RESEARCH ARTICLE

Increased plasma expression of a disintegrin and metalloproteinase with thrombospondin motifs like 4 in patients with idiopathic pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension

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Abstract

Idiopathic pulmonary arterial hypertension (IPAH) and chronic thromboembolic pulmonary hypertension (CTEPH) can result in right heart failure. We aimed to evaluate the plasma protein levels of a disintegrin and metalloproteinase with thrombospondin motifs like 4 (ADAMTSL4) and its relationship with IPAH and CTEPH. Plasma ADAMTSL4 protein levels were measured using proteomics analysis in eight patients with IPAH and nine healthy controls. ADAMTSL4 levels in pulmonary tissues were assessed using bioinformatics tools. Protein expression of ADAMTSL4 in platelet-derived growth factor (PDGF)-BB-treated primary rat pulmonary arterial smooth muscle cells (PASMCs) was detected by Western blot. Plasma ADAMTSL4 concentrations were measured in 45 patients (15 with IPAH and 30 with CTEPH) using enzyme-linked immunosorbent assay (ELISA). Correlation between ADAMTSL4 levels and clinical parameters was evaluated. In patients with IPAH, the plasma levels of ADAMTSL4 protein were significantly higher than those in healthy controls (flod change [FC] 1.85, $p < 0.05$), and mRNA expression levels were significantly elevated (log FC 0.66, $p < 0.05$). The protein expression of ADAMTSL4 was significantly increased in PDGF-BB-treated PASMCs compared to that in the control grAoup ($p < 0.05$). Plasma ADAMTSL4 protein levels in patients with IPAH (4.71 ± 0.73 ng/mL, $p < 0.01$) and CTEPH (4.22 ± 0.66 ng/mL, $p < 0.01$) were higher than in healthy controls (3.01 ± 0.46 ng/mL). Plasma ADAMATL4 protein levels had a cutoff value of 3.55 ng/mL based on the receiver operator characteristic curve and were positively correlated with mean pulmonary artery pressure (mPAP) ($r = 0.305$, $p < 0.05$). In patients with IPAH and CTEPH, elevated plasma ADAMTSL4 levels were positively associated with mPAP.

KEYWORDS
ADAMTSL4, correlation, CTEPH, IPAH, levels

Pulmonary Circulation

INTRODUCTION

Idiopathic pulmonary arterial hypertension (IPAH) and chronic thromboembolic pulmonary hypertension (CTEPH) are characterized by a progressive increase in pulmonary vascular resistance, leading to obliterative pulmonary vasculopathy and eventually, right heart failure and mortality. The mechanisms underlying pulmonary vascular remodeling in both IPAH and CTEPH remain poorly understood.[1] Due to this knowledge gap, molecular indicators are needed to aid in diagnosing and predicting the prognosis of pulmonary hypertension (PH).[2] Potential molecular indicators include chemokine CXC ligand 13, pentraxin 3, and N-terminal pro-brain natriuretic peptide (NT-proBNP); however, their diagnostic and evaluative values require large-scale validation.[3–5] Proteomics analysis offers a comprehensive approach for identifying novel indicators by examining the complete protein profiles associated with these conditions.[6–8]

A disintegrin and metalloproteinase with thrombospondin motifs like 4 (ADAMTSL4) is a member of the ADAMTSL protein family and a member of the thrombospondin type 1 repeat (TSR) superfamily of seven ADAMTSL proteins. These seven ADAMTSL proteins can be categorized into two distinct clades. ADAMTSL4 and ADAMTSL6 belong to one clade, which differs from the other clade comprising ADAMTSL1, ADAMTSL3, and ADAMTSL7. Notably, the distinguishing feature of ADAMTSL4 and ADAMTSL6 is the absence of immunoglobulin repeat regions, which are present in other proteins of the ADAMTSL family. ADAMTSL4 is widely expressed in the brain, colon, heart, kidneys, liver, lungs, pancreas, and spleen. Despite its broad expression pattern, the exact function of ADAMTSL4 remains unclear.[9–11] A low level of expression of ADAMTSL4 has been observed in the medial layer of the arterial wall, as well as in medial vascular smooth muscle cells in patients with spontaneous coronary artery dissection.[12–14] ADAMTS family proteins are important in connective tissue organization, antiangiogenesis, and cell migration and attachment,[15] and TSR-containing proteins inhibit the proliferation and migration of endothelial cells.[16]

This study aimed to compare the expression levels of ADAMTSL4 in plasma samples obtained from healthy individuals and patients diagnosed with IPAH or CTEPH and to investigate the relationship between plasma ADAMTSL4 levels and disease severity in these patients.

MATERIALS AND METHODS

Bioinformatics analysis

We used the Rstudio 2022.12.0 (Posit software, PBC) specifically utilizing the "Limma" package, to identify genes differentially expressed in the pulmonary tissues of individuals with IPAH compared with the control group. A significance threshold of an adjusted p-value <0.05 was set to determine the statistical significance of the differential expression. Expression profile data from the GSE15197 data set were downloaded from the Gene Expression Omnibus (GEO) database.

Cell acquisition and culture

Primary rat pulmonary arterial smooth muscle cells (PASMCs) were isolated from healthy Sprague-Dawley rats (6–8 weeks, 180–200 g) and cultured in Dulbecco's modified Eagle's medium/nutrient mixture F-12 containing 20% fetal bovine serum and 1% penicillin and streptomycin. After PASMCs were starved in serum-free DMEM/F-12 for 24 h, they were treated with 20 ng/mL platelet-derived growth factor (PDGF)-BB for 24 h.

Western blot

Whole proteins of treated PASMCs were extracted using the RIPA buffer supplemented with a protease inhibitor cocktail (Beyotime). The concentrations of proteins were detected using BCA assay kits. Using a 5× loading buffer, the proteins were prepared at the same concentration and heated for 10 min. Then, the proteins were separated through 10% gels and transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories). Thereafter, the membranes were blocked with 5% nonfat milk at room temperature for 1.5 h and incubated with primary antibodies (β-actin [1:5000], ADAMTSL4 [1:1000]) overnight at 4°C. They were then incubated with corresponding secondary antibodies for 1.5 h at room temperature. Finally, the protein bands were detected with a gel imaging system (Bio-Rad) using an enhanced chemiluminescence reagent.

Patients and controls

For this study, a cohort of patients with IPAH diagnosed between June 1, 2016 and November 30, 2018 was enrolled for proteomics analysis. Additionally, patients

diagnosed with either IPAH or CTEPH between June 1, 2016 and June 30, 2021 were included to analyze protein levels using the enzyme-linked immunosorbent assay (ELISA). According to standard criteria, IPAH or CTEPH was diagnosed using ventilation/perfusion scintigraphy and right-heart catheterization in all patients. The presence of CTEPH was determined using pulmonary angiography as a mandatory procedure. Patients having IPAH with secondary factors were carefully excluded.[17] We specifically excluded patients with postcapillary PH, connective tissue disease, and significant parenchymal lung disease. Blood samples were obtained from healthy volunteers included as control participants. This study was approved by the Ethics Committee of Chongqing Medical University School. Informed consent was obtained from all participants to ensure voluntary and informed participation in the study. Forty-five patients (IPAH, $n = 15$; CTEPH, $n = 30$) and healthy control participants ($n = 21$) were enrolled. Table 1 provides the demographic information and baseline characteristics.

Right-heart catheterization

A standard protocol was followed for right-heart catheterization using a jugular approach. The pressure transducer was calibrated at the midthoracic level to establish a baseline reference. During the procedure, various measurements were performed, including right atrial pressure, mean pulmonary arterial pressure (mPAP), pulmonary arterial wedge pressure (PAWP), and oxygen saturation levels in the superior vein cava, right atrium, right ventricle, and pulmonary artery. Cardiac output (CO) was measured using the thermo-dilution method, in which a series of at least three recordings were obtained. The average of these recordings was the reported value of CO. To ensure consistency, the recordings selected for calculating the average had a variation of less than 10%. Pulmonary vascular resistance (PVR) was calculated using a standard equation shown as below equation.

$$(PVR = mPAP–mPAWP/CO). \qquad (1)$$

Blood sampling

Blood samples were collected from healthy individuals and patients with IPAH or CTEPH during their first diagnostic right-heart catheterization procedure. Venous blood (2 mL) was collected, transferred to an ethylenediamine tetraacetic acid anticoagulant tube,

and mixed thoroughly. The collected samples were centrifuged at 4°C for 10 min at 3000 g to separate the blood components. Following centrifugation, the upper plasma layer was carefully transferred to a 1.5 mL centrifuge tube using a pipette. To preserve the integrity of the plasma proteins, a protease inhibitor was added to the sample and stored at −80°C for further analysis and testing.

Preparation of plasma samples with albumin removal

After thawing, 1 mL of the blood sample was centrifuged at 4°C for 1 min at 12,000 g to remove the upper lipid layer and retain the middle layer. Subsequently, the protein in the sample was diluted and filtered using a 0.22 mm membrane. A high protein abundance column (Agilent) was employed to further eliminate the high protein abundance. Proteins were quantified using the Bradford method and subsequently labeled with Cy2, Cy3, and Cy5 fluorescent dyes. The labeled samples were then added to Eppendorf tubes, mixed vigorously, and centrifuged in the inching mode. Simultaneously, an internal standard was established, and an equal volume of a two-fold sample buffer was added to the samples, followed by incubation in an ice bath for 10 min. Isoelectric focusing and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were performed, and silver nitrate staining was used to visualize the protein bands following electrophoresis.

Scanning and image analysis

Images were scanned using a Typhoon scanner and eCyder™ V6.5 software (GE Healthcare). The scanned images were subsequently analyzed. Three gel maps were generated for each film by scanning. Differential in-gel analysis was performed for each protein spot on the gel map. Through the matching process, the relative abundance of each protein spot was determined by comparing the Cy3/Cy2 and Cy5/Cy2 ratios, enabling the identification of differentially expressed proteins.

Mass spectrometry

Matrix-Assisted Laser Desorption Ionization was used to analyze the differential protein spots. Differentially expressed proteins were identified by searching the ipi HUMANv3.53 protein library using BIOWORKS.

Pulmonary Circulation

TABLE 1 Characteristics of patients and controls at baseline.

	Control	IPAH + CTEPH	IPAH	CTEPH
Age	25.71 ± 3.52	50.46 ± 16.92[a]	34.27 ± 10.63[a,b]	58.47 ± 18.09[a]
Female (%)	67	67	80	60
WHO-FC	21/0/0/0	0/12/32/0[a]	0/4/11/0[a]	0/21/8/1[a]
WHO-FC				
I, II		25 (55.6%)	4 (26.7%)	21 (70.0%)
III, IV		20 (44.4%)	11 (73.3%)	9 (30.0%)[c]
BMI (kg/m^2)	21 ± 2	23 ± 3	23 ± 3	22 ± 3
6MWD (m)	NA	347 ± 138	345 ± 130	349 ± 143
Hemodynamics				
RAP (mmHg)	NA	8 ± 6	9 ± 6	8 ± 6
PASP (mmHg)	NA	74 ± 20	80 ± 20	71 ± 20
mPAP (mmHg)	NA	48 ± 11	55 ± 11	44 ± 10[d]
CO (L/min)	NA	3.68 ± 1.09	3.92 ± 1.37	3.57 ± 0.93
PVR (wood unit)	NA	11.23 ± 7.99	14.84 ± 10.79	9.43 ± 5.53
SvO$_2$ (%)	NA	62 ± 9	64 ± 10	60 ± 9
NT-ProBNP/BNP (ng/L)				
NT-proBNP <1100/BNP < 800	NA	39 (86.7%)	14 (93.3%)	25 (83.3%)
NT-proBNP ≥1100/BNP ≥ 800	NA	6 (13.3%)	1 (6.7%)	5 (16.7%)[c]
Creatinine (μmol/L)	NA	77.82 ± 18.68	71.60 ± 17.20	80.93 ± 18.88
Uric acid (μmol/L)	NA	466.31 ± 143.63	477.33 ± 160.76	460.80 ± 136.85
Total bilirubin (μmol/L)	NA	24.42 ± 24.78	19.52 ± 15.26	26.95 ± 28.47
Albumin (g/L)	NA	36.64 ± 6.83	42.87 ± 4.91	37.97 ± 7.15
Coagulation				
PT (s)	NA	16.27 ± 7.17	12.51 ± 1.71	18.83 ± 7.93
PTR	NA	1.43 ± 0.62	1.07 ± 0.15	1.62 ± 0.68
INR	NA	1.45 ± 0.62	1.08 ± 0.15	1.63 ± 0.93
PTA (%)	NA	70.21 ± 30.66	94.21 ± 21.74	58.21 ± 27.42[d]
APTT (s)	NA	36.31 ± 13.62	29.33 ± 3.73	39.80 ± 15.39[d]
Fbg (g/L)	NA	2.71 ± 0.88	2.64 ± 0.71	2.81 ± 0.96
UCG				
LA (mm)	NA	30 ± 5	27 ± 2	30 ± 6
LV (mm)	NA	41 ± 6	37 ± 6	43 ± 5[d]
RA (mm)	NA	49 ± 11	49 ± 9	49 ± 11
RV (mm)	NA	30 ± 8	32 ± 9	30 ± 8
LVEF (%)	NA	66 ± 5	68 ± 7	65 ± 4
TAPSE (mm)	NA	14 ± 4	12 ± 3	14 ± 4

Abbreviations: APTT, activated partial thromboplastin time; BMI, body mass index; BNP, brain natriuretic peptide; CO, cardiac output; CTEPH, chronic thromboembolic pulmonary hypertension; Fbg, fibrinogen; INR, international normalized ratio; IPAH, idiopathic pulmonary arterial hypertension; LA, left atrium; LV, left ventricle; LVEF, left ventricular ejection fraction; mPAP, mean pulmonary arterial pressure; NA, not applicable; NT-ProBNP, N-terminal pro-brain natriuretic peptide; PT, prothrombin time; PTA, prothrombin activity; PTR, prothrombin time ratio; PVR, pulmonary vascular resistance; RA, right atrium; RAP, right atrial pressure; RV, right ventricle; SPAP, pulmonary artery systolic pressure; SvO$_2$, mixed venous oxygen saturation; TAPSE, tricuspid annular plane systolic excursion; UCG, ultrasonic cardiogram; WHO-FC, World Health Organization function classification; 6MWD, 6-min walk distance.

[a]$p < 0.01$ versus control group.

[b]$p < 0.01$ versus IPAH + CTEPH group.

[c]$p < 0.05$ versus IPAH group.

[d]$p < 0.05$ versus CTEPH group.

Measurements of plasma ADAMTSL4 levels

Blood samples were collected from patients diagnosed with IPAH or CTEPH during right-heart catheterization. Blood samples were obtained from the peripheral veins of healthy controls. Immediately after collection, the blood samples were mixed with ethylenediamine tetraacetic acid, centrifuged at 3000 g for 10 min at 4°C, and stored at −80°C. Serum levels of ADAMTSL4 were measured using ELISA according to the manufacturer's instructions (R&D Systems, Minneapolis, Catalog Number DCX130). ADAMTSL4 levels were measured in duplicate for each sample. The color intensity was measured using a standard ELISA Reader (Tecan Spectra Mini). Standard curves were generated using a Quantikine kit to establish the relationship between color intensity and the concentration of ADAMTSL4.

Statistical analysis

The experiments were repeated at least three times to ensure the robustness of the results. The data are expressed as mean ± standard deviation. All statistical analyses were performed using SPSS 22.0 (IBM Corporation). Differences between groups were assessed using Student's t-test and one-way analysis of variance. Receiver operating characteristic (ROC) curve analysis was performed to identify the optimal threshold value of ADAMTSL4 expression for diagnosing IPAH and CTEPH. Pearson's and Spearman's correlation analyses were conducted, adjusting for age and sex, to investigate the potential associations between ADAMTSL4

and clinical and hemodynamic measurements. Survival rate was estimated using Kaplan–Meier analysis based on plasma ADAMTSL4 levels. Statistical significance was set at $p < 0.05$.

The patients were categorized into two groups according to the World Health Organization functional classification (WHO-FC). The data pertaining to NT-proBNP and BNP levels were divided into two groups (NT-proBNP <1100 or BNP < 800, ng/L; NT-proBNP ≥ 1100 or BNP ≥ 800, ng/L),[17] and then consolidated and reanalyzed.

RESULTS

In this proteomics study, eight patients with IPAH and nine controls were compared. By conducting an image analysis of the gel-based proteome, 119 differentially expressed proteins were identified in the IPAH and control groups. Among these proteins, 66 were upregulated, and 53 were downregulated, as shown in Supporting Information: Figure S1. The IPAH group showed a significantly higher level of protein expression for ADAMTSL4 than the control group (Figure 1a). The relative quantitative values were 15440 ± 5872 in the IPAH group and 8077 ± 1279 in the control group ($p < 0.05$). The fold change (FC) in ADAMTSL4 level between the two groups was 1.85.

Bioinformatics analysis of ADAMTSL4 expression in the GSE15197 data set

The study results revealed that the IPAH group exhibited significantly higher levels of ADAMTSL4 mRNA
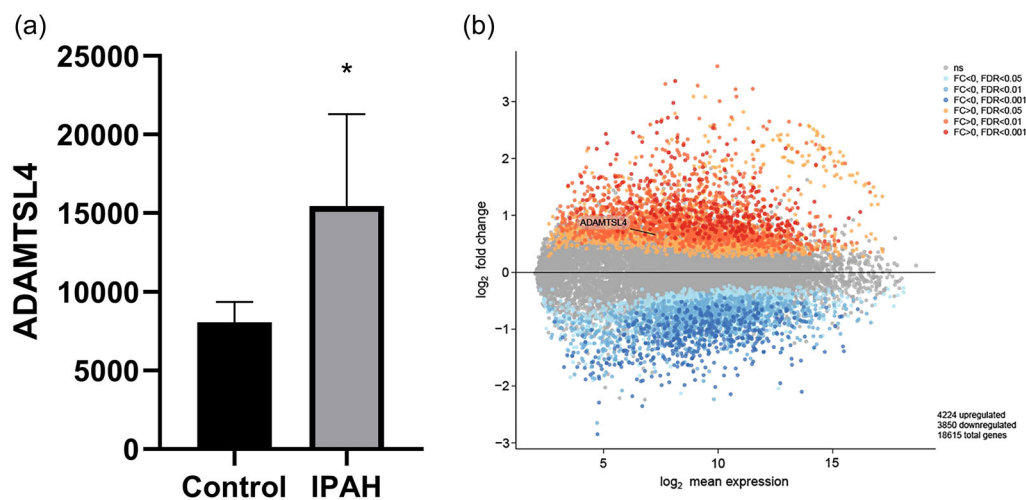
FIGURE 1 (a) An image analysis between the control and IPAH groups of the gel-based proteome, ADAMTSL4. *$p < 0.05$ versus control group. (b) Volcano plot to compare between the control and IPAH groups based on bioinformatics analysis. ADAMTSL4, a disintegrin and metalloproteinase with thrombospondin motifs like 4; IPAH, idiopathic pulmonary arterial hypertension.

expression than the control group. The relative quantitative values were 7.52 ± 0.68 versus 6.87 ± 0.73 ($p < 0.05$), and the log FC of ADAMTSL4 between the two groups was 0.66 (Figure 1b).

PDGF-BB promotes the proliferation of PASMCs and upregulates the protein expression level of ADAMTSL4

To investigate the expression of ADAMTSL4 in PASMCs, we extracted primary rat PASMCs and treated them with 20 ng/mL PDGF-BB for 24 h. We found that the protein expression level of ADAMTSL4 in PDGF-BB-treated PASMCs was significantly upregulated compared to that in the control group ($p < 0.05$) (Figure 2).

Validation of plasma ADAMTSL4 levels by ELISA

As shown in Figure 3, the plasma levels of ADAMTSL4 were elevated in patients with IPAH compared with those in the control group (4.71 ± 0.73 ng/mL vs. 3.01 ± 0.46 ng/mL, $p < 0.01$). Furthermore, plasma ADAMTSL4 levels were increased in patients with CTEPH compared with those in the control group (3.01 ± 0.46 ng/mL vs. 4.22 ± 0.66 ng/mL, $p < 0.01$). Additionally, the plasma levels of ADAMTSL4 were higher in both the IPAH and CTEPH patient cohorts than in the control group (4.38 ± 0.72 ng/mL vs. 3.01 ± 0.46 ng/mL, $p < 0.01$). Notably, no significant difference was observed between the IPAH and CTEPH groups in terms of plasma ADAMTSL4 levels.
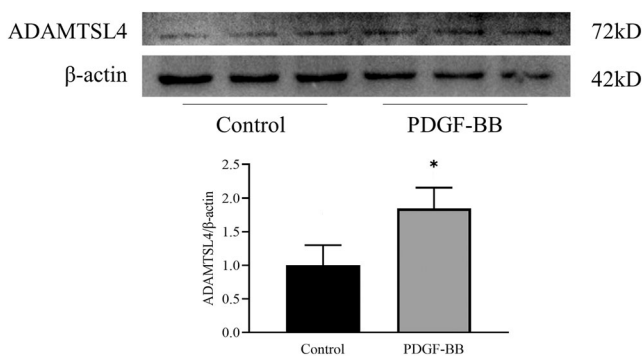

FIGURE 2 ADAMTSL4 expression in PASMCs. The protein expression of ADAMTSL4 in PDGF-BB-treated PASMCs were detected using Western blot. *$p < 0.05$ versus the control group. ADAMTSL4, a disintegrin and metalloproteinase with thrombospondin motifs like 4; IPAH, idiopathic pulmonary arterial hypertension; PASMCs, pulmonary artery smooth muscle cells; PDGF, platelet-derived growth factor.
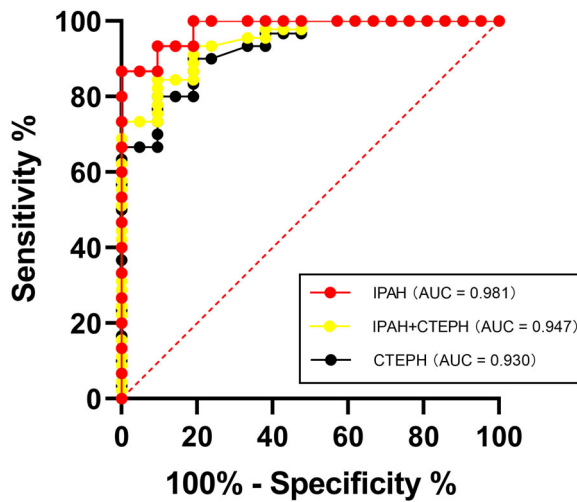
ROC curves

To determine the potential diagnostic value of ADAMTSL4 level for CTEPH, the plasma protein levels of ADAMTSL4 were examined in both the control group and patients with CTEPH. As shown in Figure 4, the area under the ROC curve (AUC) for ADAMTSL4 was 0.947 (95% confidence interval [CI]: 0.898–0.999, $p < 0.01$) for the PH group (IPAH and CTEPH combined). In the IPAH subgroup, the AUC for ADAMTSL4 was 0.981 (95% CI: 0.947–1, $p < 0.01$). Similarly, in patients with CTEPH, the AUC for ADAMTSL4 was 0.930 (95% CI: 0.866–0.995, $p < 0.01$). Using Youden's index, ADAMTSL4 plasma expression levels > 3.55 ng/mL in the PH group (IPAH + CTEPH) exhibited a sensitivity of 84.4% and a specificity of 90.5%.

Relationship between plasma ADAMTSL4 level and survival

As shown in Figure 5, Kaplan–Meier analysis revealed that patients with PH (IPAH + CTEPH) exhibited
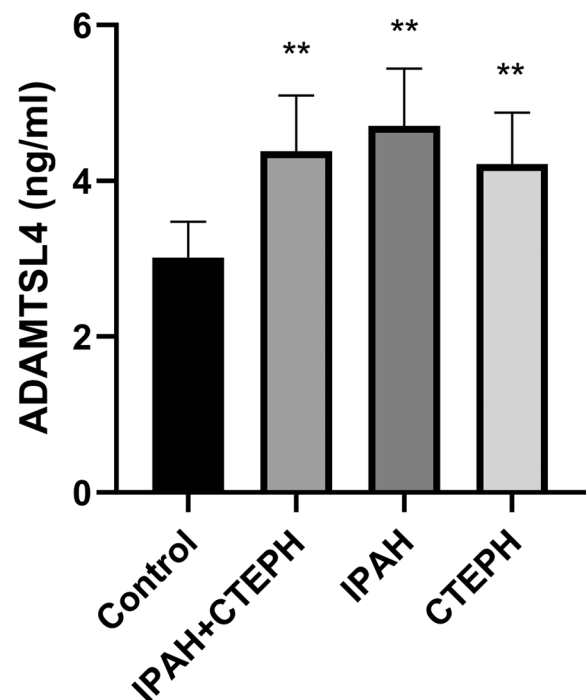
FIGURE 3 Plasma ADAMTSL4 protein levels in the control, IPAH, and CTEPH groups analyzed using ELISA. **$p < 0.01$ versus control group. ADAMTSL4, a disintegrin and metalloproteinase with thrombospondin motifs like 4; CTEPH, chronic thromboembolic pulmonary hypertension; ELISA, enzyme-linked immunosorbent assay; IPAH, idiopathic pulmonary arterial hypertension.

FIGURE 4 ROC curve for ADAMTSL4 in patients with IPAH + CTEPH. IPAH + CTEPH (AUC, 0.947; 95% confidence interval [CI]: 0.898–0.999; $p < 0.01$); IPAH (AUC, 0.981; 95% CI: 0.947–1; $p < 0.01$); and CTEPH (AUC, 0.930; 95% CI 0.866–0.995; $p < 0.01$). ADAMTSL4, a disintegrin and metalloproteinase with thrombospondin motifs like 4; AUC, area under the ROC curve; CI, confidence interval; IPAH + CTEPH, idiopathic pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension; ROC, receiver operating characteristics (curve).
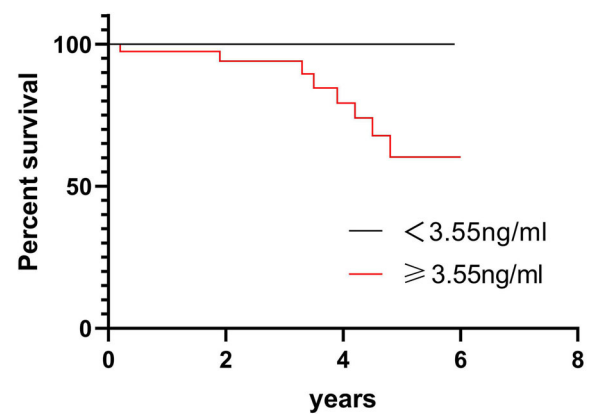

FIGURE 5 Kaplan–Meier analysis showing the probability of survival in patients with PH (IPAH + CTEPH) according to serum ADAMTSL4. ADAMTSL4, a disintegrin and metalloproteinase with thrombospondin motifs like 4; IPAH + CTEPH, idiopathic pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension; PH, pulmonary hypertension.

elevated plasma levels of ADAMTSL4. However, there was no significant difference in the ROC cutoff values between the two groups ($p = 0.19$).

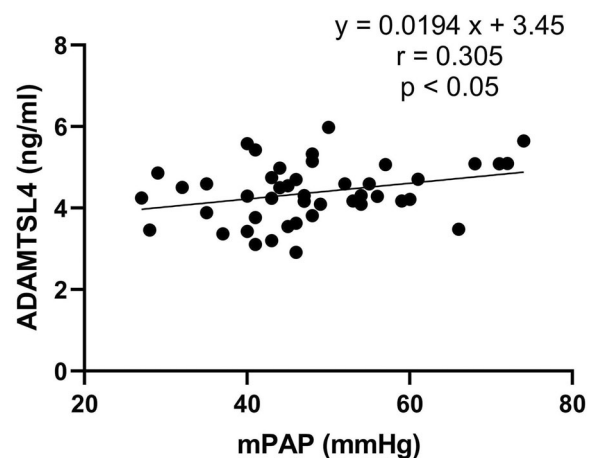## Correlations between plasma ADAMTSL4 levels and hemodynamic parameters

As shown in Figure 6, a positive correlation was observed between plasma ADAMTSL4 levels and mPAP in the PH group (IPAH and CTEPH combined) ($r = 0.305$, $p < 0.05$). However, no significant correlation was found between ADAMTSL4 levels and clinical, laboratory, or other hemodynamic parameters in either the IPAH or CTEPH group. Figure 7 shows a forest plot illustrating the association between ADAMTSL4 level and various parameters.

DISCUSSION

This is the first study to provide evidence of elevated plasma ADAMTSL4 protein expression levels in patients with IPAH by utilizing proteomics technology and to confirm these findings through ELISA. ELISA further demonstrated elevated plasma ADAMTSL4 levels in patients with CTEPH. The results of the bioinformatics
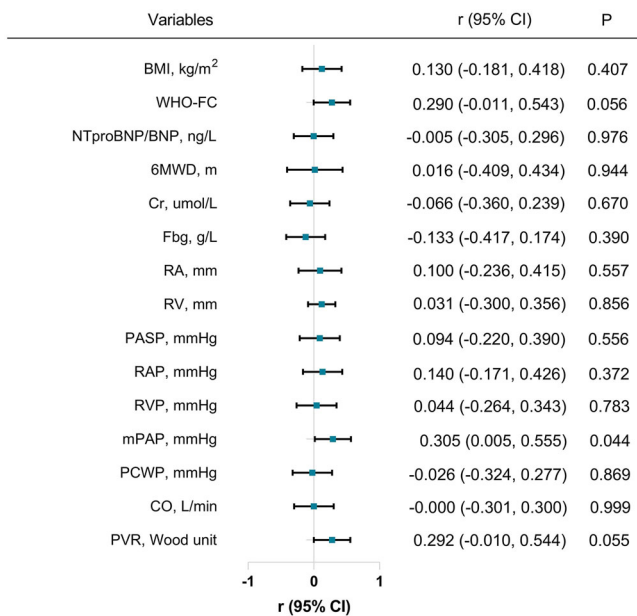

FIGURE 6 The positive correlation between plasma ADAMTSL4 levels and mPAP. ADAMTSL4, a disintegrin and metalloproteinase with thrombospondin motifs like 4; mPAP, mean pulmonary arterial pressure.

part of the study also supported these findings by indicating elevated mRNA expression levels of ADAMTSL4 in the pulmonary tissues of patients with IPAH. The results of Western blot showed that the protein expression of ADAMTSL4 was upregulated in PASMCs in vitro. Analysis of the ROC curve suggested that ADAMTSL4 can help in predicting the occurrence of IPAH and CTEPH. Furthermore, a positive correlation was observed between ADAMTSL4 levels and PVR in patients with PH (IPAH and CTEPH).

Bioinformatics is an effective and valuable tool in PH research. One such resource, the GEO, provides

Variables		r (95% CI)	P
BMI, kg/m^2		0.130 (−0.181, 0.418)	0.407
WHO-FC		0.290 (−0.011, 0.543)	0.056
NTproBNP/BNP, ng/L		−0.005 (−0.305, 0.296)	0.976
6MWD, m		0.016 (−0.409, 0.434)	0.944
Cr, umol/L		−0.066 (−0.360, 0.239)	0.670
Fbg, g/L		−0.133 (−0.417, 0.174)	0.390
RA, mm		0.100 (−0.236, 0.415)	0.557
RV, mm		0.031 (−0.300, 0.356)	0.856
PASP, mmHg		0.094 (−0.220, 0.390)	0.556
RAP, mmHg		0.140 (−0.171, 0.426)	0.372
RVP, mmHg		0.044 (−0.264, 0.343)	0.783
mPAP, mmHg		0.305 (0.005, 0.555)	0.044
PCWP, mmHg		−0.026 (−0.324, 0.277)	0.869
CO, L/min		−0.000 (−0.301, 0.300)	0.999
PVR, Wood unit		0.292 (−0.010, 0.544)	0.055

r (95% CI)
−1   0   1

FIGURE 7 The relationship between plasma ADAMTSL4 levels and clinical parameters. ADAMTSL4, a disintegrin and metalloproteinase with thrombospondin motifs like 4; BMI, body mass index; CI, confidence interval; CO, cardiac output; Cr, creatinine; Fbg, fibrinogen; mPAP, mean pulmonary arterial pressure; NTproBNP/BNP, N-terminal pro-brain natriuretic peptide/brain natriuretic peptide; PASP, pulmonary artery systolic pressure; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RA, right atrium; RAP, right atrial pressure; RV, right ventricle; RVP, right ventricle pressure; WHO-FC, World Health Organization function classification; 6MWD, 6-min walk distance.

transcriptional data pertaining to pulmonary tissue samples obtained from patients with IPAH and healthy controls, specifically identified as the GSE15197 data set.[18] In our study, we investigated the mRNA levels of ADAMTSL4 in pulmonary tissues using the "Limma" package[19] in Rstudio 2022.12.0. After identifying elevated levels of ADAMTSL4 in patients with IPAH using proteomics and bioinformatics analyses, we measured the plasma concentration of ADAMTSL4 using ELISA. After adjusting for age and sex, we performed a correlation analysis between ADAMTSL4 levels and clinical parameters. The results showed that only mPAP was significantly correlated with ADAMTSL4 expression.

We categorized the patients into two groups according to the WHO-FC: WHO-FC I and II, and WHO-FC III and IV. There was a significant difference between the IPAH and CTEPH groups. The proportion of patients with WHO-FC III-IV heart function was higher in the IPAH group.

NT-proBNP levels were assessed in some patients, and BNP levels were determined in others. Therefore, to facilitate a better comparison between the IPAH and CTEPH groups, we divided the data into two groups (NT-proBNP level <1100 ng/L or BNP < 800 ng/L; NT-proBNP ≥ 1100 ng/L or BNP ≥ 800 ng/L)[17] and consolidated and analyzed them again. The NT-proBNP/BNP ratio did not differ significantly between IPAH and CTEPH groups.

ADAMTSL4 has been extensively studied in ophthalmology.[11,20,21] However, growing evidence indicates that ADAMTSL4 might have implications in the development of coronary artery disease.[14,22] TSR-containing protein, a crucial component of ADAMTSL4, significantly inhibits the proliferation and migration of human umbilical vein endothelial cells in vitro.[16] ADAMTSL4 is a glycoprotein secreted into the extracellular space.[11] Both protein and mRNA expressions of ADAMTSL4 have been detected in the medial layer of the arterial wall, specifically in medial vascular smooth muscle cells. This expression has also been observed in other organs containing smooth muscles[12,14] including pulmonary vascular smooth muscle cells. In patients with severe PH, certain vascular lesions display cancer-like characteristics such as dysregulation of apoptosis.[23,24] Additionally, overexpression of ADAMTSL4 can alter the sensitivity of ovarian cancer cells to TNF-induced apoptosis.[25] Further investigation is required to determine whether ADAMTSL4 plays a role in proliferation, apoptosis, or other pathogenic processes associated with IPAH and CTEPH.

Our study has several limitations. First, it was a retrospective study conducted at a single center, which may restrict the generalizability of the findings. Second, the sample size for plasma ADAMTSL4 analysis was relatively small in patients with IPAH and CTEPH. Third, we lacked data on changes in plasma ADAMTSL4 levels during the follow-up period. Finally, we did not analyze the mRNA levels of ADAMTSL4 using proteomics and bioinformatics in patients with CTEPH. Consequently, a larger sample size may have yielded statistically significant associations between plasma ADAMTSL4 levels, disease severity, and survival markers.

In conclusion, elevated plasma ADAMTSL4 protein levels were observed in patients with IPAH and CTEPH. Additionally, increased transcription of ADAMTSL4 has been identified in the pulmonary tissues of patients with IPAH. Moreover, ADAMTSL4 expression was positively correlated with mPAP. The association between ADAMTSL4 expression and prognosis of IPAH and CTEPH requires further investigation.

AUTHOR CONTRIBUTIONS

CONFLICT OF INTEREST STATEMENT
The authors declare no conflict of interest.

ETHICS STATEMENT
This study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Chongqing Medical University (protocol number 2020-404). Informed consent was obtained from all the participants.

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SUPPORTING INFORMATION

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