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Circulating regulators of the wingless pathway in precapillary pulmonary hypertension

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Abstract

Background and objective: Dysregulated Wnt signalling has been implicated in pulmonary hypertension (PH). We hypothesized that plasma levels of secreted Wnt proteins would be increased in patients with precapillary PH, correlate with indices of vascular resistance and cardiac function and give information on long-term prognosis.
Methods: We measured the Wnt ligand Wnt5a and secreted Wnt antagonists Dickkopf (DKK) DKK1, DKK3, secreted frizzled-related protein 3 (sFRP3), Wnt inhibitory factor-1 (WIF1) and sclerostin (SOST) in 106 patients with precapillary PH and 40 healthy controls. A second sample was obtained after a median of 4 months (n = 52). During a median of 90 months follow-up, 67 patients died.
Results: Our main findings were (i) Precapillary PH is characterized by enhanced systemic Wnt activity as reflected by elevated plasma levels of Wnt5a and secreted antagonists irrespective of diagnostic subgroups. (ii) WIF1 and in particular Wnt5a correlated with pulmonary vascular resistance and cardiac dysfunction. (iii) High levels of Wnt5a, sFRP3, DKK3 and WIF1 were associated with poor prognosis in age- and sex-adjusted analysis (hazard ratios per log/SD change ~1.4) and for DKK3 after further adjustment with right arterial pressure, pulmonary oxygen saturation, cardiac index, N-terminal pro B-type natriuretic peptide and peak oxygen uptake (VO_2). Finally, an elevation of Wnt5a and DKK3 during follow-up was independently associated with poor prognosis.
Conclusion: Our data indicate that Wnt signalling pathways could be implicated in the pathogenesis of precapillary PH, and that some of the Wnt-related molecules (i.e., Wnt5a and DKK3) should be further investigated in these patients.

KEYWORDS
biomarkers, prognosis, pulmonary hypertension, pulmonary vascular resistance, survival, Wnt signalling

INTRODUCTION

Pulmonary hypertension (PH) is a progressive pulmonary vascular disease associated with increased pulmonary vascular resistance (PVR) that can lead to right ventricular failure and subsequent death.[1] It may develop in prevalent heart and lung disease, or it may be present in rare clinical conditions with precapillary PH, such as pulmonary arterial hypertension (PAH) and chronic thromboembolic PH (CTEPH). Current treatment modalities improve functional capacity but any diagnosis of PH still portends an adverse impact and a suggested sevenfold increase in mortality.[2]

Transcriptome profiling has identified a critical role of Wnt signalling in PH and pulmonary remodelling.[3–9] Dysregulated Wnt signalling in the context of PH includes attenuated response to vascular injury due to disrupted crosstalk between canonical and non-canonical Wnt signalling in pulmonary

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endothelial[10] and smooth muscle cells (SMC).[11,12] Furthermore, the non-canonical ligand Wnt5a may promote pulmonary fibrosis[13,14] and Wnt5a mRNA expression in bone morphogenic-2-stimulated SMCs form patients with idiopathic PAH (IPAH) correlated positively with pulmonary arterial pressure.[11] We recently demonstrated increased systemic and myocardial Wnt5a in relation to PH and right ventricular failure in patients with left ventricular heart failure.[15,16] Modulation of Wnt signalling could therefore potentially represent a novel therapeutic option in PH, and several approaches targeting these pathways are under investigation.[17,18] However, data on the regulation of Wnt pathways in clinical precapillary PH are scarce.

The Wnt pathways consist of a number of endogenous secreted pathway modulators, such as Dickkopfs (DKKs) and the secreted frizzled-related proteins (sFRPs), that regulate both canonical and non-canonical Wnt signalling by binding various ligands and receptors.[18,19] Furthermore, these antagonists often circulate at readily measurable levels and may represent a negative feedback loop to limit Wnt pathway activity and could therefore indirectly reflect activity in the Wnt signalling pathways.[19] We previously reported that secreted Wnt antagonists, such as Wnt inhibitory factor-1 (WIF1), sFRP3 and DKK1, are associated with poor outcome in acute coronary syndrome,[20] aortic stenosis[21] and systolic HF.[22,23] To the best of our knowledge, circulating levels of Wnt-related proteins have not been investigated in precapillary PH. We hypothesized that plasma levels of the Wnt ligand Wnt5a and the secreted Wnt antagonist's sFRP3, DKK1, DKK3, sclerostin (SOST) and WIF1 would be increased in patients with precapillary PH, correlate with haemodynamic measures of vascular resistance and cardiac function and could give information on long-term prognosis.

SUMMARY AT A GLANCE

We evaluated secreted Wnt proteins in precapillary pulmonary hypertension (PH) and detected enhanced Wnt5a and secreted antagonists, which correlated with cardiac dysfunction. High Dickkopf (DKK) 3 and the elevation of Wnt5a and DKK3 during follow-up were associated with poor prognosis. Our data support a role for Wnt signalling in the pathogenesis of precapillary PH.

METHODS

Study population

We studied 106 patients with precapillary PH in New York Heart Association functional classes II–IV (Table 1). The diagnosis of precapillary PH was defined in accordance with the latest international guidelines as mean pulmonary artery pressure (MPAP) ≥25 mm Hg at rest, with a normal pulmonary capillary wedge pressure (PCWP; ≤15 mm Hg) and a PVR > 3.0 WU.[24] Based on the clinical classification of the same guidelines, the study population was divided into three groups: (i) patients with IPAH, $n = 30$; (ii) patients with PAH related to associated conditions (APAH, $n = 44$) and (iii) patients with (CTEPH, $n = 32$) verified with pulmonary angiograms. All patients in the APAH and CTEPH were incident, while 28 of 30 were incident in the

TABLE 1 Characteristics of the study group

	IPAH ($n = 30$)	APAH ($n = 44$)	CTEPH ($n = 32$)	p-Value
Age, years	43.1 (14.9)	44.2 (13.7)	56.7 (12.6)*,**	0.001
Sex, male, % (n)	23 (7)	27 (12)	50 (16)*,**	0.047
RAP, mm Hg	6.6 (4.2)	6.3 (5.4)	5.8 (3.6)	0.69
MPAP, mm Hg	55 (13)	51 (16)	43 (11) *,**	0.009
PCWP, mm Hg	6.5 (3.7)	6.1 (3.0)	7.0 (3.2)	0.71
Cardiac index, L/min/m^2	1.8 (0.4)	2.2 (0.6)*	2.2 (0.7)	0.041
$PaSO_2$, mm Hg	58 (8)	59 (11)	62 (9)	0.20
$FaSO_2$, mm Hg	94.3 (3.1)	90.9 (6.5)	91.0 (4.5)	0.007
PVR, Wood units	15.9 (5.4)	13.1 (6.3)	9.3 (4.4)*,**	0.002
Peak VO_2, ml/kg/min	12 (4)	13 (6)	9 (4)	0.64
NT-proBNP, pmol/L	341 (266)	297 (312)	185 (175)	0.16
PDE-5 inhibitor, % (n)	50 (15)	50 (22)	0 (0)*,**	<0.001
PGI_2, % (n)	47 (14)	16 (7)*	3 (1)*	<0.001
Endothelin receptor antagonists, % (n)	40 (12)	21 (9)	3 (1)*,**	0.002
Dual/triple therapy,[a] % (n)	27 (8)/13 (4)	18 (8)/1 (2)	0 (0)/0 (0)*,**	<0.001

Note: Data are presented as mean (SD) for continuous data and as percentage (n=) for categorical data. The medications represent therapy at baseline at the time of blood sampling. The p-value to the right represent the test for trend determined by either Kruskal–Wallis (continuous data or chi-square [categorical data]).

Abbreviations: APAH, associated PAH; CTEPH, chronic thromboembolic pulmonary hypertension; $FaSO_2$, femoral artery oxygen saturation; IPAH, idiopathic PAH; MPAP, mean pulmonary artery pressure; NT-proBNP, N-terminal pro-brain natriuretic peptide; PAH, pulmonary arterial hypertension; $PaSO_2$, pulmonary artery oxygen saturation; PCWP, pulmonary capillary wedge pressure; PDE-5, phosphodiesterase-5; PGI_2, prostacyclin analogue; RAP, right atrial pressure; VO_2, oxygen uptake.

[a]Combination of two or three of the indicated therapies.

*$p < 0.05$ versus IPAH. **$p < 0.05$ versus APAH.

IPAH group. In these patients, blood samples were obtained before the initiation of therapy. For changes in therapy between time-points in the IPAH and APAH groups, no specific treatment algorithm was used. Targeted therapies such as prostacyclin analogues, endothelin receptor antagonists and phosphodiesterase-5 inhibitors were begun as considered appropriate according to recommendations. Patients with unsatisfactory response at 3- and 12-month controls were considered for combination therapy and increased doses of PAH-specific medication. For comparison, we also studied 40 apparently healthy controls based on disease history and C-reactive protein levels within normal limits.

Haemodynamics and cardiopulmonary exercise testing

Right-sided cardiac catheterization was performed with a thermodilution catheter through the right jugular vein, with a catheter inserted into the right femoral artery for the monitoring of arterial blood pressure and blood gases. Baseline haemodynamic variables included heart rate and right atrial pressure (RAP), MPAP and PCWP. Cardiac output was obtained using the thermodilution technique as the mean of three measurements. PVR and cardiac index (CI) were calculated using standard formulas. Oxygen saturation was measured in blood samples from the femoral ($FaSO_2$) and pulmonary arteries ($PaSO_2$). Within 24–48 h of right-sided cardiac catheterization, a symptom-limited exercise test was performed using a cycle ergometer (ER900; Jäger, Wurzburg, Germany) started at 20 W, with the pedal rate kept constant at 60 rpm, and with stepwise increments of 5–10 W/min. Peak oxygen uptake (peak VO_2) was defined as the greatest 30-s average of oxygen uptake during the last minute of exercise.

Blood sampling and biochemical analysis

Blood samples were collected from the pulmonary artery and peripheral vein (healthy controls) in chilled pyrogen-free EDTA tubes and platelet-free plasma (centrifuged within 30 min at 2000*g* for 20 min) was stored in multiple aliquots at −80°C until analyses and only thawed once prior to biochemical analysis. Circulating Wnt5a was analysed by enzyme immunoassay from Cusabio. sFRP3, DKK1, DKK3, WIF1 and SOST (validation[25]) were analysed by enzyme immunoassays from R&D Systems (Minneapolis, MN, USA). N-terminal pro B-type natriuretic peptide (NT-proBNP) was determined by an electrochemiluminescence immunoassay on a Modular platform (Roche Diagnostics, Basel, Switzerland). All intra- and inter-assay coefficients of variation were <10%.

Statistical analysis

Differences between groups were tested using Mann–Whitney *U*-test (two groups) or Kruskal–Wallis test (more than two groups). Differences in the distribution of categorical data were analysed with chi-square test. As there were differences in age and gender distribution compared to controls and in relation to aetiology among the precapillary PH groups, Wnt proteins were log transformed (as they were skewed) and differences between the groups compared by multivariate analysis of covariance with age and sex as covariates with Bonferroni adjusted post hoc tests. Change in Wnt proteins over time was analysed by the Wilcoxon paired test. Differences in change in Wnt proteins between survivors and non-survivors were analysed by comparing change values (Mann–Whitney *U*-test) and, if significant, a repeated measures analysis of variance (ANOVA) was performed with age, sex and baseline values as covariates and presented as back-transformed estimated marginal means. Associations between Wnt proteins and clinical and haemodynamic parameters were assessed by Spearman correlation.

The association of normalized Wnt protein concentrations (i.e., ln transformed/SD) and change in Wnt protein levels between baseline and follow-up with all-cause mortality were assessed by multivariable Cox proportional hazards models. For assessment of baseline levels, three models, with incremental addition of co-variates were used in addition to univariate analysis. Model 1 included age and sex; Model 2 added RAP, $PaSO_2$, CI and NT-proBNP (as the strongest predictors in univariate analysis) and Model 3 added peak VO_2. Adjustment of the association between change in Wnt proteins and all-cause mortality was performed one covariate at a time (i.e., covariates were not cumulated in the model) due to lower power. Results are presented as estimated hazards ratios and 95% CIs. Kaplan–Meier curves were constructed to visualize and evaluate (log-rank test) differences in survival according to tertiles of Wnt proteins. Two-sided probability values were considered significant at $p < 0.05$.

RESULTS

Clinical characteristics of the study population are shown in Table 1. Patients with CTEPH were older, more frequently male, had lower MPAP and PVR, and used less medication compared to patients with IPAH and APAH. For comparison of circulating Wnt proteins, we used a control group of healthy individuals ($n = 40$) that were older (63 ± 8 vs. 48 ± 15, $p < 0.001$) and had a higher proportion of males (48% vs. 33%, $p < 0.001$).

Plasma Wnt proteins are elevated in precapillary PH

Patients had increased levels of the Wnt ligand Wnt5a and the secreted Wnt antagonists sFRP3, DKK1, DKK3 and WIF1 compared to controls (Figure 1) irrespective of

diagnostic group in age- and sex-adjusted analysis, except for DKK1, which was not significantly higher in IPAH. No significant differences were observed across diagnostic groups.

Wnt5a and WIF1 are associated with pulmonary pressures and cardiac function in PAH

Correlation analysis (Table S1 in the Supporting Information) revealed that WIF1 and in particular Wnt5a was associated with pulmonary pressures and cardiac function (Figure 2). The most prominent correlations are presented in Figure 2. Thus, Wnt5a and WIF1 were positively correlated with RAP, PVR as well as neuro-hormonal activation as reflected by NT-proBNP and negatively correlated with $PaSO_2$. In addition, Wnt5a correlated negatively with CI and peak VO_2 while WIF1 was positively correlated with MPAP.

DKK3 is associated with all-cause mortality in PAH

During a median 90 months follow-up (range 0.5–193 months), 67 of 106 patients with precapillary PH died. Plasma levels of Wnt5a, sFRP3, DKK3 and WIF1 were associated with long-term all-cause mortality in univariate analyses (Table 2). After age and sex adjustment, the strongest association was found for DKK3.

Of haemodynamic and biochemical data (n = 102), $PaSO_2$, RAP, CI and NT-proBNP were the strongest predictors of long-term outcome (Figure 3A). Adjustment with these measures had no influence on the association between DKK3 and outcome (39% higher risk per SD increase), nor did further adjustment with peak VO_2 (n = 81, 44% per SD increase) (Table 2 and Figure 3B). Kaplan–Meier curves according to tertiles of DKK3 are shown in Figure 3C. As there were some missing data on covariates, an adjusted multiple imputation model was also assessed (Table S3 in the Supporting Information).

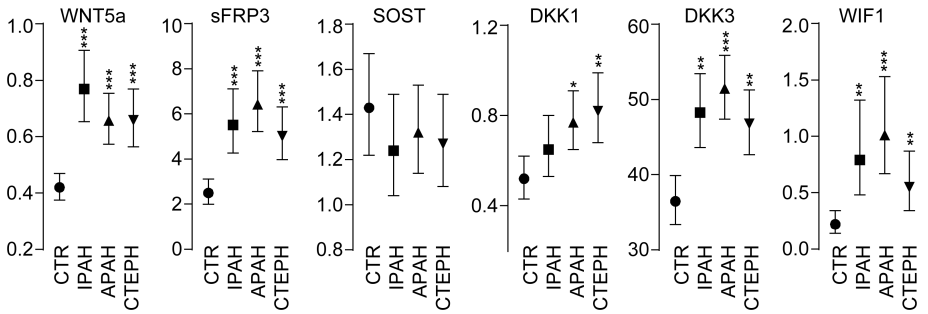

FIGURE 1 Levels of circulating Wnt proteins in precapillary pulmonary hypertension. Patients with pulmonary arterial hypertension (PAH) were classified as idiopathic PAH (IPAH, n = 30), associated PAH (APAH, n = 44) and chronic thromboembolic pulmonary hypertension (CTEPH, n = 32), and in healthy controls (CTR, n = 40). Data are given as estimated marginal means (ng/ml) adjusted for age and sex. *p < 0.05, **p < 0.01, ***p < 0.001 versus controls

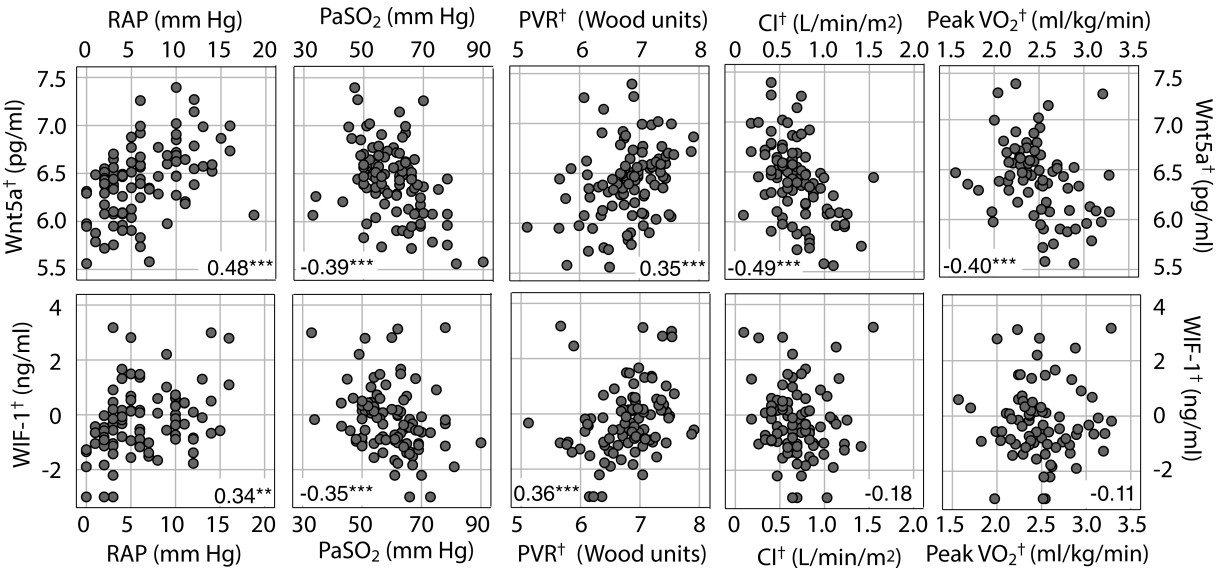

FIGURE 2 Correlation between the Wnt ligand Wnt5a, the secreted Wnt antagonist Wnt inhibitory factor-1 (WIF1) and haemodynamic and cardiopulmonary features in precapillary pulmonary hypertension. $PaSO_2$, pulmonary artery oxygen saturation; PVR, pulmonary vascular resistance; RAP, right arterial pressure. †log10 transformed. Spearman correlation: *p < 0.05, **p < 0.01, ***p < 0.001

TABLE 2 Multivariable Cox regression models for circulating Wnt proteins with step-wise adjustment for confounders and predictors of all-cause mortality in precapillary pulmonary hypertension

	Univariate		+Age, sex, creatinine		+CI, RAP, NT-proBNP, $PaSO_2$		+Peak VO_2	
	HR (95% CI)	***p***	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Wnt5a	1.22 (0.96–1.55)	0.098	1.22 (0.96–1.54)	0.099	1.00 (0.76–1.32)	0.990	0.93 (0.66–1.32)	0.691
sFRP3	1.34 (1.06–1.69)	0.010	1.39 (1.09–1.77)	0.009	1.28 (0.91–1.80)	0.155	1.31 (0.89–1.95)	0.176
SOST	0.99 (0.78–1.26)	0.951	0.99 (0.79–1.25)	0.937	1.19 (0.89–1.58)	0.238	1.10 (0.78–1.55)	0.583
DKK1	0.98 (0.76–1.25)	0.840	0.95 (0.75–1.22)	0.706	1.06 (0.81–1.39)	0.692	0.89 (0.65–1.23)	0.477
DKK3	**1.34 (1.07–1.68)**	**0.012**	**1.34 (1.05–1.71)**	**0.018**	**1.39 (1.04–1.86)**	**0.028**	**1.44 (1.02–2.04)**	**0.039**
WIF1	1.23 (0.96–1.57)	0.096	1.34 (1.06–1.71)	0.015	1.24 (0.88–1.75)	0.217	1.27 (0.84–1.92)	0.249

Abbreviations: CI, cardiac index; DKK, Dickkopf; HR, hazard ratio; NT-proBNP, N terminal pro-brain natriuretic peptide; $PaSO_2$, pulmonary artery oxygen saturation; RAP, right arterial pressure; sFRP, secreted frizzled-related protein; SOST, sclerostin; VO_2, oxygen uptake; WIF1, Wnt inhibitory factor-1.

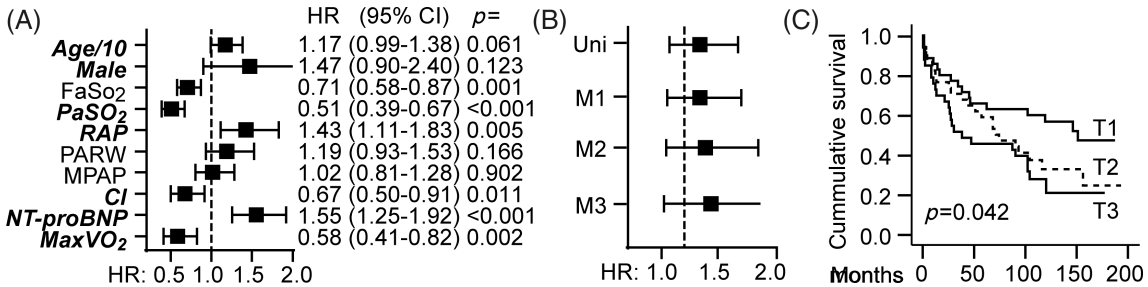

FIGURE 3 Dickkopf (DKK) 3 and all-cause mortality in precapillary pulmonary hypertension. (A) Cox regression analysis showing univariate predictors of all-cause mortality. CI, cardiac index; Fa/Pa-SO_2, femoral and pulmonary arterial oxygen saturation; MPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; RAP, right arterial pressure. (B) Cox regression analysis of the association between baseline levels of DKK3 in plasma with different levels of adjustment. Uni, unadjusted; M1, age and sex; M2, M1 + RAP, $PaSO_2$, CI and N-terminal pro B-type natriuretic peptide; M3, M2 + peak oxygen uptake (VO_2). Hazard ratios and 95% CIs are presented and are detailed in Table 2. (C) Kaplan–Meier curve showing association between tertiles of DKK3 and all-cause mortality, *p*-values are from the log-rank test. Tertile limits: T1, <43.8 ng/ml; T2, 43.8–52.8 ng/ml; T3 > 52.8 ng/ml

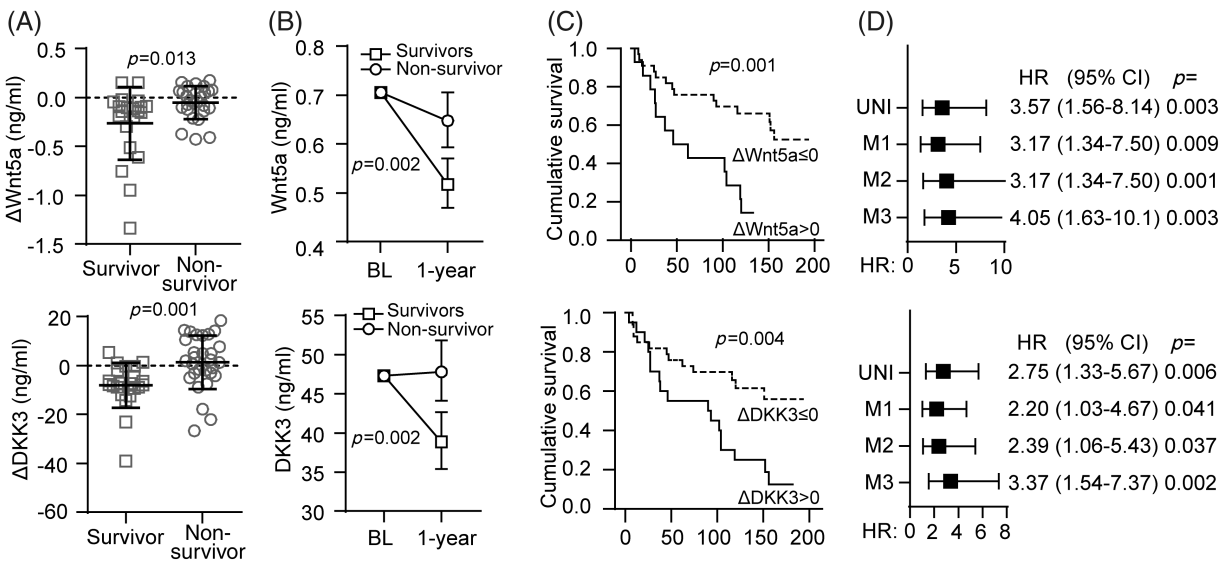

FIGURE 4 Change in Wnt5a and Dickkopf (DKK) 3 and all-cause mortality in precapillary pulmonary hypertension. (A) Temporal change in Wnt5a and Wnt inhibitory factor-1 (WIF1) in survivors and non-survivors during long-term follow-up. (B) Estimated marginal means from repeated measures analysis of variance for Wnt5a and DKK3 at baseline and follow-up (median 4 months) in survivors and non-survivors during long-term follow-up with baseline levels, age and sex as covariates. (C) Kaplan–Meier curves of patients who increased or decreased in Wnt5a and WIF1. (D) Cox regression analysis showing univariate and adjusted associations with long-term survival. Due to limited follow-up samples, the adjusted models were not added but evaluated independently: M1: Age and sex; M2: Right arterial pressure, $PaSO_2$, cardiac index and N-terminal pro B-type natriuretic peptide; M3: peak oxygen uptake (VO_2)

In contrast to DKK3, the association between sFRP3, WIF1 and in particular Wnt5a, and all-cause mortality was markedly attenuated and no longer associated with outcome following adjustment with haemodynamic data.

Change in levels of Wnt proteins and all-cause mortality

A follow-up sample was obtained from 52 (49%) patients with precapillary PH (median follow-up for sampling 4 months, range 1–31 months). Of these, 30 died (58%) during a median 108 months follow-up (range 4–186 months). As shown in Table S2 in the Supporting Information, plasma Wnt5a, SOST and sFRP3 decreased. A larger decrease in Wnt5a ($p = 0.013$) and particular in DKK3 ($p = 0.001$) was observed during follow-up in survivors compared to non-survivors (Figure 4A and Table S4 in the Supporting Information). Repeated measures ANOVA confirmed the significant interactions between survival status and temporal change adjusting for differences in sex, age and baseline levels (Figure 4B). The Kaplan–Meier curves in Figure 4C, dichotomizing Wnt5a and DKK3 levels according to increase or decrease, show poorer prognosis in patients who increased during follow-up, with a 3.6 and 2.8 times higher risk of death in univariate Cox regression analysis (Figure 4D) and these associations remained significant following adjustment for age and sex as well as haemodynamic data.

DISCUSSION

We found marked upregulation in circulating levels of several Wnt proteins in precapillary PH including the non-canonical ligand Wnt5a and the secreted antagonist's sFRP3, DKK1, DKK3 and WIF1. Of these, Wnt5a has been extensively studied in chronic lung disease[3] but its role in PAH and CTEPH is unclear. Thus, both enhancing[26,27] and attenuating[28] effects of Wnt5a have been found in pulmonary artery SMC proliferation and right ventricular hypertrophy, potentially reflecting different experimental models for PAH (i.e., hypoxia[26,27] vs. bleomycin-induced PAH[28]). More recently, Yuan et al. reported that endothelial loss of Wnt5a reduced pericyte recruitment and contributed to impaired regeneration of small vessels in PAH,[29] indicating that increased Wnt5a could reflect a compensatory response to prevent regression and small vessel loss in PH. Still, the correlation with pulmonary pressures, impaired cardiac function and mortality in precapillary PH support our findings in clinical heart failure and postcapillary PH.[15,16] Wnt5a primarily activates non-canonical Wnt signalling but may influence canonical and could play different roles during the progression of the disease. Further evidence for enhanced non-canonical signalling in PH has been shown by Laumanns et al. who demonstrated up-regulation of several members of the Wnt/planar cell polarity pathway, including the non-canonical ligand Wnt11, in remodelling vessels in the lungs from IPAH patients.[9] Interestingly, Wnt5a and Wnt11 are frequently co-expressed and work together,[30,31] and when dimerized may even been promote canonical signalling in experimental models.[31] Thus, while activation of some Wnt pathways may preserve vascular homeostasis in response to injury, others could promote excessive vascular remodelling during later stages of the disease and accelerate progression to end-stage right ventricular failure, which may be the case in our patients with manifest precapillary PH.

In addition to the receptor context, the outcome of Wnt signalling is regulated by secreted antagonists.[19,32] A major finding was the strong association of DKK3 with all-cause mortality both at baseline and during longitudinal testing. Moreover, in contrast to Wnt5a, DKK3 did not correlate with pulmonary pressures and cardiac function, suggesting that increased DKK3 is not merely a secondary phenomenon to deterioration of haemodynamic parameters. Through the activation of canonical, and inhibition of non-canonical pathways, DKK3 attenuates cardiac dysfunction and ventricular remodelling following experimental myocardial infarction (MI)[33,34] In clinical studies, plasma DKK3 was inversely related to carotid artery intima-media thickness and progression of carotid atherosclerosis[35] and decreased DKK3 levels were observed in unstable angina.[36] Whether this discrepancy reflects different pathogenic roles of DKK3 in precapillary PH and atherosclerosis, or whether increased DKK3 is restricted to more advanced disease as in the present study, is not clear. Nonetheless, whereas the pathogenic role of DKK3 in PAH and CTEPH will have to be clarified, it could be hypothesized that high DKK3 in precapillary PH could represent a counteracting mechanism, reflecting the degree of activation of Wnt pathways in the pulmonary vasculature, without being merely a secondary phenomenon to impaired myocardial function.

In conclusion, our data indicate that Wnt signalling pathways could be implicated in the pathogenesis of precapillary PH, and that some of the Wnt-related molecules (i.e., Wnt5a and DKK3) should be further investigated in these patients.

CONFLICT OF INTEREST

The authors have nothing to disclose.

HUMAN ETHICS APPROVAL DECLARATION

The investigation conforms to the principles outlined in the Declaration of Helsinki. The Regional Committees for Medical Research Ethics—South East Norway approved the study (project: 6.2008.1198-S-08373d, 2008/10604), and informed consent was obtained from each subject.

AUTHOR CONTRIBUTIONS

Arne Andreasseen: Conceptualization; data curation; investigation; writing-original draft; writing-review & editing. **Aurelija Abraityte:** Investigation; methodology; writing-review & editing. **Hilde Norum:** Investigation; writing-review & editing. **Lars Gullestad:** Conceptualization; data curation; investigation; writing-review & editing. **Pål Aukrust:** Conceptualization; project administration; writing-original draft; writing-review &

editing. **Sharanga Varathalingam:** Data curation; investigation; writing-review & editing. **Thor Ueland:** Conceptualization; data curation; formal analysis; investigation; methodology; writing-original draft; writing-review & editing.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

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

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