

ORIGINAL ARTICLE



Multimodal Framework of Left Heart-Pulmonary Vascular Remodeling Underlying Right Ventricular Failure in PH-HFpEF

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BACKGROUND: Right ventricular (RV) dysfunction in pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF) leads to adverse outcomes, yet the mechanisms underlying RV failure remain incompletely defined. We aimed to develop a multimodal framework integrating vascular mechanics and myocardial transcriptomics for a mechanistic understanding of RV dysfunction in PH-HFpEF.

METHODS: In a 2-step study, a predominantly retrospective PH-HFpEF cohort (n=48) underwent comprehensive assessment with clinical evaluation, echocardiography, cardiac magnetic resonance imaging (MRI), and invasive cardiopulmonary exercise testing. Based on cardiac MRI-derived RV ejection fraction <45%, the PH-HFpEF cohort was stratified into a normal RV function group (n=29) and an RV dysfunction group (n=19). A prospective subset underwent pulmonary vascular mechanics (impedance and wave intensity analysis, n=17), 4-dimensional flow cardiac MRI (n=15), and endomyocardial biopsy with long-read RNA sequencing (n=10).

RESULTS: PH-HFpEF participants with RV dysfunction had worse 1-year outcomes (mortality or first heart failure hospitalization; hazard ratio, 8.2 [95% CI, 2.5–25.4]) and exhibited multisystem limitations (abnormal cardiac reserve, pulmonary vascular, and ventilatory function). Compared with the normal RV subgroup, the RV dysfunction subgroup had impaired left ventricular longitudinal strain on cardiac MRI. Pulmonary vascular mechanics demonstrated increased proximal pulmonary arterial stiffness (characteristic impedance), increased RV energy expenditure, and abnormal distal vascular reflections with exercise, indicating segmental pulmonary vascular remodeling. Four-dimensional flow MRI revealed disturbed flow patterns and trends toward increased viscous energy loss across the left heart and pulmonary circulation. Global gene differences were minimal, likely reflecting the limited statistical power for detecting individual differentially expressed genes in this modest cohort; however, pathway analysis revealed upregulation of RNA metabolism and downregulation of mitochondrial pathways in the RV dysfunction subgroup. Long-read sequencing further identified selective isoform expression in key cardiac genes, highlighting differential regulation in PH-HFpEF with RV dysfunction.

CONCLUSIONS: This integrative methodological framework of vessel-specific wave mechanics and myocardial transcriptomics advances the mechanistic understanding of left heart-pulmonary vascular remodeling in PH-HFpEF.

Key Words: biopsy ■ exercise test ■ gene expression profiling ■ heart failure ■ hypertension, pulmonary ■ magnetic resonance imaging

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WHAT IS NEW?

- Right ventricular (RV) dysfunction in pulmonary hypertension due to heart failure with preserved ejection fraction occurs across both isolated post-capillary PH and combined pre-/postcapillary PH, demonstrating that conventional pulmonary vascular resistance-based classification does not entirely capture disease severity. RV dysfunction is associated with multisystem limitations (cardiac, pulmonary vascular, respiratory, and skeletal muscle).
- Invasive wave mechanics (impedance and wave separation analyses) and noninvasive 4-dimensional flow cardiac magnetic resonance imaging reveal unique patterns of impaired left ventricular strain, proximal pulmonary artery stiffness, RV energy inefficiency, and abnormal distal pulmonary vascular reflections in pulmonary hypertension due to heart failure with preserved ejection fraction, providing insights into segmental abnormalities across the left heart-pulmonary circulation axis.
- Pulmonary hypertension due to heart failure with preserved ejection fraction phenogroups exhibit distinct pathway signatures. Long-read RNA sequencing reveals pathway-level shifts (RNA processing ↑, mitochondrial function ↓) associated with RV dysfunction.

WHAT ARE THE CLINICAL IMPLICATIONS?

- These findings offer a methodological framework of vessel-specific pulmonary vascular abnormalities for more refined patient classification beyond conventional pulmonary vascular resistance-based methods. This may improve identification of individuals at higher risk of RV failure who may benefit from closer monitoring or clinical management.
- The integrative approach highlights transcript expression differences for a handful of genes that could reveal potential therapeutic targets, supporting the development of biology-informed, phenotype-specific interventions for pulmonary hypertension due to heart failure with preserved ejection fraction and RV dysfunction.

Heart failure (HF) with preserved ejection fraction (HFpEF) is the most rapidly growing form of HF, driven by an aging population and increasing rates of comorbidities.¹ In addition to myocardial dysfunction and stiffness, remodeling of the pulmonary vasculature precipitates pulmonary hypertension (PH) in >83% of HFpEF cases.^{2,3} Pulmonary vascular disease further exacerbates heterogeneity in the already complex multisystem disease of HFpEF, resulting in right ventricular (RV) failure and increased mortality.⁴ RV dysfunction likely results from the combined effects of intrinsic myocardial disease and superimposed pulmonary vascular remodeling, leading to excess afterload and impaired RV-pulmonary arterial (PA) coupling.⁵ Despite its prognostic

Nonstandard Abbreviations and Acronyms

4D	4-dimensional
cmMRI	cardiac magnetic resonance imaging
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
iCPET	invasive cardiopulmonary exercise testing
LV	left ventricular
PA	pulmonary artery
PH	pulmonary hypertension
PVR	pulmonary vascular resistance
RNA-seq	RNA sequencing
RV	right ventricular

importance, the mechanisms driving RV failure in PH-HFpEF remain incompletely defined, highlighting a critical need for mechanistic investigation.⁵

The pathobiological nature of pulmonary vascular disease in PH-HFpEF is poorly characterized by clinical right heart catheterization, which only captures the steady component of RV afterload with pulmonary vascular resistance (PVR).^{6–9} PVR is commonly used to categorize PH-HFpEF into isolated postcapillary PH (with PVR ≤2 Woods units) or combined pre-/postcapillary PH (with PVR >2 Woods units).¹⁰ While these categories identify a higher-risk PH-HFpEF phenotype (combined pre-/postcapillary PH),¹¹ PVR remains a simplistic measure that does not inform vessel-specific pulmonary vascular remodeling.^{8,9} This limitation, and the inability of PVR to better phenotype the heterogeneous disease of PH-HFpEF, is reflected in multiple failed clinical trials.^{12–14}

To develop more effective, individualized therapies that prevent RV failure in PH-HFpEF, several critical challenges must first be addressed: (1) poor phenotypic and structural characterization of PH-HFpEF with limited data (rest clinical hemodynamics [eg, PVR] and echocardiography),^{7,15} (2) anatomic heterogeneity of pulmonary vascular disease (large pulmonary arteries, small arterioles and capillaries, and venules/veins),^{6,9} and (3) unknown biological mechanisms associated with the transition from a low-risk (no RV failure) to a high-risk (with RV failure) PH-HFpEF state.¹⁶ We hypothesize that PH-HFpEF arises from a coupled myocardial (left heart) and pulmonary vascular remodeling that drives RV failure (Figure 1A). Using a multimodal framework incorporating vascular mechanics, cardiopulmonary 4-dimensional (4D) flow MRI, and myocardial transcriptomics, this study aims to establish a methodological platform for deep phenotyping. This integrative approach can advance the understanding of left heart-pulmonary vascular remodeling

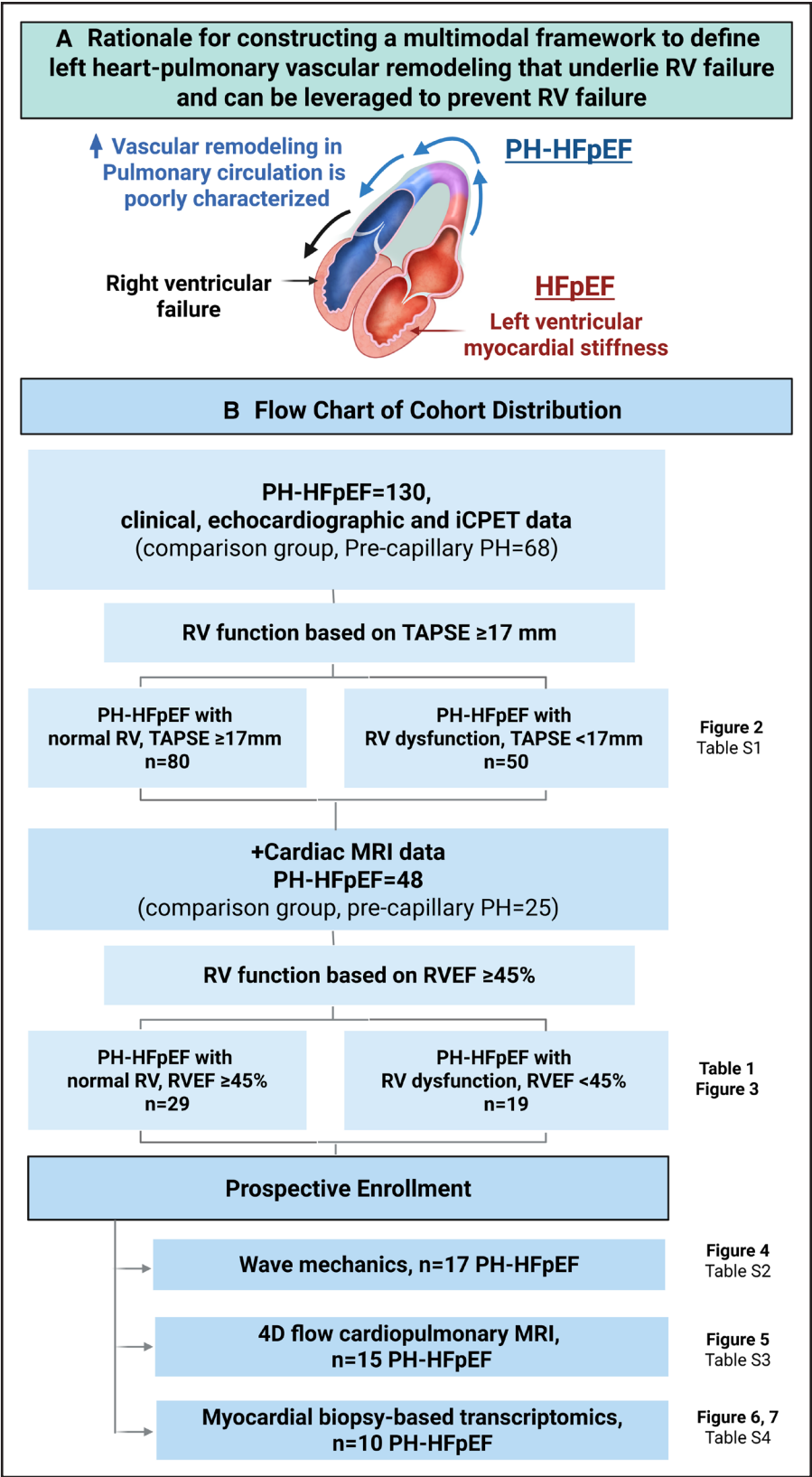


Figure 1. Study rationale and cohort distribution. Study rationale (A) and cohort distribution (B). HFpEF indicates heart failure with preserved ejection fraction; iCPET, invasive cardiopulmonary exercise testing; MRI, magnetic resonance imaging; PH, pulmonary hypertension; RV, right ventricular; RVEF, right ventricular ejection fraction; and TAPSE, tricuspid annular plane systolic excursion. Created in BioRender. Raza, F. (2026) <https://BioRender.com/hftah4y>.

underlying RV failure and may potentially inform future precision-based therapies in PH-HFpEF.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Population

As summarized in Figure 1B, this 2-step study included analyses of: (1) a larger PH-HFpEF patient group, including retrospective and prospective cohorts, and (2) a prospective cohort of PH-HFpEF study participants.

For the larger, predominantly retrospective cohort, participants with PH-HFpEF (study group, $n=130$) and precapillary PH (comparison group, $n=68$) were included if they underwent comprehensive assessments including clinical evaluations, echocardiography, and invasive cardiopulmonary exercise testing (iCPET). A subset of these participants also underwent cardiac magnetic resonance imaging (cMRI) within 6 months of the iCPET study, including 48 with PH-HFpEF and 25 with precapillary PH. This subset with cMRI data constituted the primary analysis cohort and included participants enrolled prospectively (Table). On cMRI, an RV ejection fraction $<45\%$ was selected as a metric for RV dysfunction based on prior literature in both PH due to HFpEF¹⁷ and PA hypertension.¹⁸ All PH-HFpEF participants met the criteria for HFpEF and secondary PH according to the European Society of Cardiology/European Respiratory Society 2022 PH guidelines,¹⁰ with HF symptoms and a left ventricular (LV) ejection fraction $\geq 50\%$ on echocardiography. Similarly, all participants in the precapillary PH group met the European Society of Cardiology/European Respiratory Society 2022 PH guidelines.¹⁰ For all participants, adverse clinical outcomes (HF hospitalization and death) were recorded for 1 year after the iCPET study.

The second prospective arm of the study included modular participation of study participants. Seventeen PH-HFpEF and 5 precapillary PH participants underwent invasive ventricular-vascular mechanics studies with exercise and RV pressure-volume loops.^{7–9} Among these participants, 15 PH-HFpEF (and 3 precapillary PH participants) underwent 4D flow cardiopulmonary MRI (within 48 hours of the iCPET study), and 10 PH-HFpEF participants underwent endomyocardial biopsy.

Given our focus on pulmonary vascular remodeling, precapillary PH represents an appropriate comparison group, as remodeling is primarily confined to the arterial compartment and targeted pulmonary vascular therapies are well established. All participants provided written informed consent in accordance with the University of Wisconsin-Madison institutional review board approval (ID: 2019-1184). The study complied with the Declaration of Helsinki.

Invasive Cardiopulmonary Exercise Test

iCPET was performed using a recumbent stationary ergometer, as previously reported.^{3,19–23} Briefly, right heart catheterization was performed with a balloon-tipped catheter through a 7-Fr venous sheath via internal jugular access, and a 4-Fr radial arterial catheter was placed for arterial blood gas analyses. Breath-by-breath expired gas exchange analysis was performed with

an Ultima CardiO2 MedGraphics metabolic cart. Cardiac output was measured at all stages with the direct Fick principle. Hemodynamic data were acquired at rest and at different stages of exercise (every 25 W; peak-exercise data reported). Specific CPET metrics were recorded: peak oxygen consumption and the slope of minute ventilation/carbon dioxide production.

Cardiac MRI

cMRI was performed on all participants to assess cardiac structure and function. Imaging was conducted using a 3.0T MRI scanner (Signa Premier, GE Healthcare, Waukesha, WI) at the University of Wisconsin-Madison. Standard cine imaging sequences were obtained to evaluate LV and RV volumes, ejection fraction, and myocardial mass. LV and RV function were specifically assessed using short-axis and axial cine balanced steady-state free precession views to quantify RV volumes and ejection fraction, as previously described.⁷ Scan parameters included reconstructed temporal resolution=35 to 45 ms, repetition time=3.1 ms, echo time=1.1 ms, field of view=40×40 cm, acquired spatial resolution=1.79×1.79 mm, reconstructed spatial resolution=1.56×1.56 mm, slice thickness=8 mm, and reconstructed cardiac phases=20.

Integrative Cardiopulmonary 4D Flow MRI

Imaging was performed using a 3.0T MRI scanner (GE SIGNA Premier), with a radially undersampled 4D flow cMRI protocol.²⁴ This technique allows for comprehensive, retrospective, and detailed visualization and quantification of blood flow dynamics in the vasculature and cardiac chambers of the heart. The 4D flow MRI data were preprocessed following established guidelines, accounting for noise correction, background phase correction, and velocity antialiasing.²⁵ A 3-dimensional PC angiogram was then generated to manually segment the pulmonary veins, pulmonary arteries, left atrium, and aorta using commercial software (Mimics Innovation Suite 20.0; Materialize, Leuven, Belgium). The manual segmentations were then exported to flow-quantification software (EnSight 2022 R2; Ansys, Canonsburg, PA), where cut planes were placed at standardized regions of interest for each vessel (aorta, pulmonary artery, and pulmonary veins) and cardiac chambers (left atrium and LV), followed by creating flow streamline visualizations. The cut planes were then processed using custom in-house software in MATLAB (MathWorks, Natick, MA).

Impedance and Wave Intensity Analysis

For quantification of impedance and wave intensity analysis, simultaneous PA pressure (via a Millar catheter in the PA during iCPET) and RV outflow tract pulse wave Doppler-based flow (obtained via Doppler echocardiography) were collected at rest and during low-workload exercise (25 W), as previously described.^{7–9,20} These simultaneous pressure-flow data were postprocessed using a time-domain method to generate a PQ loop (pressure-flow).^{8,9,26} This loop was then used to derive metrics that specify vessel-specific and cardiac cycle-specific abnormalities, as well as ventricular-vascular interactions in the pulmonary circulation and RV.^{8,9} These metrics included characteristic impedance (a measure of proximal PA stiffness),^{78,27} compression waves during systole (which reflect RV force generation and proximal wave reflections from the pulmonary

Table. PH-HFpEF Subgroups and Comparison Precapillary PH Group

	PH-HFpEF with normal RV, n=29	PH-HFpEF with RV dysfunction, n=19	Precapillary PH, n=25
PH-HFpEF phenotype			
lpcPH	16	4	
CpcPH	13	15	
Precapillary PH subtype			
Idiopathic PAH			9
Scleroderma PAH			8
WHO group III PH			4
CTEPH			4
Age, y	64.9±9.3*	69.9±9.8†	61.0±11.6
Female, n (%)	16 (55)*	5 (26)†	14 (58)
BMI, kg/m ²	36.1±8.2*	31.8±5.5	28.7±6.2
Creatinine, mg/dL	1.1±0.4*	1.4±0.5†	1.0±0.4
Comorbidities			
Diabetes	10 (34)	7 (17)	7 (29)
Hypertension	27 (93)	17 (89)†	12 (50)‡
COPD	3 (10)	3 (16)	2 (8)
Coronary artery disease	7 (24)	11 (58)	5 (21)
Chronic kidney disease-3	6 (21)	6 (32)	3 (12)
Interstitial lung disease	1 (3)	1 (5)	5 (21)
Atrial fibrillation	8 (10)	13 (68)†	2 (8)
Pulmonary embolism	7 (24)	0 (0)	7 (29)
Scleroderma	1 (3)	2 (10)	5 (21)
OSA	11 (38)	11 (58)	7 (29)
Echocardiogram			
LVEF, %	63±3	61±5	60±7
LVMI, g/m ²	83±17*	108±49†	77±24
Eccentricity index	1.0±0.2*	1.2±0.3	1.4±0.5‡
RV:LV ratio	0.8±0.1	1.0±0.4	1.1±0.4‡
RV base/apex systole	2.3±0.6*	1.7±0.2	1.8±0.5‡
RV FAC, %	36±8*	31±12	29±8‡
TAPSE, mm	21±7	20±7	18±3
LAVI, mL/m ²	30±9*	42±16†	26±10
Mitral E, cm/s	85±26	81±26	69±20
E' medial, cm/s	6.3±3.0	6.9±3.6	7.2±3.6
E' lateral, cm/s	8.6±2.9	8.8±3.6	8.8±2.5
E/E' avg	12±6	11±8	10±4
Epicardial fat, mm	6.9±2.7	6.5±2.4	5.3±2.0
Rest hemodynamics			
Heart rate, beats per minute	72±12	69±12	72±10
Right atrial pressure, mmHg	13±4	12±4	11±4
mPAP, mmHg	32±8	31±10	39±10‡
PAWP, mmHg	18±4	17±4†	13±2‡
Fick cardiac output, L/min	5.8±1.6*	4.8±1.1	4.5±0.9‡
Fick cardiac index, L/min per m ²	2.7±0.6*	2.3±0.5	2.3±0.4‡
PVR, WU	2.7±1.8	3.1±1.8†	5.8±2.5
PAC, mL/mmHg	4.8±1.9*	3.6±1.2	2.4±1.0‡

(Continued)

Table. Continued

	PH-HFpEF with normal RV, n=29	PH-HFpEF with RV dysfunction, n=19	Precapillary PH, n=25
Exercise hemodynamics			
Heart rate, beats per minute	103±17	94±23	107±19
Right atrial pressure, mm Hg	21±5	21±4	18±5
mPAP, mm Hg	49±11	50±13	59±14
PAWP, mm Hg	30±5	32±6†	19±2‡
Fick cardiac output, L/min	10.6±3.4*	8.4±3.6	9.0±2.3‡
Fick cardiac index, L/min per m ²	4.9±1.4*	3.9±1.6†	4.7±1.1‡
PVR, WU	2.4±2.4	2.5±1.9	5.3±2.4
PAC, mL/mm Hg	3.2±1.7	2.6±1.3	1.8±0.9‡
mPAP/CO slope, mm Hg/L per min	5.1±4.6*	10.0±10.3	6.4±3.9
PAWP/CO slope, mm Hg/L per min	4.0±5.9*	8.2±8.0†	1.6±1.3‡
Cardiopulmonary exercise test			
Work, W	69±38	61±26	62±35
Peak VO ₂ , mL/kg per min	16.2±4.9*	12.2±4.7	15.6±5.5
Peak VO ₂ (%predicted)	60±17*	49±18	55±21
V _E /VCO ₂ slope	32±5*	38±8	38±10‡
OUES, mL/min per log(L/min)	1.53±0.37*	1.21±0.41	1.24±0.36‡
Peak O ₂ -pulse, mL/beat	10.6±2.6	10.5±3.0	8.6±2.1‡
Cardiac MRI			
RVEF, %	53±6*	38±6	43±13
RV EDVi, mL/m ²	69±16*	92±18	83±28
RV ESVi, mL/m ²	33±10*	58±15	51±28‡
LVEF, %	60±7†	57±5†	54±11‡
LV EDVi, mL/m ²	63±14	74±26	63±16
LV ESVi, mL/m ²	27±9	39±24	30±16
LV strain longitudinal, %	−12.8±2.6*	−9.2±5.0†	−12.0±4.8
LV strain circumferential, %	−16.8±2.3*	−13.2±3.3†	−16.0±4.9
LV strain radial, %	27.9±5.8*	20.3±7.1†	27.0±10.5
RV strain longitudinal, %	−17.4±5.5*	−12.2±4.8†	−13.6±7.2‡
RV strain circumferential, %	−13.9±3.3*	−10.3±2.6	−11.3±6.3
RV strain radial, %	22.9±7.6*	15.4±5.3	18.8±12.6
LA longitudinal strain, %	21.2±16.4	18.4±7.8†	24.9±9.9
LA EF, %	41±16	38±17†	54±11‡
RA longitudinal strain, %	18.2±9.7	14.4±10.5	19.1±12.5
RA EF, %	42±16	35±15	42±10
Epicardial fat, mL	54±36	44±15	39±23
Septal angle	140±14*	143±17	144±15

Adjusted *P* values using the Benjamini-Hochberg method. BMI indicates body mass index; CO, cardiac output; COPD, chronic obstructive pulmonary disease; CpcPH, combined pre-/postcapillary PH; CTEPH, chronic thromboembolic pulmonary hypertension; E', mitral tissue Doppler; EDVi, end-diastolic volume index; EF, ejection fraction; ESVi, end-systolic volume index; FAC, fractional area change; lpcPH, isolated postcapillary PH; LA, left atrium; LAVI, left atrial volume index; LV, left ventricular; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; Mitral E, mitral early diastolic velocity; mPAP, mean pulmonary artery pressure; MRI, magnetic resonance imaging; OSA, obstructive sleep apnea; OUES, oxygen uptake efficiency slope; PAC, pulmonary artery compliance; PAH, pulmonary arterial hypertension; PAWP, pulmonary artery wedge pressure; PH-HFpEF, pulmonary hypertension due to heart failure with preserved ejection fraction; PVR, pulmonary vascular resistance; RA, right atrium; RV, right ventricular; RVEF, right ventricular ejection fraction; TAPSE, tricuspid annular plane systolic excursion; V_E/VCO₂, ventilatory efficiency of carbon dioxide; VO₂, oxygen consumption; WHO, World Health Organization; and WU, Woods unit.

**P*<0.05 normal RV vs RV dysfunction PH-HFpEF.

†*P*<0.05 RV dysfunction PH-HFpEF vs precapillary PH.

‡*P*<0.05 normal RV PH-HFpEF vs precapillary PH.

circulation), and decompression waves during diastole (representing RV relaxation and distal vascular reflections from pulmonary venular/venous segments).^{9,28–32} We have previously described these methods in detail.^{8,9}

Endomyocardial Biopsy Collection

Endomyocardial septal biopsies (Figure S1) were collected from a subset of participants (PH-HFpEF=10) to define differential gene and transcript expression associated with the high-risk state of RV failure (versus the low-risk state with preserved RV function) in PH-HFpEF (procedural details are provided in the Supplemental Material).

Long-Read RNA-Seq and Bioinformatic Analysis

To assess gene expression and transcript isoform usage between normal RV and dysfunctional RV phenogroups, full-length RNA sequencing (RNA-Seq) was performed using PacBio long-read technology. Total RNA was converted into full-length cDNA libraries (Kinnex Full-Length RNA Kit) and sequenced on a PacBio Revio platform (30-hour movie), with primary processing conducted using SMRT Link, v13.1, to generate high-quality full-length reads. Reads were quantified using IsoQuant (v3.6.1) with GRCh38 (Ensembl v113),³³ employing minimap2 (v2.28) for alignment to obtain transcript- and gene-level count matrices.³⁴ Transcript models were classified and filtered using SQANTI3 (v5.5)³⁵ to remove artifacts (eg, intraprimering and RT-switching), followed by rescue of valid reads to annotated or high-confidence novel isoforms. Differential transcript usage was analyzed using DRIMSeq (v1.34.0)³⁶ with Dirichlet-multinomial modeling and stageR for 2-stage false discovery rate control ($\alpha=0.05$), after filtering low-abundance features. Differential gene and transcript expression were assessed using DESeq2 (v1.42.1)³⁷ following standard normalization and dispersion estimation workflows, with low-expression features excluded. Principal component analysis was performed on the top 1000 most variable genes using log₂-transformed normalized counts to evaluate global transcriptomic differences. Gene set enrichment analysis was conducted using clusterProfiler (v4.10.0),³⁸ ranking genes by DESeq2 Wald statistics to identify enriched Gene Ontology pathways, while overrepresentation analysis was additionally applied to genes with >0.1% novel isoform usage. For gene set enrichment analysis, Gene Ontology enrichment, and pathway analysis, *P* values were adjusted using the Benjamini-Hochberg method, and significance was defined as an adjusted $P<0.05$.³⁹ All code for the bioinformatics analysis is available here: <https://github.com/stewart-lab/IsoQuant>.

Statistical Analysis

Baseline characteristics were presented as mean $\pm$ SD for continuous variables and as frequencies and proportions (%) for categorical variables. For continuous variables, comparisons among groups were conducted using the ANOVA test (or Wilcoxon rank-sum test for nonnormally distributed data). To account for multiple comparisons, false discovery rate correction was applied using the Benjamini-Hochberg method.³⁹ Differences in categorical data were assessed using the χ^2 test or the Fisher exact test, as appropriate. Kaplan-Meier survival curves were created to estimate survival rates within

each group. Differences between groups were evaluated using the log-rank test. In addition, multivariate Cox proportional hazards regression, adjusted for age and sex, was used to identify and quantify the association between predictors and the hazard of an event. The analyses were conducted in GraphPad Prism, version 10.4.2, R, version 4.4.2, and Python, version 3.9.7.

RESULTS

Multisystem Limitations Associated With RV Failure in PH-HFpEF

In the larger PH-HFpEF data set ($n=130$; Figure 1), using tricuspid annular plane systolic excursion ≥ 17 mm as the optimal cutoff per guidelines, 80 participants had normal RV function and 50 had RV dysfunction. As shown in Figure 2 and Table S1, PH-HFpEF participants with RV dysfunction had limitations across multiple domains, including reduced aerobic capacity and cardiac reserve, abnormal pulmonary vascular and respiratory function, along with reduced peripheral oxygen utilization by skeletal muscles.

Unified Left Heart-Pulmonary Vascular Contributors to RV Dysfunction in PH-HFpEF

A subset of the larger PH-HFpEF cohort had cardiac MRI data, enabling in-depth morphological characterization of biventricular structure and function. This subset included 48 PH-HFpEF participants and 25 precapillary PH participants in the comparison group (Figure 1B). Using RVEF $<45\%$ ^{17,18} as a marker of RV dysfunction, the comparison of PH-HFpEF subgroups (normal RV, $n=29$, versus RV dysfunction, $n=19$) provided insights into contributors of RV dysfunction in PH-HFpEF (Figure 3; Table).

When comparing PH-HFpEF subgroups with normal RV function ($n=29$) and RV dysfunction ($n=19$), there was heterogeneous distribution of conventional postcapillary PH subtypes (Ipc- versus Cpc-PH; Figure 3A), indicating that RV dysfunction is not confined to high-risk Cpc-PH. RVEF and RV longitudinal strain were lower in the RV dysfunction subgroup, as expected (Figure 3B and 3C). Compared with the normal RV subgroup, the RV dysfunction subgroup had lower LV longitudinal strain (Figure 3D) and lower PA compliance (Figure 3E), indicating unified contributions of left heart and pulmonary vascular remodeling, potentially contributing to RV dysfunction. As expected, the RV dysfunction subgroup had worse exercise capacity, ventilatory efficiency, and exercise cardiac reserve (Figure 3F through 3H), which contributed to better prediction of adverse outcomes (compared with conventional postcapillary PH subtypes of Ipc- versus Cpc-PH; Figure 3I and 3J). The adverse outcomes included mortality or first HF hospitalization

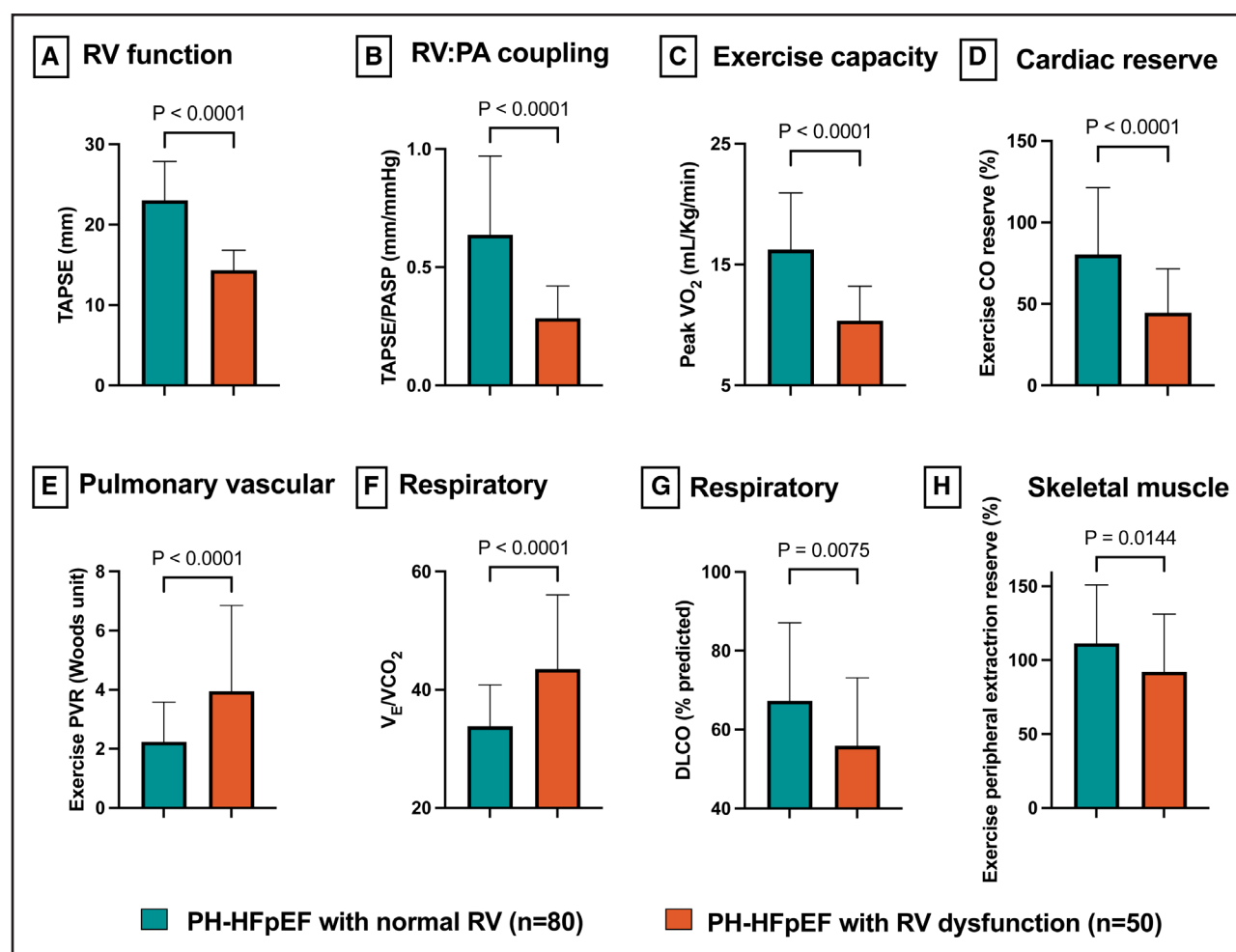


Figure 2. Multisystem limitations associated with right ventricular (RV) failure in pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF).

RV function (A) and RV:PA coupling (B) in predefined groups, and among these subgroups, global exercise capacity (C), exercise cardiac reserve (D), pulmonary vascular remodeling (E), respiratory function (F and G), and Skeletal muscle extraction with exercise (H). CO indicates cardiac output; DLCO, diffusion lung capacity of carbon monoxide; PA, pulmonary artery; PASP, pulmonary arterial systolic pressure; PVR, pulmonary vascular resistance; TAPSE, tricuspid annular plane systolic excursion; V_E/VCO_2 , slope of minute ventilation/carbon dioxide production; and VO_2 , oxygen consumption.

(1 event counted per patient, either mortality or first HF hospitalization). The isolated postcapillary PH (n=18) group had 2 incidents of HF hospitalization, while the combined pre-/postcapillary PH group (n=30) had 2 deaths and 10 HF hospitalizations. In comparison, the normal RV function group (n=29) had 3 incidents of HF hospitalization and the RV dysfunction group (n=19) had 2 deaths and 9 HF hospitalizations.

Pulmonary Vascular Mechanics Among PH-HFpEF Subgroups

To better characterize the mechanical properties of the pulmonary circulation beyond clinical hemodynamics, we performed pulmonary vascular wave mechanics analysis, which revealed distinct patterns among PH-HFpEF subgroups and the precapillary PH comparison group (Figure 4A; Table S2). During the 25 W exercise stage, the

PH-HFpEF subgroup with RV dysfunction had increased characteristic impedance (proximal PA stiffness; Figure 4B) and forward compression wave in systole (ventricular energy expenditure and force generation, which may indicate energy inefficiency; Figure 4C).^{9,28,29} Among the decompression waves (also known as diastolic waves), RV relaxation is captured by the forward wave, while distal pulmonary vascular reflections are captured by the backward wave and diastolic reflection index (ratio of diastolic forward:backward wave; Figure 4D).^{9,31,32} Although statistically nonsignificant, the RV dysfunction PH-HFpEF subgroup had worsening distal vascular reflections with exercise, while distal vascular reflections in the normal RV PH-HFpEF and precapillary PH groups decreased with exercise. Taken together, these metrics of wave mechanics provide more meaningful insights beyond the pressure-volume loop-based global metric of RV afterload (Figure 4E).

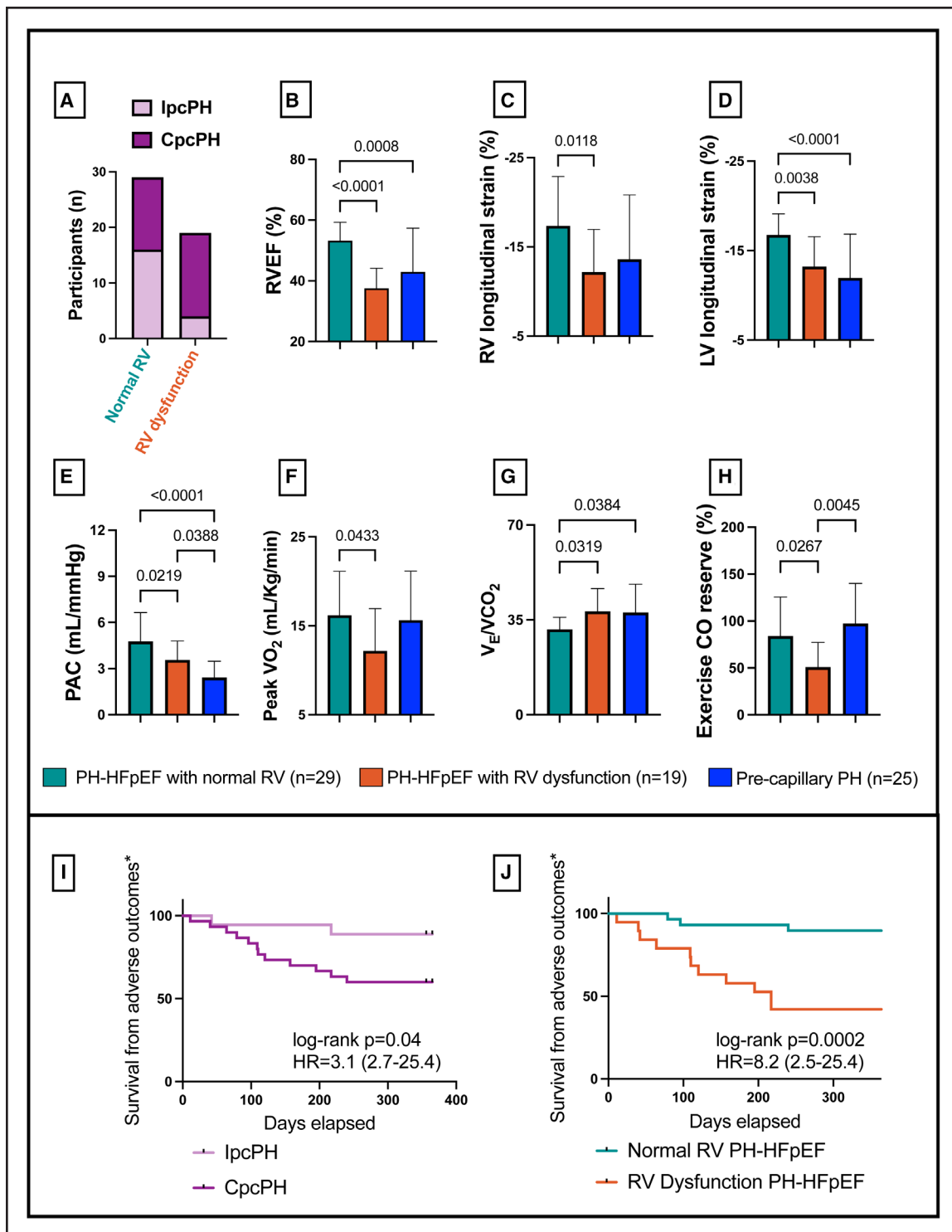


Figure 3. Unified left heart-pulmonary vascular contributors to right ventricular (RV) dysfunction in pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF).

Among subgroups of normal RV vs RV dysfunction, distribution of lpcPH and CpcPH (A), cardiac MRI based RVEF (B) and RV longitudinal strain (C). Among these PH-HFpEF subgroups, RV dysfunction subgroup had decreased LV longitudinal strain (D), pulmonary arterial compliance (E), peak exercise capacity (F), ventilatory efficiency (G), and global exercise cardiac reserve (H). Survival curves among lpcPH vs CpcPH (I) are less discriminatory of adverse outcomes compared to RV dysfunction (J). *Mortality or first heart failure (HF) hospitalization. Only 1 event was counted per patient (either mortality or HF hospitalization). Isolated postcapillary PH (lpcPH), n=18 (2 HF hospitalizations) vs combined pre-/postcapillary PH (CpcPH), n=30 (2 deaths and 10 HF hospitalizations). Normal RV, n=29 (3 HF hospitalizations) vs RV dysfunction, n=19 (2 deaths and 9 HF hospitalizations). CO indicates cardiac output; HR, hazard ratio; LV, left ventricular; PAC, pulmonary artery compliance; RVEF, right ventricular ejection fraction; V_E/VCO₂, ventilatory efficiency for carbon dioxide; and VO₂, oxygen consumption.

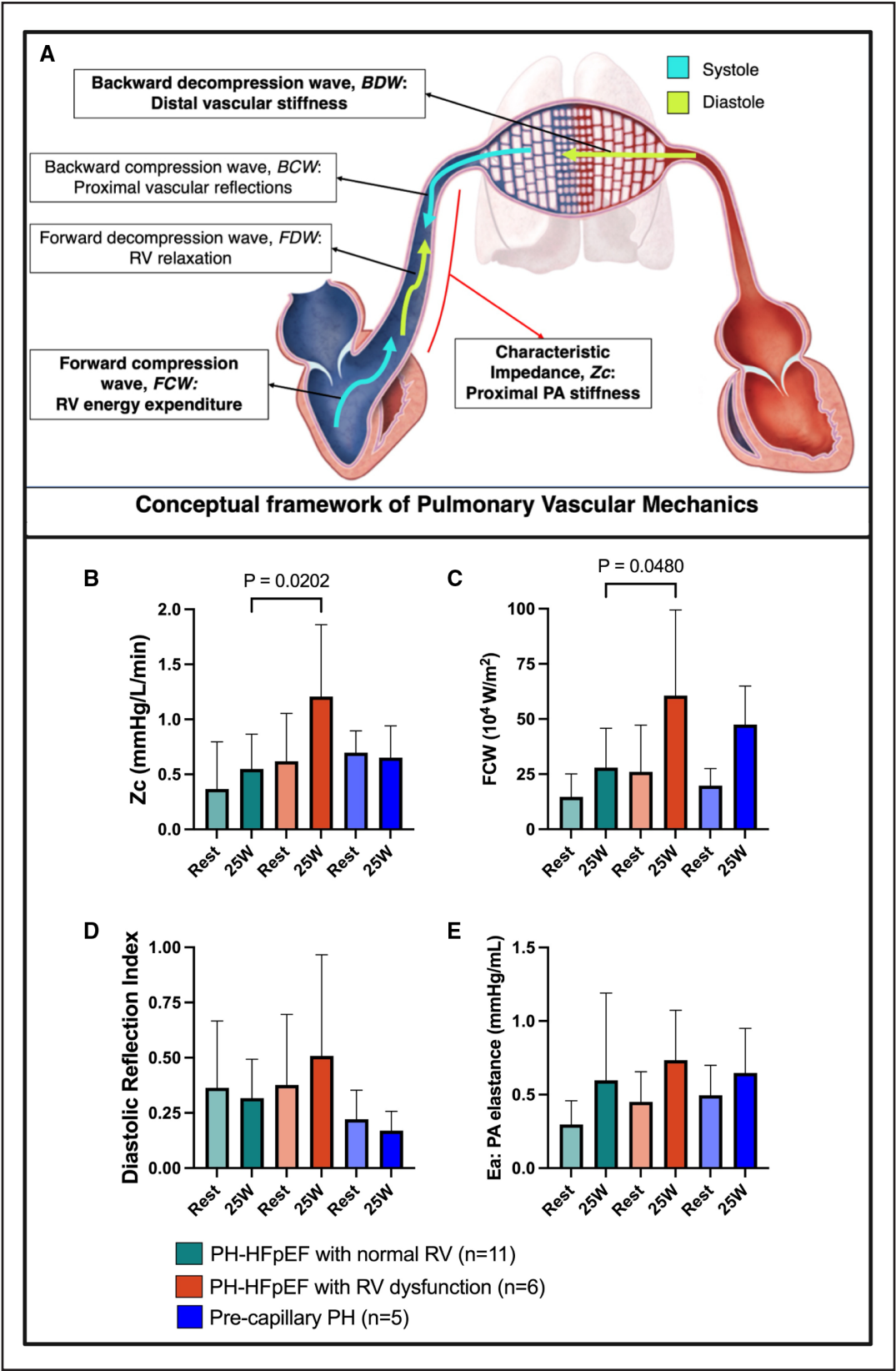


Figure 4. Invasive pulmonary vascular mechanics.

Conceptual framework (A) and key metrics among pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF) subgroups and the precapillary comparison group (B through E). Diastolic reflection index=backward decompression wave/forward decompression wave. Ea indicates arterial elastance; PA, pulmonary artery; and RV, right ventricular. Created in BioRender. Raza, F. (2026) <https://BioRender.com/hx2jc5i>.

Cardiopulmonary 4D Flow MRI-Based Ventricular and Pulmonary Vascular Mechanics (Morphological) Among PH-HFpEF Subgroups

To develop noninvasive, innovative approaches for capturing vessel-specific alterations in the left heart and pulmonary circulation, we performed 4D flow cardiac MRI (Figure 5 and Table S3). A representative PH-HFpEF participant with normal RV had laminar flow (Figure 5A and 5B), compared with disturbed flow in a PH-HFpEF participant with RV dysfunction (Figure 5C and 5D). Moreover, the PH-HFpEF subgroup with RV dysfunction trended toward increased energy loss and decreased energy efficiency^{40–43} in the LV (Figure 5E and 5F), left inferior pulmonary vein (Figure 5G and 5H), and pulmonary artery (Figure 5I and 5J). While these trends are statistically nonsignificant, they indicate the ability of cardiopulmonary 4D flow MRI to define in-depth abnormalities across the left heart-pulmonary circulation axis.

Transcriptomic Signatures of Dysfunctional RV PH-HFpEF Reveal RNA Metabolism and Mitochondrial Dysfunction

To define molecular signatures underlying normal RV (n=6) and dysfunctional RV (n=4) PH-HFpEF phenogroups, we performed PacBio long-read RNA-seq on endomyocardial biopsies from a subset of 10 patients (aggregate characteristics summarized in Table S4; Figure 6A). PacBio long-read RNA-seq generated high-quality data across all samples, with ≈70 million total reads per biopsy, >98% classified as polyadenylated, and mean read lengths of ≈1500 bp, indicating robust transcript coverage and alignment (Table S5). Principal component analysis revealed substantial overlap between normal RV and dysfunctional RV phenogroups, suggesting broadly similar global transcriptomic profiles (Figure 6B). Notably, sample distribution in the principal component analysis suggested potential sex-related heterogeneity; however, given the small sample size and the descriptive nature of the analysis, no definitive conclusions regarding sex differences can be drawn (Figure 6B). Consistent with the principal component analysis results, differential expression analysis identified only a limited number of significantly altered genes (n=5) between phenogroups. While statistical power is not high enough to detect many individual differentially expressed genes between phenogroups, pathway-level changes are readily detectable. Gene set enrichment analysis showed that pathways related to RNA processing, transport, and transcriptional regulation were enriched in the RV dysfunctional group (Figure 6C). In contrast, pathways associated with mitochondrial function and oxidative phosphorylation were significantly downregulated in the RV dysfunctional group (Figure 6D).

Modest Differential Isoform Expression Between RV Dysfunctional and RV Normal PH-HFpEF Phenogroups

To further characterize phenogroup differences, we leveraged long-read RNA-seq to quantify differential transcript expression in myocardial samples from RV dysfunctional and RV normal PH-HFpEF patients. Comparison of gene-level expression changes revealed largely concordant profiles between subgroups, with most genes exhibiting minimal shifts in both isoform expression and usage. Only a small subset of genes (6) demonstrated significant differences in transcript expression, independent of overall gene expression changes (Figure 7A). We have demonstrated the splicing pattern and transcript usage of 3 genes (*GJA5*, *GATD3*, and *PFPK*) as representatives (Figure 7B through 7D).

Consistent with this, the distribution of alternative splicing event types was highly similar between phenogroups. The most frequent events included alternative polyadenylation, exon elongation (>50 bp), and intron retention, indicating a shared repertoire of dominant splicing mechanisms (Figure 7E and 7F). Among 6424 genes with transcript abundance ≥10 TPM, the majority (84.3%, n=5416) expressed exclusively reference transcripts. Within the remaining genes expressing at least 1 novel isoform, the proportion of novel transcript usage followed a similar distribution in both phenogroups, spanning low to high usage fractions rather than clustering in a single dominant category (Figure S2A). Gene Ontology enrichment analysis of genes with novel transcript usage identified pathways related to RNA processing, cytoskeletal organization (including actin filament-based processes), sarco/endoplasmic reticulum function, and cell-cell junctions (Figure S2B and S2C). These enrichments were consistent across phenogroups and were not strongly associated with gene expression levels, suggesting shared biological processes underlying alternative splicing activity (Figure S2B and S2C).

DISCUSSION

In this study, we demonstrate that RV dysfunction is associated with multisystem limitations in PH-HFpEF. Right heart dysfunction in PH-HFpEF is likely due to contributors of coupled left heart-pulmonary vascular remodeling. Most importantly, to define specific mechanisms that contribute to right HF, this novel study described integrative multimodal methods of invasive pulmonary vascular mechanics and noninvasive 4D flow cardiopulmonary MRI. These integrative methods provide a framework to define regional contributions of left heart and pulmonary vascular remodeling localized to distinct vascular segments (vessel-specificity: PA versus venous) in PH-HFpEF. Finally, we reported myocardial biopsy-based unique differences in PH-HFpEF, which shed light on

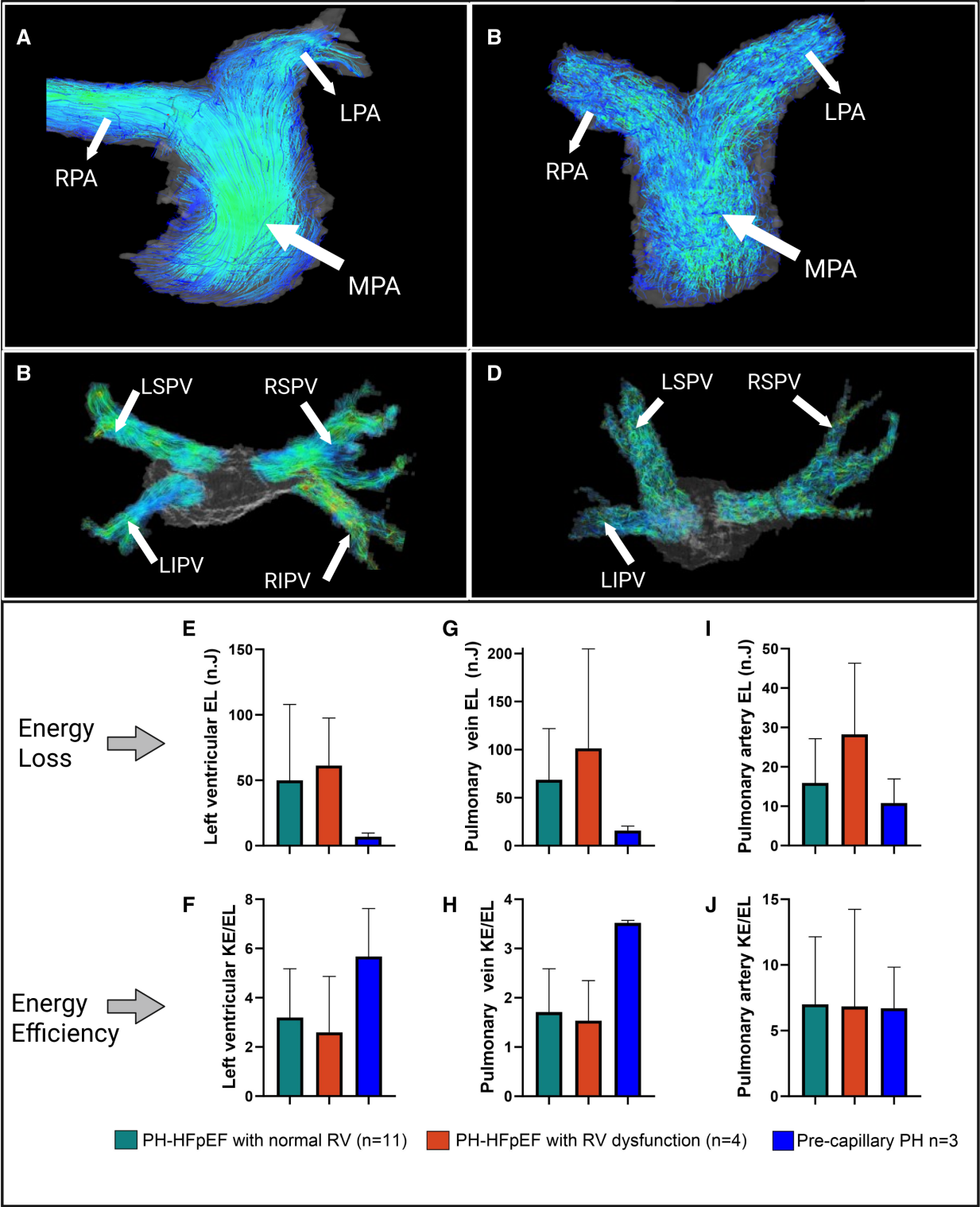


Figure 5. Cardiopulmonary magnetic resonance imaging (MRI) with 4-dimensional (4D) flow. Representative qualitative comparison of 4D flow streamline visualizations in normal right ventricular (RV; laminar flow, **A** and **B**) vs RV dysfunction (disturbed flow, **C** and **D**) in pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF). Key metrics among PH-HFpEF subgroups and the precapillary comparison group in the left ventricular (**E** and **H**), left inferior pulmonary vein (**F** and **I**), and pulmonary artery (**G** and **J**). EL indicates viscous energy loss; E, kinetic energy; LIPV, left inferior pulmonary vein; LPA, left pulmonary artery; LSPV, left superior pulmonary vein; MPA, main pulmonary artery; RIPV, right inferior pulmonary vein; RPA, right pulmonary artery; and RSPV, right superior pulmonary vein. Created in BioRender. Raza, F. (2026) <https://BioRender.com/g171wb3>.

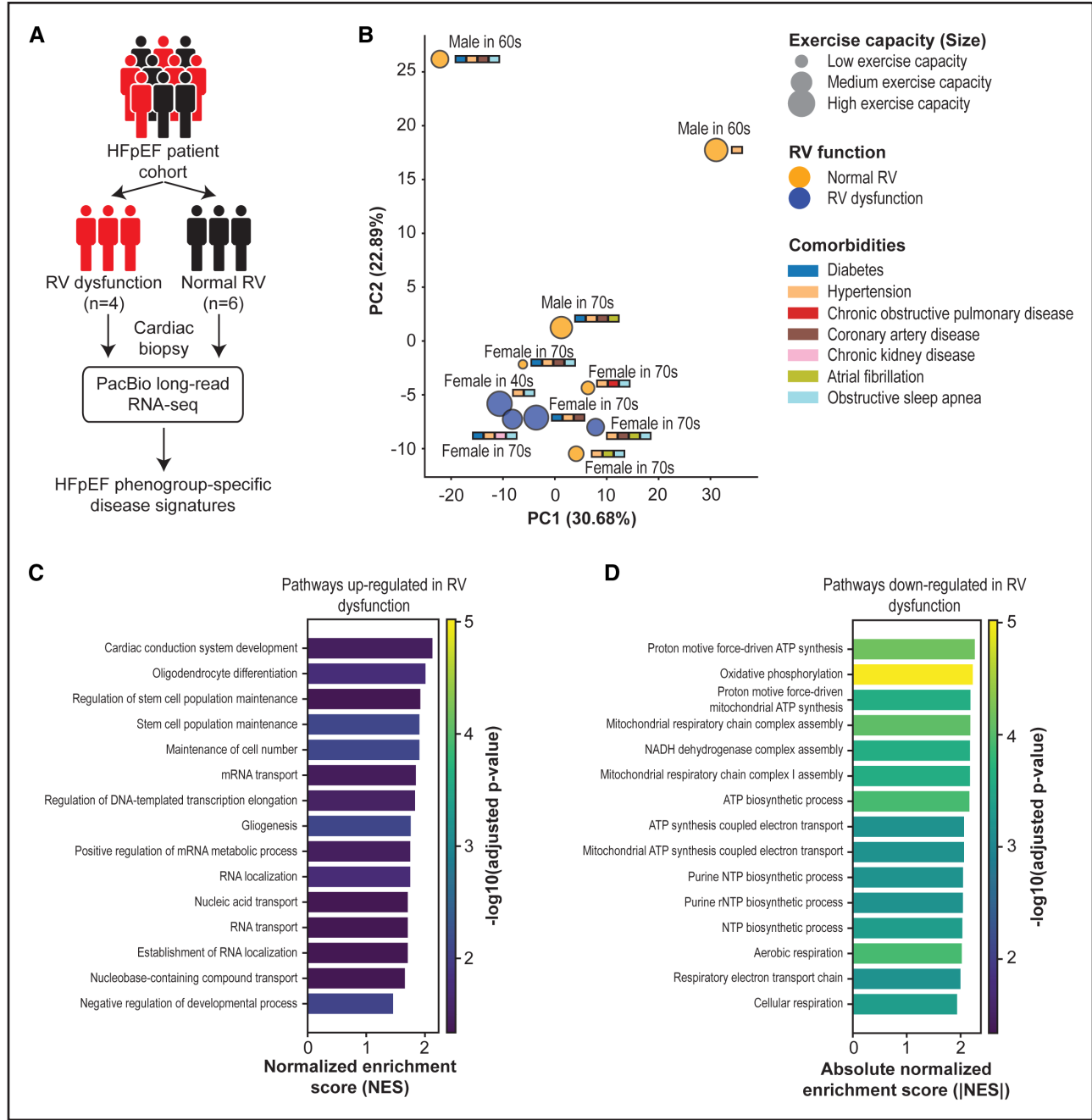


Figure 6. Long-read RNA sequencing (RNA-seq) analysis of processes affected in the right ventricular (RV) dysfunction vs normal RV pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF) subgroups. **A**, Schematic overview of transcriptomic analysis of patients in the RV dysfunction and normal RV PH-HFpEF subgroups using third-generation long-read RNA-seq. **B**, Principal component analysis showing overlap of individuals in the RV dysfunction and normal RV PH-HFpEF subgroups. A technical replicate is present for each sample. Bar plots showing biological process terms up (**C**) and downregulated (**D**), as determined by Gene Set Enrichment Analysis (GSEA) analysis in the RV dysfunction PH-HFpEF compared with the normal RV subgroup.

biological mechanistic differences between the normal RV and RV dysfunction subgroups. These findings are a first step toward creating a geographic patient-specific map of abnormalities in the left heart-pulmonary circulation axis and links to unique biological features.

RV failure is well known to be a high-risk feature in PH-HFpEF.^{4,5} Our reports of multisystem limitations associated with RV failure (Figure 2) and unified contributions

of left heart-pulmonary vascular remodeling toward RV failure (Figure 3) add to the significance of right HF in PH-HFpEF. More importantly, the current study presents a multimodal innovative framework to understand mechanisms of RV failure in PH-HFpEF. First, invasive pulmonary vascular mechanics inform on vessel-specificity of pulmonary vascular remodeling (Figure 4). As a significant contributor to RV afterload, characteristic

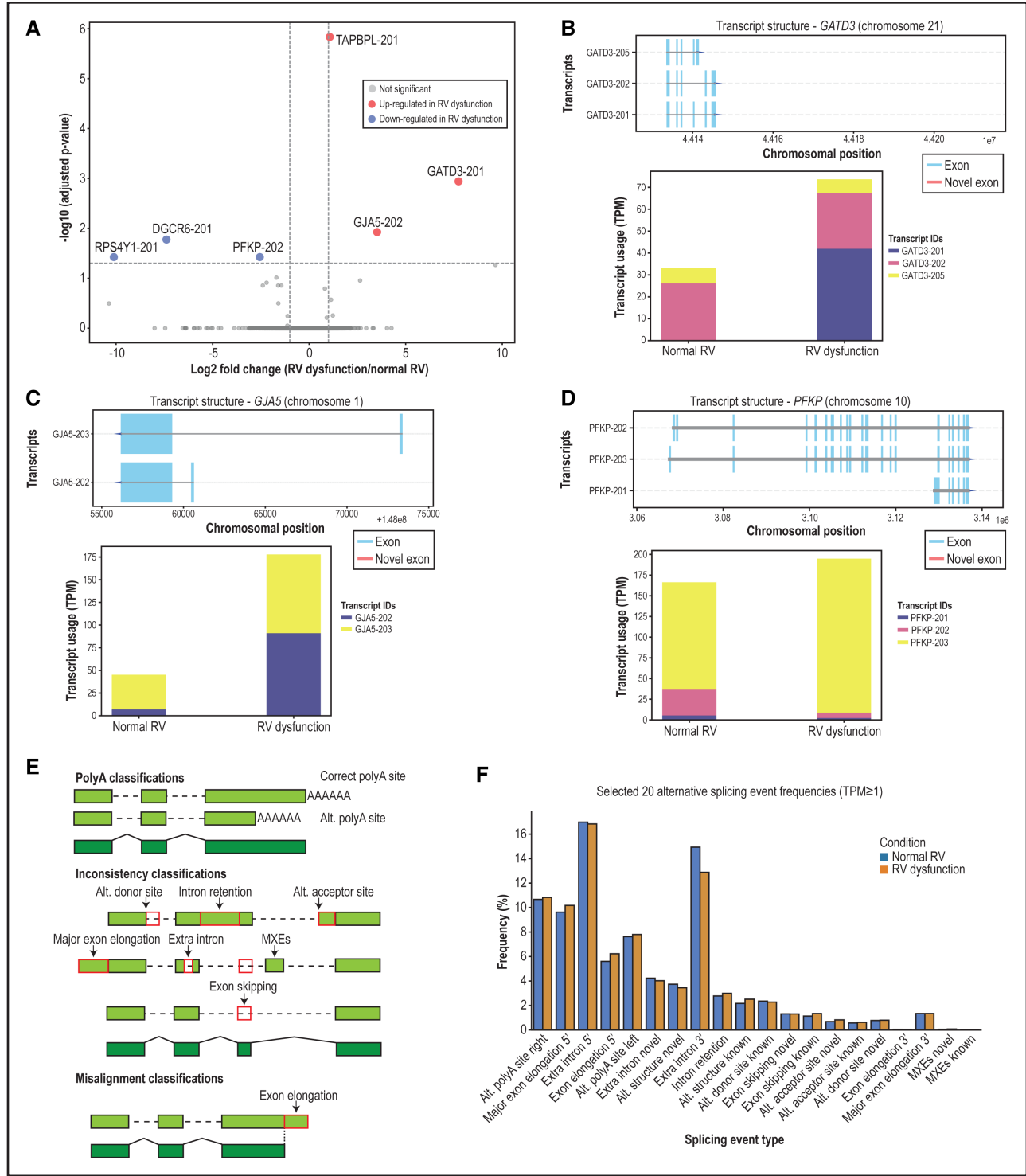


Figure 7. Long-read RNA sequencing analysis of alternative transcript usage in right ventricular (RV) dysfunction vs normal RV pulmonary hypertension due to heart failure with preserved ejection fraction (PH-HFpEF) subgroups.

A, Volcano plot showing the log₂-fold change in gene expression between RV dysfunction and normal RV phenogroups (x-axis) and -log₁₀ adjusted *P* value (y-axis). **B** through **D**, Transcript structure and usage plots highlighting differential transcript usage for *GATD3* (**B**), *GJA5* (**C**), and *PFKP* (**D**) in RV dysfunction compared with normal RV PH-HFpEF subgroups. **E**, Schematic illustrating different types of splicing events identified. **F**, Bar graph showing the frequency of selected splicing events (TPM ≥ 1) observed in both RV dysfunction and normal RV phenogroups. NIC indicates novel in catalog.

impedance captures proximal PA stiffness.^{8,26,44} Among wave intensity metrics, the diastolic reflection index (and backward decompression wave) captures distal vascular

reflections, which may indicate diseased distal vasculature^{9,31,32} (eg, pulmonary venules, similar to prior reports of pulmonary venular hypertrophy in combined pre-/

postcapillary PH⁶). These reflections are uniquely different from precapillary PH with a similar PVR. Second, our findings of noninvasive 4D flow cardiopulmonary MRI-based metrics (viscous energy loss and kinetic energy), albeit preliminary in nature, provide a framework for future studies to localize changes across the pulmonary circulation-left heart axis.^{25,40,45,46} These future studies can define the clinical relevance of pulmonary vascular wave mechanics metrics as key differentiating variables, using data-driven approaches such as least absolute shrinkage and selection operator modeling.

Although prior studies have identified a prognostic role of RV failure in HFpEF,⁵ few have investigated the molecular features that distinguish these subgroups and define the contributions of differential transcript expression toward RV failure. In this study, we used long-read RNA-seq for the comprehensive transcriptomic profiling of myocardial biopsies from patients with RV dysfunctional or RV normal PH-HFpEF. This study aimed to uncover molecular features associated with disease severity. Our data revealed that genes that were up- or downregulated between the 2 phenogroups were enriched in pathways predominantly related to mRNA turnover/stability (upregulated in RV dysfunctional) and mitochondrial metabolic processes (downregulated in RV dysfunctional). These findings align with growing evidence that RNA metabolism and mitochondrial dysfunction are central features of HF pathogenesis, particularly in HFpEF, and may contribute to phenotypic divergence between these HFpEF subgroups. These transcriptional signatures likely reflect a cellular stress response aimed at adapting to increased demand or injury but may ultimately drive maladaptive remodeling in the myocardium.

Notably, ≈94% of expression (≥10 TPM) corresponded to reference isoforms, indicating that most transcript-level differences were driven by quantitative changes in expression rather than widespread generation of novel isoforms. However, alterations in alternative polyadenylation, exon elongation, and intron retention were still observed and could contribute to functional diversity and regulation in these phenogroups. Despite the relatively modest prevalence of novel isoforms, genes with nonzero novel splicing rates disproportionately affect pathways adjacent to HFpEF pathogenesis.

To investigate the functional implications of genes with differential transcript expression, we leveraged the literature analysis tool SKiM-GPT⁴⁷ and manual review. This analysis highlighted significant phenogroup-enriched transcript expression, including *GATD3* (glutamine amidotransferase class 1 domain containing 3), *GJA5* (gap junction protein alpha 5), and *PFKP* (phosphofructokinase, platelet). *GATD3* is a mitochondrial protein (MitoCarta3.0), and we find that the isoform GATD3-201 is expressed higher in patients with RV dysfunction relative to patients with normal RV (Figure 7B), suggesting that this isoform may contribute to mitochondrial dysfunction that underlies

RV dysfunction. *GJA5* codes for the gap junction alpha 5 protein, also known as connexin 40, which has been shown to be upregulated in ischemic ventricles.⁴⁸ Remodeling of gap junction organization, and thus changes in connexin expression, can contribute to arrhythmia and other cardiac dysfunctions.^{48,49} We find the specific isoform GJA5-202 to be upregulated in RV dysfunctional patients (Figure 7C), supporting the hypothesis that reorganization of connexin expression is a marker of cardiac dysfunction. Finally, *PFKP* is a member of the phosphofructokinase A protein family and is involved in the regulation of glycolysis. Because progression of HFpEF is highly correlated with decreased exercise tolerance, elevated glycolysis can be induced to compensate for the loss of oxygen and some *PFKPs* have been targeted to alleviate hypoxia-associated myocardial injury by being induced.⁵⁰ Here, the isoform PFKP-202 is downregulated in patients with RV dysfunction, and, while not significant, the isoform PFKP-203 is higher in the RV dysfunction phenogroup (Figure 7D), suggesting that this change in PFKP isoform expression may be indicative of cardiac dysfunction.

The current study advances the field in 3 specific ways. First, deep morphological and exercise hemodynamics-based characterization defined high-risk features of cMRI-based poor LV strain and worse PA compliance in the PH-HFpEF subgroup with RV dysfunction. Second, and more importantly, we have reported a framework (invasive wave mechanics and morphological cMRI) for in-depth characterization of pulmonary vascular disease and a pipeline to link it with biological myocardial changes. Third, for the first time, transcriptomic profiling implicated differences in RNA metabolism and mitochondrial function linked to RV dysfunction in PH-HFpEF. In the future, larger studies are needed to define biological mechanisms that link to specific segments of the pulmonary vascular bed, which are studied at rest and under stress (physiological stress of exercise and vasodilation) to define structural/fixed versus vasoreactive changes.

Limitations

This study has several limitations. First, the sample size is modest, particularly in the prospective and transcriptomic subsets, limiting statistical power for subgroup and molecular analyses. Second, the retrospective design of the primary cohort introduces potential selection bias, especially in patients who underwent advanced testing such as cMRI. Third, findings from pulmonary vascular mechanics and 4D flow MRI, while mechanistically informative, are based on small subsets and should be considered hypothesis-generating. Fourth, transcriptomic analyses were performed in a limited number of myocardial biopsy samples, which may not fully capture biological heterogeneity. Finally, causal relationships between vascular remodeling, myocardial changes, and RV failure cannot be definitively be established in the current study.

Conclusions

In PH-HFpEF, RV dysfunction is associated with multi-system impairment and arises from coupled left heart-pulmonary vascular remodeling. Using a multimodal framework integrating invasive exercise hemodynamics and vascular mechanics, cardiac MRI including 4D flow imaging, and myocardial transcriptomics, we demonstrate that RV dysfunction is characterized by poor LV myocardial strain, increased proximal PA stiffness, and abnormal distal vascular reflections. Finally, gene and transcript profiles suggest that changes in RNA metabolism and oxidative phosphorylation accompany the evolution from normal RV to dysfunctional RV disease states in PH-HFpEF.

ARTICLE INFORMATION

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Author Contributions

Dr Raza conceived the study, led the project, collected all data, oversaw analyses, and wrote/revised the manuscript. Dr Gregorich oversaw myocardial biopsy biobanking, supported transcriptomics analyses, and contributed to manuscript writing. J. Freeman led transcriptomics analyses and figure creation. Dr Moore performed differential transcript usage analysis. T.W. Houston performed 4-dimensional (4D) flow magnetic resonance imaging analyses. Drs Garcia-Arango, Chen, and Sahai collected cardiac MRI data and analyzed myocardial strain. Dr Lechuga performed impedance and wave intensity analyses. Dr El Shaer collected invasive cardiopulmonary exercise testing data. K. Cui and Dr Park performed clustering analyses. K. Jones managed project operations and data management. W. Tu conducted all other statistical analyses. Dr Runo contributed to study conception and manuscript writing. Dr Schulte analyzed myocardial biopsy data. Dr Nagpal oversaw the cardiac MRI protocol and data analyses. Dr Ge conceived the transcriptomics analyses and contributed to manuscript writing. Dr Wieben led 4D flow MRI analyses. Dr Stewart co-led transcriptomics analyses. Dr Chesler led vascular mechanics analyses. Dr Guo conceived the study, led transcriptomics analyses, and interpretation, and wrote the manuscript.

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Disclosures

None.

Supplemental Material

Supplemental Methods

Tables S1–S5

Figures S1–S2

References 33–38,51

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