype including IPAH, as well as APAH (CTD-APAH) classification. Abnormal hemodynamics in the PAHB cohort were significant for higher right mean atrial pressures (RAP) (coefficient = 1.34, $p < 0001$) for each log-unit increase in IGFBP4. There was a tendency towards similarly elevated mPAP in the PAHB cohort, however, this did not achieve statistical significance (coefficient = 0.78, $p = 0.07$).

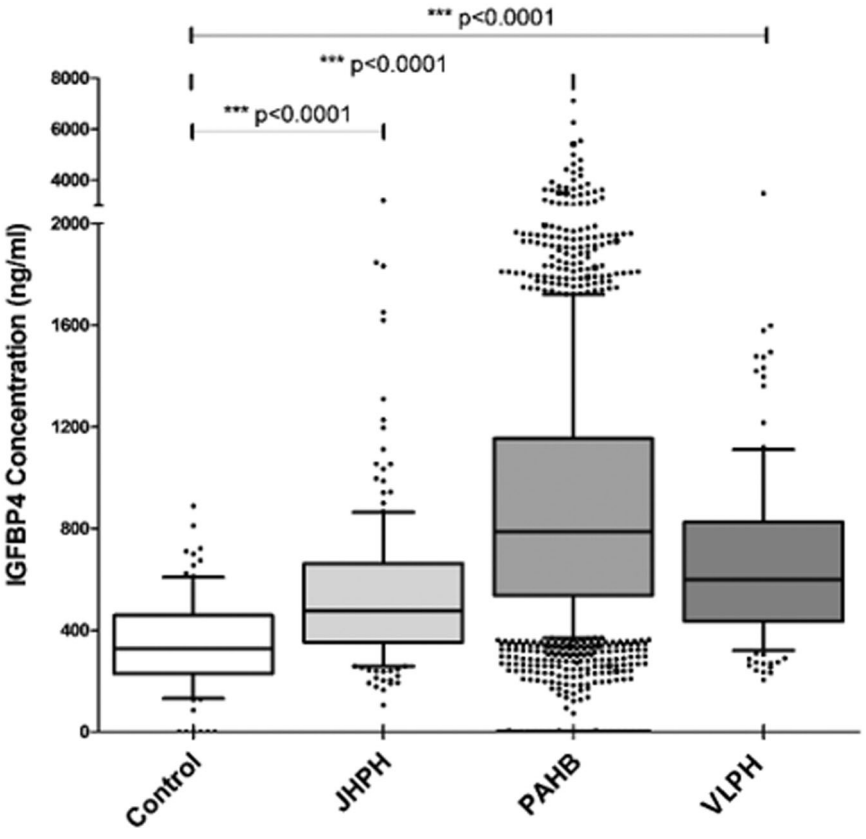


**FIGURE 1** Box and whisker plot of serum IGFBP4 concentration (ng/mL) between study cohorts versus controls. Boxes represent the interquartile range (IQR: 25%–95%) and horizontal lines are the median. Outliers are indicated with solid dots.

**TABLE 2** Comparison between PAH biomarkers across cohorts.

| | Control cohort $N = 90$ | JHPH cohort $N = 127$ | PAHB cohort $N = 2579$ | VLPH cohort[a] $N = 127$ | $p$-value[b] |
|---|---|---|---|---|---|
| Biomarker | | | | | |
| NT-proBNP, pg/mL<br>mean ± *SD* (median, IQR) | 281 ± 225<br>(212; 127–375) | 2474 ± 4245<br>(780; 250–2827) | 2331 ± 4897<br>(675; 230–2161) | 625 ± 790<br>(321; 106–827) | <0.001 |
| IGFBP4, ng/mL<br>mean ± *SD* (median, IQR) | 346 ± 187<br>(329; 229–454) | 548 ± 348<br>(475; 353–663) | 948 ± 635<br>(788; 542–1158) | 690 ± 434<br>(593; 440–826) | <0.001 |

*Note*: All data presented as mean ± *SD*, median and IQR.

Abbreviations: IQR, interquartile range; JHPH, Johns Hopkins pulmonary hypertension cohort; NT-proBNP, N-terminal brain natriuretic peptide pro-hormone; PAH, pulmonary arterial hypertension.

[a]Biomarker levels in the VLPH cohort correspond to baseline visit.

[b]Kruskal–Wallis rank sum test.

**TABLE 3** Associations (adjusted for age and sex) between IGFBP4 and clinical variables in test and verification cohorts.

| | JHPH | | | PAHB | | |
|---|---|---|---|---|---|---|
| **Linear regression** | **Coefficient** | **95% CI** | ***p*-value** | **Coefficient** | **95% CI** | ***p*-value** |
| Demographics | | | | | | |
| BSA, $m^2$ | 0.0040 | −0.089, 0.096 | 0.9 | 0.043 | ***0.026, 0.060*** | ***<0.001*** |
| 6MWD, m | −34.43 | −78.77, 9.92 | 0.1 | ***−31.41*** | ***−41.80, −21.02*** | ***<0.001*** |
| Hemodynamics | | | | | | |
| RAP, mmHg | 1.09 | −0.50, 2.68 | 0.2 | ***1.34*** | ***0.99, 1.70*** | ***<0.001*** |
| mPAP, mmHg | ***6.21*** | ***1.25, 11.18*** | ***0.01*** | 0.78 | −0.076, 1.64 | 0.07 |
| PCWP, mmHg | −0.19 | −1.42, 1.04 | 0.8 | 0.24 | −0.025, 0.50 | 0.08 |
| PVR, Wood units | ***2.76*** | ***0.87, 4.66*** | ***0.005*** | 0.15 | −0.23, 0.53 | 0.4 |
| Cardiac output, L/min | ***−0.87*** | ***−1.47, −0.27*** | ***0.005*** | 0.064 | −0.068, 0.20 | 0.3 |
| **Logistic regression** | **Coefficient** | | ***p*-value** | **Coefficient** | | ***p*-value** |
| NYHA-FC[a] | | | | | | |
| Class II | −0.53 | −1.89, 0.83 | 0.4 | −0.013 | −0.29, 0.27 | 0.3 |
| Class III | 0.51 | −0.82, 1.85 | 0.4 | ***0.28*** | ***−0.0001, 0.57*** | ***0.05*** |
| Class IV | | | | ***0.73*** | ***0.33, 1.12*** | ***<0.001*** |
| REVEAL 2.0 | | | | | | |
| Class II | 0.61 | −1.99, 3.22 | 0.6 | ***1.34*** | ***0.50, 2.17*** | ***0.003*** |
| Class III | | | | ***1.72*** | ***0.83, 2.62*** | ***<0.001*** |

*Note*: All data presented as beta coefficient, 95% confidence interval, and $p$-value. Bold and italic values stand for statistically significant at $p < .05$. See Tables 1 and 2 for all other abbreviations.

Abbreviations: CI, confidence interval; JHPH, Johns Hopkins pulmonary hypertension cohort; NYHA-FC, New York heart association functional class; PAHB, pulmonary arterial hypertension Biobank.

[a]Multinomial logistic regression performed for dichotomous variables in the PAHB cohort only.

Associations between IGFBP4 and functional class (New York Heart Association Functional Class) were assessed in all cohorts. Initially, and given distribution of classifications across cohorts, NYHA-FC was dichotomized as class I/II versus III/IV with logistic regression demonstrating a 0.93 (JHPH), 0.36 (PAHB), and 0.33 (VLPH) increased log likelihood of patients being classified in a higher functional class with increased IGBP4 concentration. This relationship was further assessed for increased granularity via multinomial logistic regression in the PAHB cohort, defining class I as the base outcome. Logarithmic increases in IGFBP4

were associated with a 0.42 ($p = 0.003$) and 0.88 ($p < 0.001$) increased relative log odds of being classified as classes III and IV, respectively.

PAH severity, and associated mortality risk, was also assessed through use of the REVEAL 2.0 Lite risk score, a validated predictive algorithm for 1-year survival in PAH.[23–25] All variables for score calculation, however, were only available in the PAHB cohort. Within the cohort, complete data was available for approximately half of the subjects ($n = 1326$, 51%) with the majority of the cohort (99%) classified as low risk based on REVEAL risk score ≤5. After adjustment for age and sex, and using low risk as base outcome, logarithmic increases in IGFBP4 were associated with a 1.34 ($p = 0.003$) increased relative log odds of being classified in a higher classification (moderate risk).

The PAHB cohort was used to evaluate differences in hemodynamic data, biomarker levels, and associations with PAH severity according to PAH subtype (see Table 4 and Table S2). Across PAH subgroups in the PAHB cohort, the CTD-APAH subgroup demonstrated the highest overall IGFBP4 concentration (median = 876 ng/mL) and CHD-APAH the lowest (median = 632 ng/mL).

Linear regressions adjusted for age and sex were performed for the major PAH subtypes, IPAH, CTD-APAH, and CHD-APAH. Effect on 6MWD was greatest in the IPAH subgroup, with an approximate 44-m decrease with each log-unit increase in IGFBP4 (coefficient = −43.7, $p < 0.001$). Elevated mean right atrial pressures were also associated with log-unit increases in IGFBP4 across all PAH subtypes. The IPAH subgroup specifically demonstrated the highest change, with a 2 mmHg increase in RAP with log-unit increase in IGFBP4 (coefficient = 2.0, $p < 0.001$). Log-unit increases in IGFBP4 were associated with increase in mPAP in both the CTD-APAH and IPAH subtypes, with the former demonstrating both the higher effect (coefficient = 1.9, $p = 0.005$) as well as a significant increase in PVR (coefficient = 0.69, $p = 0.02$).

**TABLE 4** PAHBiobank study participants by PAH classification.

| | Overall | CTD-APAH | CHD-APAH | IPAH | *p*-value[a] |
|---|---|---|---|---|---|
| Demographics | | | | | |
| Subjects, *n* | 2579 | 790 | 182 | 1095 | |
| Age, years | 55 (15) | 59 (14) | 47 (16) | 55 (15) | <0.01 |
| Sex, *n* female (%) | 2012 (79) | 711 (90) | 141 (77) | 859 (78) | <0.01 |
| Race, *n* Caucasian (%) | 2062 (80) | 603 (76) | 154 (84) | 891 (81) | NS |
| NYHA FC, *n* I/II/III/IV (%III/IV) | 140/652/1094/ 175 (61%) | 29/175/342/ 40 (64%) | 12/48/79/ 11 (50%) | 61/266/473/ 89 (63%) | NS |
| 6MWD, m | 346 (135) | 323 (147) | 376 (121) | 349 (135) | <0.01 |
| Deaths, *n* (%) | 513 (20) | 204 (26) | 33 (18) | 174 (16) | <0.01 |
| Biomarker values | | | | | |
| NT-proBNP, pg/mL (median, IQR) | 661 (227–2102) | 913 (353–2864) | 725 (318–1980) | 546 (189–1702) | <0.01 |
| IGFBP4, ng/mL (median, IQR) | 779 (526–1149) | 876 (587–1275) | 632 (459–938) | 784 (526–1119) | <0.01 |
| Hemodynamics | | | | | |
| RAP, mmHg | 9 (5) | 8.5 (5) | 8 (5) | 9 (6) | <0.01 |
| mPAP, mmHg | 49 (14) | 44 (11) | 57 (18) | 50 (14) | <0.01 |
| PAWP, mmHg | 10 (4) | 10 (4) | 10 (4) | 10 (4) | NS |
| PVR, Wood units | 10 (6) | 8.5 (5) | 12.5 (8) | 10.5 (6) | <0.01 |
| Cardiac output, L/min | 4.6 (1.8) | 4.6 (1.6) | 4.6 (2) | 4.5 (1.6) | NS |
| Cardiac index, L/min/m$^2$ | 2.7 (1.1) | 2.8 (0.8) | 3 (1.7) | 2.6 (0.9) | <0.01 |

*Note*: Data expressed as mean (*SD*) unless otherwise specified. All *p* values reflect comparisons between CTD-PAH, CHD-PAH, and IPAH. See Tables 1 and 2 for other abbreviations.

Abbreviations: CTD-PAH, connective tissue disease-associated pulmonary arterial hypertension; IPAH, idiopathic PAH; PAHB, pulmonary arterial hypertension Biobank.

[a]Kruskal–Wallis rank sum test.

Similar to the overall cohort assessment, logarithmic increases in IGFBP4 were associated with a 0.37 ($p = 0.007$), 0.68 (0.03), and 0.63 ($p < 0.001$) increased relative log odds of being in a higher NYHA functional classification. Multinomial logistic regressions for NYHA-FC by PAH subtype were also performed. Increased log odds of being classified in classes III and IV was significant in the IPAH subgroup only (0.64, $p = 0.005$; 1.30, $p < 0.001$).

Finally, REVEAL 2.0 risk classification by PAH subtype was assessed. After adjustment for age and sex, and using low risk as base outcome, logarithmic increases in IGFBP4 were associated with a 2.51 ($p = 0.008$) increased relative log odds of being classified in a higher REVEAL 2.0 risk category.

Similar relationships regarding PAH severity were evaluated in the VLPH cohort in both a baseline assessment, as well as a follow-up longitudinal component. After adjustment for age and sex (Table 5), a significant negative association between IGFBP4 and 6MWD was observed in the baseline assessment with a 54-m decrease in 6MWD for each log-unit increase in IGFBP4 (coefficient = −53.89, $p = 0.02$). This negative relationship was noted to persist on longitudinal assessment with a 25-m decrease in 6MWD on follow-up assessment (coefficient = −24.9, $p = 0.004$). Otherwise a higher RAP (coefficient = 5.45, $p = 0.05$) was noted on baseline assessment of the VLPH cohort. This association, however, did not persist on longitudinal assessment. There was, however, a significant increase in mPAP with each log-unit increase in IGFBP4 in follow-up (coefficient = 3.24, $p = 0.03$).

Regarding associations between IGFBP4 and NYHA-FC in the VLPH cohort, a higher IGFBP4 was not noted to correlate with a higher functional class or REVEAL score on baseline assessment. A significant association, however, was noted in longitudinal evaluation with age and sex-adjusted logistic regression analysis revealing log-unit increases in IGFBP4 were associated with higher functional class (coefficient = 0.334, $p = 0.02$).

To assess IGFBP4 variation with clinical changes over time, IGFBP4 concentrations were assessed in VLPH subjects with clinical improvement ($N = 15$, Figure S2), as described above. Overall, IGFBP4 appeared to have a decreasing trend in subjects who had evidence of hemodynamic improvement in mPAP and PVR, in the absence of declining cardiac output. The presence of two outliers with remarkable increases in IGFBP4 over time, along with an overall small number of subjects showing significant improvement, diminished the statistical significance of this trend.

**TABLE 5** Associations (adjusted for age and sex) between IGFBP4 and clinical variables of the VLPH cohort (baseline and longitudinal).

| | VLPH (baseline) | | | VLPH (longitudinal) | | |
|---|---|---|---|---|---|---|
| | **Coefficient** | **95% CI** | ***p*-value** | **Coefficient** | **95% CI** | ***p*-value** |
| Demographics | | | | | | |
| BMI, kg/m$^2$ | 2.56 | 0.035, 5.09 | 0.09 | −0.12 | −0.66, 0.46 | 0.7 |
| 6MWD, m | ***−53.89*** | ***−98.91, −9.77*** | ***0.02*** | ***−24.9*** | ***−41.74, −8.06*** | ***0.004*** |
| Hemodynamics | | | | | | |
| RAP, mmHg | ***5.45*** | ***1.50, 9.40*** | ***0.05*** | 0.82 | −0.53, 2.17 | 0.3 |
| mPAP, mmHg | 1.08 | −6.56, 8.73 | 0.8 | ***3.24*** | ***0.22, 6.26*** | ***0.03*** |
| PCWP, mmHg | 1.22 | −3.16, 5.60 | 0.5 | −0.53 | −2.06, 0.99 | 0.6 |
| PVR, Wood units | −0.77 | −4.37, 2.83 | 0.5 | 2.30 | −3.06, 7.65 | 0.4 |
| Cardiac output, L/min | 0.76 | −0.15, 1.68 | 0.2 | −0.096 | −0.47, 0.28 | 0.6 |
| **Categorical** | **Spearman Rho** | | ***p*-value[a]** | **Coefficient** | | ***p*-value[b]** |
| NYHA -FC | ***0.241*** | | ***0.04*** | ***0.334*** | | ***0.02*** |

*Note*: All data presented as beta coefficient, 95% confidence interval, and *p*-value. Bold and italic values stand for statistically significant at $p < .05$. See Tables 1 and 2 for all other abbreviations.

Abbreviations: 6MWD, 6-min walk distance; BMI, body mass index; CI, confidence interval.

[a]Spearman correlation for baseline values.

[b]Logistic regression performed for longitudinal values.

## IGFBP4 and survival in PAH

The relationship between IGFBP4 and mortality was assessed using Kaplan–Meier survival analysis in both the test and verification cohorts as well as the VLPH cohort, all of which were censored at 5 years (Figures 2 and 3, Table S3). Across all cohorts, IGFBP4 above the median value was associated with an increased risk of death in unadjusted analysis. A Cox multivariate proportional hazards model adjusted for significant clinical variables (age, sex, NYHA-FC, and 6MWD) also demonstrated increased risk of death associated with IGFBP4 levels above the median for all cohorts: JHPH hazards ratio (HR) 2.76 (95% confidence interval [CI]: 1.2–6.3; $p = 0.01$), PAHB HR 1.85 (95% CI: 1.39–2.45; $p < 0.001$), VLPH HR 3.76 (95% CI: 1.5–9.4; $p = 0.005$).

Kaplan–Meier survival analysis was also performed in the PAHB cohort stratified by PAH subtype (Figure S1). Above median IGFBP4, respective to each subtype, revealed significant differences in survival functions. When evaluating interactions within subgroups (Table S2), however, only the CTD-PAH demonstrated a statistically significant difference in hazards ratio. This relationship was further assessed with adjusted Cox proportional hazards function, revealing decreased survival with IGFBP4 above median when model was adjusted for age and sex (HR: 1.79, 95% CI: 1.34–2.40, $p < 0.001$). The model was also adjusted for other potential confounding hemodynamic variables including baseline MAP, PAP, CO, and PVR with a persistence in decreased survival (HR: 1.65, 95% CI: 1.21–2.24, $p = 0.001$). When adjusting for NYHA-FC and 6MWD the model was no longer significant (HR: 1.40, 95% CI: 0.93–2.10, $p = 0.1$).

Lastly, Kaplan–Meier survival analysis and Cox proportional hazards for NTproBNP as the current diagnostic standard were also performed across all cohorts (Figures 2b, 2d, and 3b, Table S3).

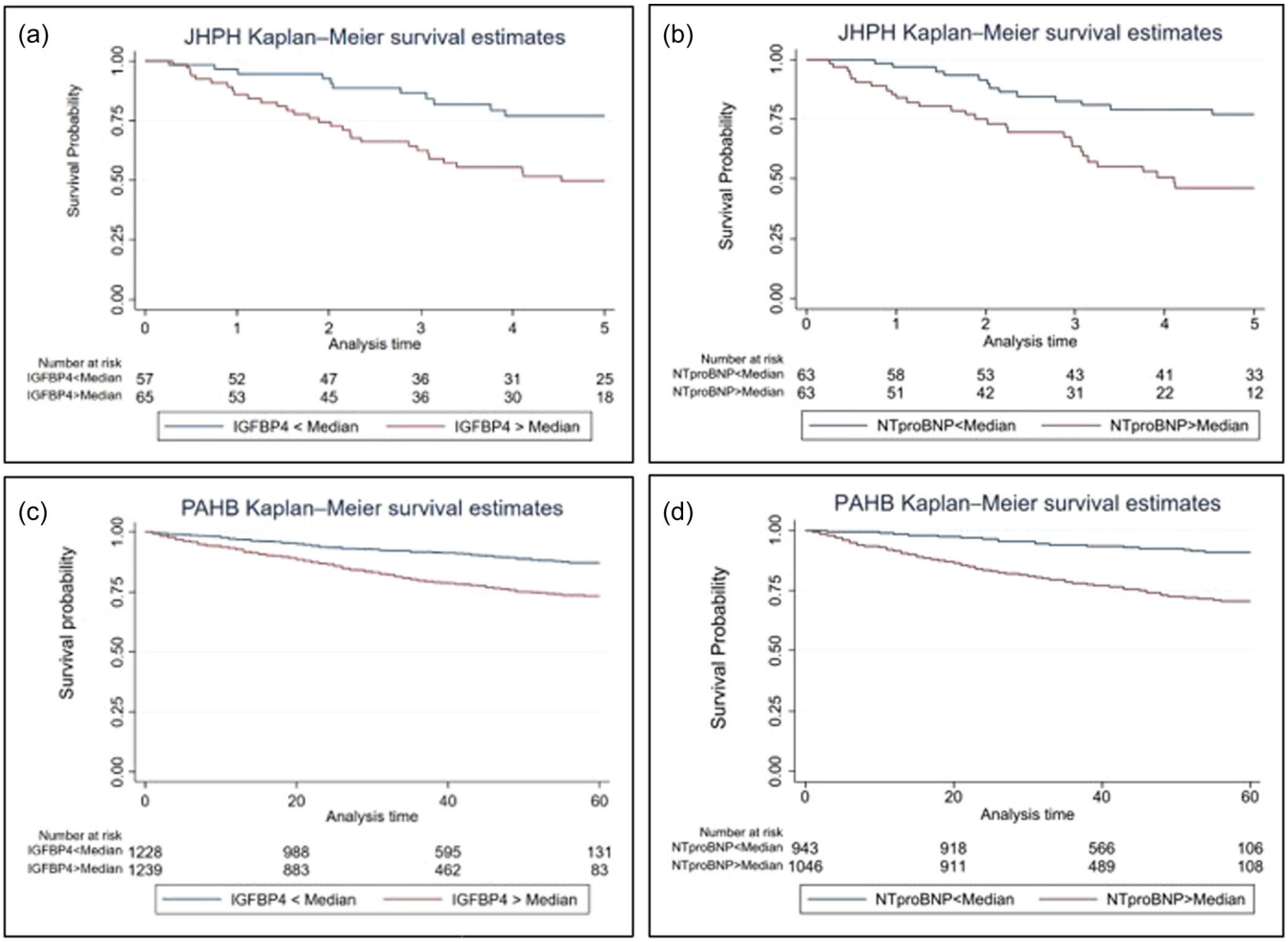


**FIGURE 2** Kaplan–Meier survival models adjusted for age, sex, NYHA functional class and 6MWD, censored at 5 years. JHPH survival analysis for (a) IGFBP4 and (b) NTproBNP. PAHB cohort survival analysis for (c) IGFBP4 and (d) NTproBNP. 6MWD, 6-min walk distance; NT-proBNP, N-terminal brain natriuretic peptide pro-hormone; NYHA, New York heart association.

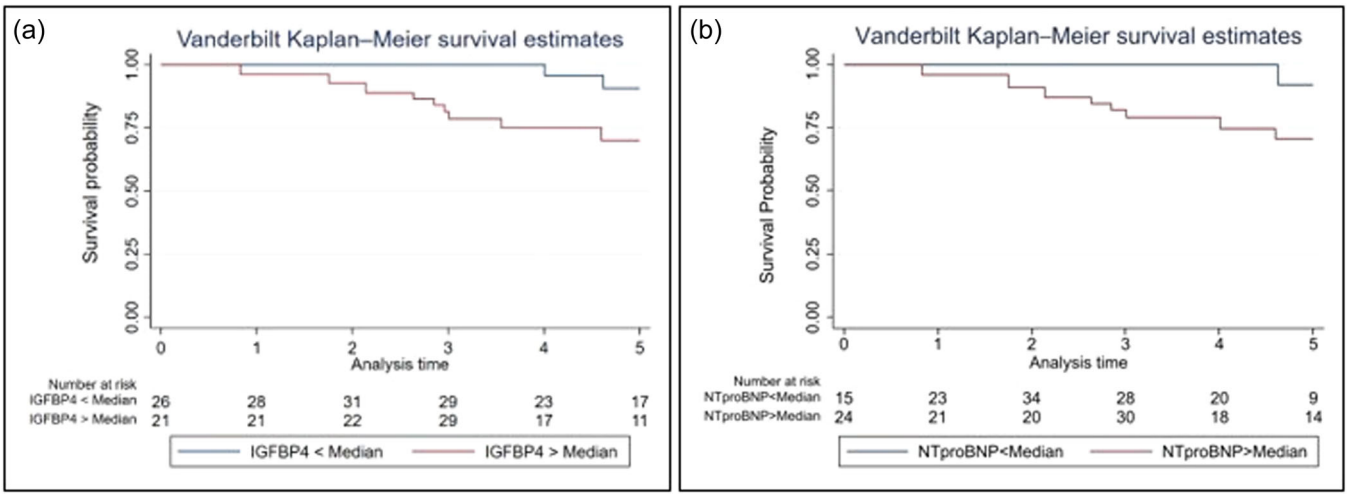


**FIGURE 3** Kaplan–Meier survival model adjusted for age, sex, and NYHA functional class in the VLPH cohort censored at 5 years. (a) IGFBP4 (b) NTproBNP. NT-proBNP, N-terminal brain natriuretic peptide pro-hormone.

Survival outcomes between above-median IGFBP4 and NTproBNP were comparable with both demonstrating statistically significant differences in survival functions by Log-rank test. Hazards ratios were also of comparable magnitude across cohorts.

## IGFBP4 is expressed and released by both PAEC and PASMC

The messenger RNA (mRNA) expression levels of IGFBP4 in cultured PAEC and PASMC was measured to assess whether pulmonary arterial cells were a source of IGFBP4 in lung tissues. A total of 10 PAEC and 13 PASMC separate cell lines of PH subjects were analyzed. As shown in Figure S3, the IGFBP4 mRNA expression in PASMC was significantly higher than in PAEC.

Conditioned media obtained from exposure to both PAEC and PASMC was also assessed for IGFBP4 concentration. IGFBP4 was detected in the media from both PAEC and PASMC (14 and 22 cells, respectively) and, similar to the cellular mRNA findings, the media levels were significantly higher in PASMC (Figure S4).

## DISCUSSION

This study proposed to establish the relationship between IGFBP4 levels, severity and survival in patients with PAH. With the use of three independent PAH cohorts (including a longitudinal cohort) we demonstrated that elevated IGFBP4 levels are able to discriminate between PAH cohorts and controls. Also, after adjustment for age and sex, logarithmic increases in IGFBP4 were shown to be associated with adverse hemodynamics, worse clinical PAH severity, and decreased survival. It was also demonstrated by subgroup analysis of PAH classifications that, despite higher overall IGFBP4 concentrations in the CTD-PAH subgroup, a higher risk of death and higher risk classification was observed on the IPAH subgroup.

IGFBP4 is a member of a large family of six binding proteins for IGF1 and IGF2.[7,8] IGFBPs prevent circulating IGF degradation, facilitating delivery to IGF cell surface receptors IGF signaling. IGFBP4 is a multifunctional protein that has IGF-independent activities in addition to its IGF binding role.[26]

Mainly studied in the setting of neoplasms, IGFBP4 inhibits IGF-1's effect on cellular mitosis and proliferation while playing IGF-independent roles as an antiangiogenic, antitumorigenic, and cardiogenic growth factor.[27] Mechanistically, IGFBP4 hypermethylation (with its associated downregulated expression) results in disruption of its growth modulatory effects. IGFBP4 plays a significant role in growth as IGFBP4-knockout mice are smaller than wild-type littermates.[28] This has been described as paradoxical, given the fact that loss of IGFBP4's binding to IGF ligands would be expected to result in hyperplasia given underregulated IGF ligand to IGF-1R binding. However, the IGFBP4 growth defect likely results from the loss of IGFBP4 specific targeting of IGF1.

IGFBP4 has been studied in multiple cardiopulmonary diseases. For example, IGFBP4 has been shown to robustly protect against myocardial infarction via limiting early-stage DNA damage occurrence and ischemic injury.[29] There is also a significant role of IGFBP4 in hypoxia signaling with increased expression

demonstrated both in-vitro via assessment of glioma cells with inhibited IRE1 activity (310) and in plasma of obstructive sleep apnea patients[30] with its associated hypoxemic changes. IGFBP4 has also been shown to promote the differentiation of cardiomyocytes by inhibition of Wnt signaling in an IGF-independent manner.[13] This was evidenced by IGFBP4-knockdown in Xenopus embryos which resulted in cardiac defects attributable to impaired cardiomyogenesis. IGFBP4 was also evaluated in the prototypic fibrosing disease, systemic sclerosis (SSc) whereby silencing IGFBP4 expression (leading to overall decreased levels) resulted in increased ECM production and fibrosis.[31] In contrast, elevated levels were noted in broncheoalveolar lavage fluid analysis of patients with idiopathic pulmonary fibrosis (IPF) and hypersensitivity pneumonitis (HP) compared to controls.[32]

Our study demonstrated significantly increased concentration and mRNA expression of IGFBP4 in analysis of PSMC conditioned media from pulmonary hypertension subjects. This highlights the lung as a potential site for IGFBP4 secretion and may also offer further insight into mechanisms leading to PH progression. Though data is somewhat conflicting, is likely that IGFBP4 plays dual roles in different phases of the fibrotic response, modulating both angiogenesis and fibrosis. It is also possible that increased IGFBP4 secretion may be a protective response, with physiologic changes thereafter correlating with disease severity.

The exact role that IGFBP4 plays in PAH pathophysiology is, therefore, still unknown. Our study demonstrated a significantly increased IGFBP4 gene expression and release in PASMCs compared to PAECs. IGFBP4 has effects on vascular smooth muscle cell (VSMC) growth as oxidized LDLs can upregulate Pregnancy-associated plasma protein-A (PAPPa) synthesis in VSMCs.[33] This protease effectively cleaves IGFBP4 and liberates active IGF-I which stimulates VSMC migration and proliferation (mechanisms known to be fundamental in neointimal hyperplasia).[34] Our study set out to establish associations between IGFBP4 and this disease, with increased expression noted to be associated with increased morbidity and mortality in three different patient cohorts, one of them followed longitudinally. Specifically, for the latter, associations with increased main pulmonary artery arterial pressures remained significant after adjustment on longitudinal assessments. Whether this speaks to primary vascular changes versus lung parenchymal fibrotic changes is yet to be established.

Hemodynamic and biomarker differences between PAH subgroups evaluated may also offer some insight into underlying pathophysiology. Particularly, a higher overall IGFBP4 concentration was noted in the CTD-PAH subgroup compared to other forms of acquired PAH (CHD-PAH) as well as IPAH. Given that rise in IGFBP4 concentration was related to higher mPAP (although rise is not proportional to level), it would be worth investigating whether IGFBP4 in this patient population becomes functionally inhibited. By previously elucidated mechanisms, this could lead either lung parenchymal changes, pulmonary vascular remodeling, or both. It is interesting that CHD-PAH is noted to have the lowest IGFBP4 concentration and higher PVR and mPAP of subgroups evaluated. Whether this is related to increased left-to-right atrial shunting (prototyping etiology for CHD-PAH) and feedback inhibitory expression of IGFB4 is yet to be determined and may be subject of future studies.

There are inherent limitations in this study. The JHPH and Vanderbilt cohorts are single-centered with intrinsic restrictions regarding generalizability according to the distribution of patients included. The PAHB cohort, however, represents a large multicenter registry with data abstracted in standardized fashion. Given lack of significant differences between cohorts, particularly as it relates to patient demographics and clinical severity, this limitation may be overcome. Another limitation is the type of controls used for comparison. Ideally, disease and age-matched controls (e.g., patients with connective tissue disease without PAH serving as controls for CTD-APAH) would be available to draw the most accurate and unbiased conclusions regarding change in IGFBP4 concentrations and its association to altered hemodynamics and disease severity in PAH. From all cohorts studied, however, only healthy controls had been selected for enrollment. Future studies including disease and age-matched controls may aid in further describing the IGF-axis' role in PAH. Regarding both the PAHB and VLPH cohort, there were some covariates missing or incomplete. For example, 36 patients had incomplete mortality data. However, sensitivity analysis demonstrated a minimal impact on the overall result. Serum collection time was also usually not contemporaneous with the assessments of other clinical variables which may somewhat limit interpretation of data. Also, not all participants in the VLPH cohort have IGFBP4 measurement at their first longitudinal visit. This has an impact regarding interpretation of survival analysis as participants enter the risk set later (when the first measurement was obtained, not at time 0, which is the first visit). Thus, individuals entered and left the risk set at various points on the graph (and of course, if they have repeat measures, they leave the risk set for their previous IGFBP4 level and enter the risk set for their new IGFBP4 level at every new measurement).

## CONCLUSIONS

To summarize, IGFBP4 is presented as a potential new circulating PAH biomarker associated with disease severity and survival and provides valuable clinical prognostic information. These associations were observed in multiple cohorts which were heterogenous in PAH mechanism, and one of which included longitudinal data. However, due to the retrospective nature of data collection for these cohorts, interpretation may still be limited. Prior studies have elucidated IGFBP4's role in pulmonary pathobiology but its direct association to PAH remains to be clearly established. Whether IGF-mediated or independent mechanisms are responsible, further improved understanding of this new pathway is needed to support the possible utility development for PAH clinical care.

## AUTHOR CONTRIBUTIONS

**Guillermo Torres, Jun Yang, Megan Griffiths, and Allen D. Everett**: Planned the project, analyzed the data and wrote the manuscript. **Guillermo Torres, Jun Yang, Stephanie Brandal, and Megan Griffiths**: Performed the experiments and interpreted the results. **Dhananjay Vaidya, Rachel Damico, Jun Yang**: Performed statistical analysis. **Melanie K. Nies, Rachel Damico, Catherine E. Simpson, Todd M. Kolb, Stephen C. Mathai, Michael W. Pauciulo, William C. Nichols, David D. Ivy, Eric D. Austin, Paul M. Hassoun, and Allen D. Everett**: Recruited subjects and performed research. All authors reviewed, revised, and approved the manuscript for submission.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## ETHICS STATEMENT

Each study cohort was approved by the Institutional Review Board at its respective participating institution, including Johns Hopkins University, Cincinnati Children's Hospital Medical Center, and Vanderbilt University, with all subjects' consents.

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## REFERENCES

1. Humbert M, Gerry Coghlan J, Khanna D. Early detection and management of pulmonary arterial hypertension. Eur Respir Rev. 2012;21(126):306–12.
2. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, Souza R. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53(1):1801913.
3. Hassoun PM, Mouthon L, Barberà JA, Eddahibi S, Flores SC, Grimminger F, Jones PL, Maitland ML, Michelakis ED, Morrell NW, Newman JH, Rabinovitch M, Schermuly R, Stenmark KR, Voelkel NF, Yuan JXJ, Humbert M. Inflammation, growth factors, and pulmonary vascular remodeling. JACC. 2009;54(1 Suppl):S10–9.
4. Budhiraja R, Tuder RM, Hassoun PM. Endothelial dysfunction in pulmonary hypertension. Circulation. 2004;109(2):159–65.
5. Nir A, Lindinger A, Rauh M, Bar-Oz B, Laer S, Schwachtgen L, Koch A, Falkenberg J, Mir TS. NT-pro-B-type natriuretic peptide in infants and children: reference values based on combined data from four studies. Pediatr Cardiol. 2009;30(1):3–8.
6. Panagopoulou V, Deftereos S, Kossyvakis C, Raisakis K, Giannopoulos G, Bouras G, Pyrgakis V, Cleman MW. NTproBNP: an important biomarker in cardiac diseases. Curr Top Med Chem. 2013;13(2):82–94.
7. Haywood NJ, Slater TA, Matthews CJ, Wheatcroft SB. The insulin like growth factor and binding protein family: novel therapeutic targets in obesity & diabetes. Mol Metab. 2019;19:86–96.
8. Collett-Solberg PF, Cohen P. Genetics, chemistry, and function of the IGF/IGFBP system. Endocrine. 2000;12(2):121–36.
9. Bach LA. What happened to the IGF binding proteins? Endocrinology. 2018;159(2):570–8.
10. Wang GK, Hu L, Fuller GN, Zhang W. An interaction between insulin-like growth factor-binding protein 2 (IGFBP2) and

integrin α5 is essential for IGFBP2-induced cell mobility. J Biol Chem. 2006;281(20):14085–91.
11. Schedlich LJ, Le Page SL, Firth SM, Briggs LJ, Jans DA, Baxter RC. Nuclear import of insulin-like growth factor-binding Protein-3 and -5 is mediated by the importin β subunit. J Biol Chem. 2000;275(31):23462–70.
12. Azar WJ, Azar SHX, Higgins S, Hu JF, Hoffman AR, Newgreen DF, Werther GA, Russo VC. IGFBP-2 enhances VEGF gene promoter activity and consequent promotion of angiogenesis by neuroblastoma cells. Endocrinology. 2011;152(9):3332–42.
13. Zhu W, Shiojima I, Ito Y, Li Z, Ikeda H, Yoshida M, Naito AT, Nishi J, Ueno H, Umezawa A, Minamino T, Nagai T, Kikuchi A, Asashima M, Komuro I. IGFBP-4 is an inhibitor of canonical Wnt signalling required for cardiogenesis. Nature. 2008;454(7202):345–9.
14. Xi G, Wai C, DeMambro V, Rosen CJ, Clemmons DR. IGFBP-2 directly stimulates osteoblast differentiation. J Bone Miner Res. 2014;29(11):2427–38.
15. Yang J, Griffiths M, Nies MK, Brandal S, Damico R, Vaidya D, Tao X, Simpson CE, Kolb TM, Mathai SC, Pauciulo MW, Nichols WC, Ivy DD, Austin ED, Hassoun PM, Everett AD. Insulin-like growth factor binding protein-2: a new circulating indicator of pulmonary arterial hypertension severity and survival. BMC Med. 2020;18:268.
16. Nies MK, Yang J, Griffiths M, Damico R, Zhu J, Vaydia D, Fu Z, Brandal S, Austin ED, Ivy DD, Hassoun PM, Van Eyk JE, Everett AD. Proteomics discovery of pulmonary hypertension biomarkers: insulin-like growth factor binding proteins are associated with disease severity. Pulm Circ. 2022;12(2).
17. 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(2):208–18.
18. Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. Nature Biotechnol. 2015;33:290–5.
19. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by rna-seq. Nature Methods. 2008;5(7):621–8.
20. Jack F, Frazee AC, Collado-Torres L, et al. Ballgown: flexible, isoform-level differential expression analysis. R package version 2.6.0; 2016.
21. Frazee AC, Pertea G, Jaffe AE, Langmead B, Salzberg SL, Leek JT. Ballgown bridges the gap between transcriptome assembly and expression analysis. Nature Biotechnol. 2015;33(3):243–6.
22. Pertea M, Kim D, Pertea GM, Leek JT, Salzberg SL. Transcript-level expression analysis of RNAseq experiments with HISAT, StringTie and Ballgown. Nat Protoc. 2016;11:1650–67.
23. Benza RL, Gomberg-Maitland M, Miller DP, Frost A, Frantz RP, Foreman AJ, Badesch DB, McGoon MD. The REVEAL Registry risk score calculator in patients newly diagnosed with pulmonary arterial hypertension. Chest. 2012;141(2):354–62.
24. Benza RL, Miller DP, Gomberg-Maitland M, Frantz RP, Foreman AJ, Coffey CS, Frost A, Barst RJ, Badesch DB, Elliott CG, Liou TG, McGoon MD. Predicting survival in pulmonary arterial hypertension: insights from the registry to evaluate early and long-term pulmonary arterial hypertension disease management (REVEAL). Circulation. 2010;122(2):164–72.
25. Benza RL, Kanwar MK, Raina A, Scott JV, Zhao CL, Selej M, Elliott CG, Farber HW. Development and validation of an abridged version of the REVEAL 2.0 risk score calculator, REVEAL lite 2, for use in patients with pulmonary arterial hypertension. Chest. 2021;159(1):337–46. https://doi.org/10.1016/j.chest.2020.08.2069
26. Clemmons DR. Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol Cell Endocrinol. 1998;140(1–2):19–24. https://doi.org/10.1016/s0303-7207(98)00024-0
27. Moreno MJ, Ball M, Rukhlova M, Slinn J, L'Abbe D, Iqbal U, Monette R, Hagedorn M, O'Connor-McCourt MD, Durocher Y, Stanimirovic DB. IGFBP-4 anti-angiogenic and anti-tumorigenic effects are associated with anti-cathepsin B activity. Neoplasia. 2013;15(5):554–67.
28. Ning Y, Schuller AGP, Conover CA, Pintar JE. Insulin-like growth factor (IGF) binding protein-4 is both a positive and negative regulator of IGF activity in vivo. Mol Endocrinol. 2008;22(5):1213–25. https://doi.org/10.1210/me.2007-0536
29. Wo D, Peng J, Ren D, Qiu L, Chen J, Zhu Y, Yan Y, Yan H, Wu J, Ma E, Zhong TP, Chen Y, Liu Z, Liu S, Ao L, Liu Z, Jiang C, Peng J, Zou Y, Qian Q, Zhu W. Opposing roles of Wnt inhibitors IGFBP-4 and Dkk1 in cardiac ischemia by differential targeting of LRP5/6 and β-catenin. Circulation. 2016;134:1991–2007.
30. Alterki A, Al Shawaf E, Al-Khairi I, Cherian P, Sriraman D, Hammad M, Thanaraj TA, Ebrahim MAK, Al-Mulla F, Abu-Farha M, Abubaker J. The rise of IGFBP4 in people with obstructive sleep apnea and multilevel sleep surgery recovers its basal levels. Dis Markers. 2021;2021:1219593. https://doi.org/10.1155/2021/1219593
31. Su Y, Nishimoto T, Hoffman S, Nguyen XX, Pilewski JM, Feghali-Bostwick C. Insulin-like growth factor binding protein-4 exerts antifibrotic activity by reducing levels of connective tissue growth factor and the C-X-C chemokine receptor 4. FASEB BioAdvances. 2019;1:167–79.
32. Günther A, Schmidt R, Nix F, Yabut-Perez M, Guth C, Rosseau S, Siebert C, Grimminger F, Morr H, Velcovsky H, Seeger W. Surfactant abnormalities in idiopathic pulmonary fibrosis, hypersensitivity pneumonitis and sarcoidosis. Eur Respir J. 1999;14(3):565–73. https://doi.org/10.1034/j.1399-3003.1999.14c14.x
33. Boyer MJ, Kimura Y, Akiyama T, Baggett AY, Preston KJ, Scalia R, Eguchi S, Rizzo V. Endothelial cell-derived extracellular vesicles alter vascular smooth muscle cell phenotype through high-mobility group box proteins. J Extracell Vesicles. 2020;9(1):1781427. https://doi.org/10.1080/20013078.2020.1781427
34. Bayes-Genis A, Schwartz RS, Lewis DA, Overgaard MT, Christiansen M, Oxvig C, Ashai K, Holmes DR, Conover CA. Insulin-like growth factor binding protein-4 protease produced by smooth muscle cells increases in the coronary artery after angioplasty. Arterioscler Thromb Vasc Biol. 2001;21(3):335–41. https://doi.org/10.1161/01.atv.21.3.335

35. Minchenko DO, Kharkova AP, Halkin OV, Karbovskyi LL, Minchenko OH. Effect of hypoxia on the expression of genes encoding insulin-like growth factors and some related proteins in U87 glioma cells without IRE1 function". Endocr Regul. 2016;50(no.2):43–54. https://doi.org/10.1515/enr-2016-0008

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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