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**Short Communication**

# BMPR-II, caspase-3, HIF-1α, and VE-cadherin profile in Down syndrome children with and without congenital heart disease and pulmonary hypertension

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## Abstract

Several cellular markers have been identified as effective in detecting vascular remodeling recently. The reduced activity of bone morphogenetic protein receptor type-II (BMPR-II), commonly observed in Down syndrome, results in insufficient production of vascular endothelial cadherin (VE-cadherin). This, in turn, increases hypoxia-inducible factor-1α (HIF-1α) levels and leads to excessive production of caspase-3. The aim of this study was to compare the plasma levels of BMPR-II, VE-cadherin, HIF-1α, and caspase-3 between pediatric Down syndrome with and without congenital heart disease (CHD) and pulmonary hypertension (PH). This was to investigate the role of these biomarkers in the pathogenesis of PH associated or not associated with CHD. A cross-sectional study was conducted on Down syndrome children aged two months to five years at a tertiary hospital between January and December 2023. The children were classified into four groups: CHD with PH, CHD without PH, non-CHD with PH and normal heart. Plasma levels of BMPR-II, caspase-3, HIF-1α, and VE-cadherin were measured using ELISA and compared based on the presence or absence of CHD and PH using Kruskal-Wallis followed by post hoc Bonferroni tests. Elevated plasma HIF-1α levels were observed in all patients with PH, with significantly higher levels in those with CHD-PH. Elevated levels of caspase-3 were also observed among children with PH groups, with the highest levels observed in the non-CHD PH group. Plasma levels of BMPR-II and VE-cadherin were elevated in PH, with significantly higher levels in the non-CHD PH group compared to other groups.

**Keywords**: BMPR-II, caspase-3, HIF-1α, VE-cadherin, pulmonary hypertension

## Introduction

Down syndrome, the most common chromosomal abnormality resulting from trisomy 21, has a prevalence ranging from 1:700 to 1:1000 live births [1]. Down syndrome is often accompanied by various anomalies, with congenital heart disease (CHD) being the most frequent, affecting 40–60% of individuals [2,3]. Comorbidities such as upper respiratory tract obstruction, CHD, and obstructive sleep apnea can contribute to hypoxic conditions [3] and this condition could trigger the activation of the p38 mitogen-activated protein kinases (MAPK) pathway, hypoxia-inducible factor-1α (HIF-1α), and reactive oxygen species (ROS) [4]. These hypoxic conditions also reduce bone morphogenetic protein receptor type-II (BMPR-II) signaling, as BMPR-II functions



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optimally under oxygenated environments [3,4]. Evidence of *BMPR-II* gene (encodes BMPR-II) mutations has been observed in trisomy 21 patients with pulmonary hypertension (PH) associated with CHD [5]. BMPR-II deficiency or mutations in its gene (*BMPR-II*) have also been identified in CHD cases without PH [6].

Children with Down syndrome and CHD have a higher risk of PH (90%) compared to those without Down syndrome (40%) [7]. Left-to-right shunts resulting from heart defects, particularly in cases of faster progression in ventricular septal defects accompanied by pulmonary hypoplasia and gastroesophageal reflux, lead to an imbalance between vasoconstrictor and vasodilator agents [8]. This imbalance induces endothelial stress, which in turn triggers irreversible remodeling of the pulmonary arteries, ultimately leading to the development of PH [7,8].

PH is characterized by endothelial dysfunction, often linked to genetic causes such as *BMPR-II* gene mutations, including nonsense, missense, and frameshift mutations [8,9]. These mutations prevent *BMPR-II* gene from being expressed on the cell surface, disrupting its signaling pathway [9]. Under conditions of hypoxia, BMPR-II is unable to bind to its ligand, preventing the formation of mothers against decapentaplegic homolog 4 (SMAD4) and adequate production of vascular endothelial cadherin (VE-cadherin), which compromises monolayer integrity [10]. BMPR-II pathway inactivation activates the p38-MAPK pathway, stimulating p53, Bcl2-associated X protein (BAX), and caspase-3, leading to excessive apoptosis [11]. Additionally, BMPR-II deficiency activates tumor necrosis alpha (TNF-α), further increasing caspase-3 activity and reducing VE-cadherin levels, which fail to sufficiently inhibit apoptosis [12]. Endothelial dysfunction leads to increased production of endothelin-1 and thromboxane-A2, while decreasing nitric oxide, vasoactive peptide, and prostaglandin E2 levels [10,11].

Although *BMPR-II* gene mutations are the most common genetic cause of PH and have been frequently identified in CHD and Down syndrome, only 10–27% of individuals with the mutations progress to PH [13-14]. This indicates that *BMPR-II* gene mutations may not play a predominant role in the pathogenesis of PH [15-17]. However, in Down syndrome, BMPR-II is still recognized as having a role in the development of PH, though further research is needed to clarify its exact contribution [17]. The aim of this study was to compare the levels of BMPR-II, VE-cadherin, HIF-1α, and caspase-3 in Down syndrome's plasma to explain the role of these biomarkers in the pathogenesis of PH, whether related with CHD or not.

# Methods

## Study design and setting

A cross-sectional study was conducted on Down syndrome children aged two months to five years who were hospitalized at Dr. Moewardi Hospital, Surakarta, Indonesia, from January to December 2023. The patients were consecutively selected from all eligible criteria. All of the patients had an echocardiography assessment and were categorized into four groups: CHD with PH, CHD without PH, non-CHD with PH, and normal heart. A total of 5 mL of blood samples were collected and the ELISA tests were conducted to measure the levels of BMPR-II, VE-cadherin, HIF-1α, and caspase-3.

## Sampling strategy and criteria

A consecutive sampling method was employed to include all participants meeting the eligibility criteria during the study period. The inclusion criteria focused on children aged two months to five years with clinical manifestations of Down syndrome. These manifestations included midfacial hypoplasia with mongoloid faces, hypertelorism, flat nasal bridges, oblique palpebral fissures, epicanthal folds, ear abnormalities, macroglossia, mandibular hypoplasia, simian creases, fifth median phalanx dysplasia, muscle hypotonia, and joint hyperflexibility. Exclusion criteria included individuals with post-closure of heart defects via open-heart surgery or device occluder installation; those with clinical signs of right heart failure (such as anasarca, edema, hepatomegaly, elevated jugular venous pressure, or massive ascites); those with Eisenmenger syndrome (characterized by a right-to-left shunt or central cyanosis); and whose with critical CHDs, including truncus arteriosus, hypoplastic left heart syndrome, and transposition of the great arteries. The minimum sample size was calculated using an unpaired numerical

comparative analysis for more than two groups, requiring nine children per group, resulting in a total sample size of 36 children.

## Study procedure and measurements

Down syndrome patients whose parents provided consent to participate underwent echocardiographic examinations. Age, body weight, and height data were collected and nutritional status was categorized based on weight/height z-scores from the World Health Organization (WHO) standards according to the following criteria: normal (z-score between +2SD and -2SD), poor (z-score between -2SD and -3SD), and malnutrition (z-score < -3SD).

PH was diagnosed via echocardiography using the parasternal short-axis view to measure the right ventricular outflow tract (RVOT) acceleration time, which was converted using the formula: mPAP = 79 – (0.45 × RVOT acceleration time (cm/s)). PH is confirmed when mean pulmonary arterial pressure (mPAP) is ≥25 mmHg at rest or >30 mmHg during activity, with normal mPAP typically <15 mmHg [18]. All anatomical heart defects were categorized, such as atrial septal defect, ventricular septal defect, patent ductus arteriosus, and atrioventricular septal defect. The severity of PH was categorized by estimated pulmonary artery systolic pressure (PASP) from tricuspid regurgitation based on the formula: PASP = 4 (tricuspid regurgitation $V_{max})^2$ + right atrial pressure. The PH was categorized as follows: normal (PASP <30 mmHg), mild PH (30–40 mmHg), moderate PH (40–60 mmHg) and severe PH (>60 mmHg) [19].

A total of 5 mL of the venous blood sample was collected from each individual and was centrifuged at 1000 rpm for 15 minutes at 2–8°C. The serum was then stored in a -80°C freezer before analysis. The levels of BMPR-II, VE-cadherin, caspase-3, and HIF-1α were measured using the Human BMPR-II, VE-cadherin, caspase-3, HIF-1α enzyme-linked immunosorbent assay (ELISA) kits (all from EIAab, Wuhan, China) following the manufacture instructions.

Symptom data were collected from parents at the time of the examination. Functional heart diagnosis was categorized using the modified Ross criteria based on anamnesis and physical examination (**Table 1**). Functional heart diagnosis was classified as follows: Class I (no heart failure) with a score of 0–2, Class II (mild heart failure) with a score of 3–6, Class III (moderate heart failure) with a score of 7–9, and Class IV (severe heart failure) with a score of 10–12.

All samples were then categorized into four groups: (1) CHD-PH: CHD with PH; (2) CHD non-PH: CHD without PH; (3) non-CHD PH: non-CHD with PH; and (4) normal heart.

Table 1. Modified Ross score used to classify the functional heart diagnosis

| Component | Score | | |
|---|---|---|---|
| | 0 | +1 | +2 |
| History | | | |
| Diaphoresis | Head only | Head and body at exertion | Head and body at rest |
| Tachypnea | Rare | Several times | Frequent |
| Breathing physical examination | Normal | Retractions | Dyspnea |
| Respiratory rate based on age (breaths/min) | | | |
| 0–1 year | <50 | 50–60 | >60 |
| 1–6 year | <35 | 35–45 | >45 |
| 7–10 year | <25 | 25–35 | >35 |
| 11–14 year | <18 | 18–28 | >28 |
| Heart rate based on age (beats/min) | | | |
| 0–1 year | <160 | 160–170 | >170 |
| 1–6 year | <105 | 105–115 | >115 |
| 7–10 year | <90 | 90–100 | >100 |
| 11–14 year | <80 | 80–90 | >90 |
| Hepatomegaly size (cm) | <2 | 2-3 | >3 |

## Study variables

The independent variables in this study were the types of diagnosis among Down syndrome children (CHD-PH, CHD non-PH, non-CHD PH, and normal heart). The dependent variables of the study were the levels of BMPR-II, caspase-3, HIF-1α, and VE-cadherin. In addition, some co-variates were also measured in this study, including age, nutritional status, symptoms related to the heart problem, severity of the PH, type of CHD and functional heart classification.

### Statistical analysis

Continuous variables were reported as mean ± standard deviation (SD), while categorical variables were presented as percentages. A Shapiro-Wilk normality distribution test was examined in all groups. Kruskal-Wallis was used for comparison among groups, followed by post-hoc analyses using the Bonferroni test. Statistical analysis was performed using SPSS version 20 software (IBM SPSS, New York, USA) with $p<0.05$ considered statistically significant.

## Results

### Characteristics of the patients

A total of 36 children with Down syndrome were included in this study, with nine children in each diagnosis group. The clinical characteristics of the children included in this study are presented in **Table 2**. The age distribution ranged from 0–12 months (25%), 12–36 months (36%), and 36–60 months (39%), with no significant difference ($p=0.389$). Poor nutritional status was observed in 47.2% of patients, while normal nutrition and malnutrition were more prevalent in the CHD group ($p=0.015$). Failure to thrive was the primary symptom in 66.7% of patients and there was a significant difference among groups ($p<0.001$). Echocardiographic results showed mild PH in 47.2% of patients and moderate PH in 2.8% of patients ($p=0.379$). CHD types included atrial septal defect in 25%, ventricular septal defect in 11.1%, patent ductus arteriosus in 8.3%, and atrioventricular septal defect in 5.6% ($p<0.001$). Functional heart classification revealed that 78% of patients had no heart failure, while mild, moderate, and severe PH were observed in 11%, 2.8%, and 2.8% of patients, respectively ($p=0.401$) (**Table 2**).

Table 2. Clinical characteristics of children with Down syndrome included in the study

| Variable | Total | | Diagnosis | | | | | | | | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CHD-PH (n=9) | | CHD non-PH (n=9) | | Non-CHD PH (n=9) | | Normal (n=9) | | |
| | n | % | n | % | n | % | n | % | n | % | |
| Ages | | | | | | | | | | | 0.389 |
| 0–12 months | 9 | 25.0 | 3 | 33.3 | 4 | 44.4 | 0 | 0.0 | 2 | 22.2 | |
| 12–36 months | 13 | 36.1 | 2 | 22.2 | 2 | 22.2 | 5 | 55.6 | 4 | 44.4 | |
| 36–60 months | 14 | 38.8 | 4 | 44.4 | 3 | 33.3 | 4 | 44.4 | 3 | 33.3 | |
| Nutritional state | | | | | | | | | | | 0.015 |
| Normal | 13 | 36.1 | 3 | 33.3 | 4 | 44.4 | 5 | 55.6 | 1 | 11.1 | |
| Poor | 17 | 47.2 | 4 | 44.4 | 1 | 11.1 | 4 | 44.4 | 8 | 88.9 | |
| Malnutrition | 6 | 16.7 | 2 | 22.2 | 4 | 44.4 | 0 | 0.0 | 0 | 0.0 | |
| Symptoms | | | | | | | | | | | <0.001 |
| None | 10 | 27.8 | 0 | 0.0 | 1 | 11.1 | 1 | 11.1 | 8 | 88.9 | |
| Dyspnea | 1 | 2.8 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 1 | 11.1 | |
| Cyanotic | 1 | 2.8 | 1 | 11.1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | |
| Failure to thrive | 24 | 66.7 | 8 | 88.9 | 8 | 88.9 | 8 | 88.9 | 0 | 0.0 | |
| Pulmonary hypertension | | | | | | | | | | | 0.379 |
| Mild | 17 | 47.2 | 8 | 88.9 | 0 | 0.0 | 9 | 100.0 | 0 | 0.0 | |
| Moderate | 1 | 2.8 | 1 | 11.1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | |
| Severe | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | |
| Type of CHD | | | | | | | | | | | <0.001 |
| None | 18 | 50.0 | 0 | 0.0 | 0 | 0.0 | 9 | 100.0 | 9 | 100.0 | |
| VSD | 4 | 11.1 | 1 | 11.1 | 3 | 33.3 | 0 | 0.0 | 0 | 0.0 | |
| ASD | 9 | 25.0 | 7 | 77.8 | 2 | 22.2 | 0 | 0.0 | 0 | 0.0 | |
| PDA | 3 | 8.3 | 0 | 0.0 | 3 | 33.3 | 0 | 0.0 | 0 | 0.0 | |
| AVSD | 2 | 5.6 | 1 | 11.1 | 1 | 11.1 | 0 | 0.0 | 0 | 0.0 | |
| Functional heart classification | | | | | | | | | | | 0.401 |
| No heart failure | 28 | 77.8 | 6 | 66.7 | 6 | 66.7 | 8 | 88.9 | 8 | 88.9 | |
| Mild heart failure | 5 | 13.8 | 2 | 22.2 | 2 | 22.2 | 0 | 0.0 | 1 | 11.1 | |
| Moderate heart failure | 2 | 5.5 | 0 | 0.0 | 1 | 11.1 | 1 | 11.1 | 0 | 0.0 | |
| Severe heart failure | 1 | 2.9 | 1 | 11.1 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | |

ASD: atrial septal defect; AVSD: atrioventricular septal defect; CHD: congenital heart disease; CHD-PH: congenital heart disease with pulmonary hypertension; CHD non-PH: congenital heart disease without pulmonary hypertension; Non-CHD PH: non-congenital heart disease with pulmonary hypertension; PDA: patent ductus arteriosus; VSD: ventricular septal defect

## Comparisons of BMPR-II, caspase-3, HIF-1α, and VE-cadherin

Significant differences in plasma BMPR-II levels were observed among groups ($p$<0.001) (**Table 3**). Non-CHD PH patients had the highest plasma BMPR-II levels, with a median value of 24.24 pg/mL, followed by CHD-PH patients (median 14.15 pg/mL), normal patients (median 0.149 pg/mL), and CHD non-PH patients (median 0.136 pg/mL).

Plasma caspase-3 levels also demonstrated significant differences among groups ($p$<0.001) (**Table 3**). Non-CHD PH patients had the highest plasma caspase-3 levels, with a median value of 21.74 ng/mL, followed by CHD-PH patients, CHD non-PH patients, and normal patients (**Table 3**).

Patients with CHD-PH exhibited the highest HIF-1α levels, with a median value of 5.89 ng/mL, followed by non-CHD PH patients (median 3.86 ng/mL), CHD non-PH patients (median 1.64 ng/mL), and normal patients (median 0.75 ng/mL) (**Table 3**). The present study found that the levels of HIF-1α were significantly different among four groups ($p$=0.006) (**Table 3**).

Significant differences in VE-cadherin levels were also noted across patient groups ($p$<0.001) (**Table 3**). Non-CHD PH patients exhibited the highest VE-cadherin expression levels, with a median value of 22.40 ng/mL, followed by CHD-PH, CHD non-PH and normal groups (**Table 3**).

Table 3. Comparisons of BMPR-II, caspase-3, HIF-1α, and VE-cadherin levels among groups

| Indicator | Group | | | | $p$-value |
|---|---|---|---|---|---|
| | CHD-PH Median (min-max) | CHD non-PH Median (min-max) | Non-CHD PH Median (min-max) | Normal Median (min-max) | |
| BMPR-II (pg/mL) | 14.15 (3.03–28.28) | 0.13 (0.05–0.41) | 24.24 (8.09–33.34) | 0.14 (0.03–0.58) | <0.001 |
| Caspase-3 (ng/mL) | 5.12 (0.27–7.28) | 0.98 (0.17–6.90) | 21.74 (4.21–44.43) | 0.42 (0.20–1.00) | <0.001 |
| HIF-1α (ng/mL) | 5.89 (1.12–11.26) | 1.64 (0.65–3.77) | 3.86 (1.69–14.86) | 0.75 (0.41–5.31) | 0.006 |
| VE-cadherin (ng/mL) | 19.30 (11.40–58.40) | 5.90 (2.50-12.20) | 22.40 (8.60–62.00) | 5.60 (2.60–8.40) | <0.001 |

BMPR-II: bone morphogenetic protein receptor type II; CHD-PH: congenital heart disease with pulmonary hypertension; CHD non-PH: congenital heart disease without pulmonary hypertension; HIF-1α: hypoxia-inducible factor-1α; non-CHD PH: non-congenital heart disease with pulmonary hypertension; VE-cadherin: vascular endothelial cadherin

## Post-hoc analysis of BMPR-II, caspase-3, HIF-1α, and VE-cadherin profile

Post-hoc analysis tests revealed significant differences in mean plasma levels of BMPR-II, caspase-3, HIF-1α, and VE-cadherin among groups (**Figure 1**). For BMPR-II, significant differences were observed between CHD-PH and both CHD non-PH ($p$<0.001) and normal patients ($p$<0.001) (**Figure 1A**). Although a difference was noted between CHD-PH and non-CHD PH patients, it was not statistically significant ($p$=0.070). Significant differences were also found between CHD non-PH and non-CHD PH patients ($p$<0.001), while no significant difference was detected between CHD non-PH and normal patients ($p$=0.895). Significant differences were also identified between non-CHD PH and normal patients ($p$<0.001) (**Figure 1A**).

For caspase-3, significant differences were found between CHD-PH and both non-CHD PH ($p$=0.005) and normal patients ($p$=0.019) (**Figure 1B**). A significant difference was observed between CHD non-PH and non-CHD PH patients ($p$=0.001), while no significant difference was noted between CHD non-PH and normal patients ($p$=0.453). Significant differences were also identified between non-CHD PH and normal patients ($p$<0.001) (**Figure 1B**).

Regarding HIF-1α, significant differences were identified between CHD-PH and CHD non-PH ($p$=0.038), as well as between CHD-PH and normal patients ($p$=0.012) (**Figure 1C**). Significant differences were also found between CHD non-PH and non-CHD PH patients ($p$=0.009), but not between CHD non-PH and normal patients ($p$=0.270). Significant differences were also present between non-CHD PH and normal patients ($p$=0.015) (**Figure 1C**).

For VE-cadherin, significant differences were detected between CHD-PH and both CHD non-PH ($p$<0.001) and normal patients ($p$<0.001) (**Figure 1D**). A non-significant difference was

observed between CHD-PH and non-CHD PH patients ($p$=0.233). Significant differences were noted between CHD non-PH and non-CHD PH patients ($p$<0.001), but no significant difference was found between CHD non-PH and normal patients ($p$=0.626). Significant differences were also identified between non-CHD PH and normal patients ($p$<0.001) (**Figure 1D**).

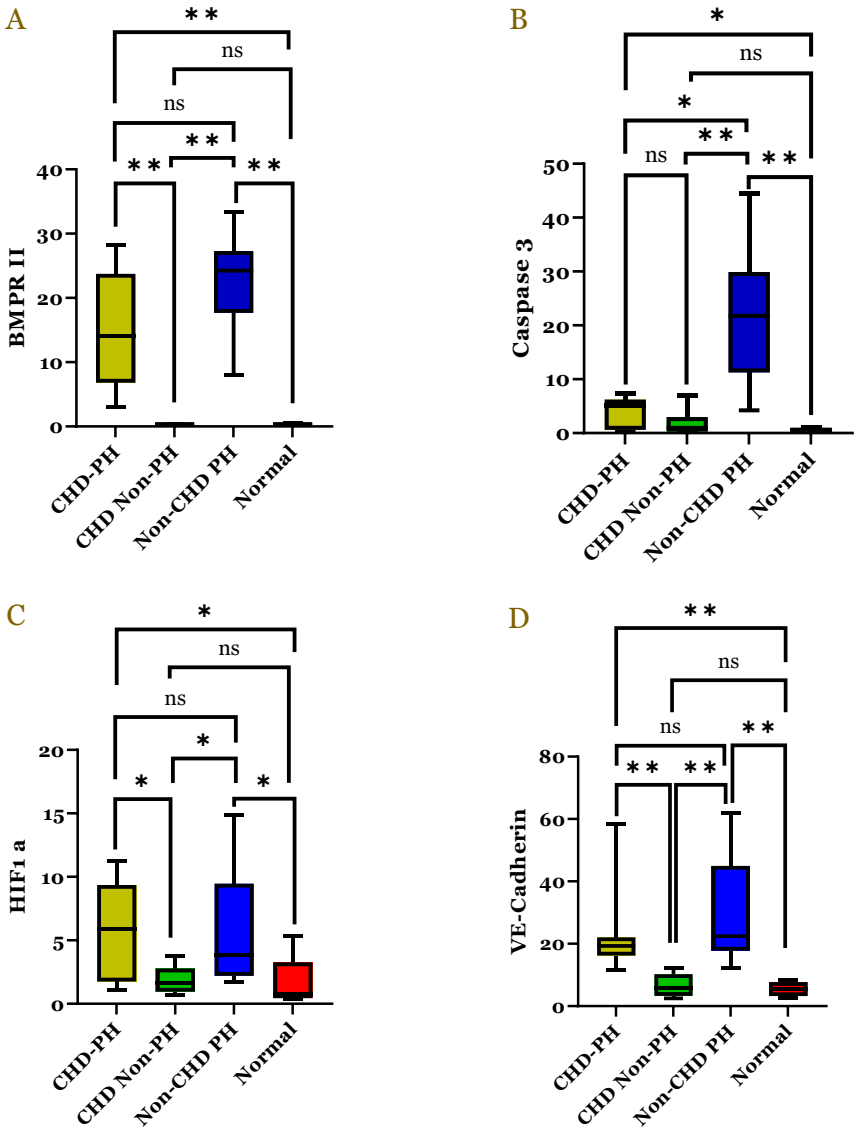


Figure 1. Post-hoc analysis showing the comparisons of the levels of Bone morphogenetic protein receptor type II (BMPR-II) (A), caspase-3 (B), hypoxia-inducible factor 1-alpha (HIF-1α) (C), and vascular endothelial cadherin (VE-cadherin) (D) between diagnosis groups of children with Down syndrome. *statistically significant at $p$<0.05, **statistically significant at $p$<0.001. CHD-PH: congenital heart disease with pulmonary hypertension; CHD non-PH: congenital heart disease without pulmonary hypertension; non-CHD PH: non-congenital heart disease with pulmonary hypertension.

## Discussion

The pathogenesis of PH in children with Down syndrome remains incompletely understood [2]. This condition is closely associated with cardiovascular malformations and pulmonary hypoplasia [1-3]. In addition to these structural anomalies, several developmental factors contribute to the condition, including chronic hypoxic states (such as those resulting from obstructive sleep apnea syndrome), dysregulation of vascular vasodilation, recurrent infections, persistent inflammation, and endothelial dysfunction [2,3,17]. Endothelial dysfunction, characterized by impaired expression of BMPR-II signaling pathways, is a key factor in the pathogenesis of PH in patients with Down syndrome [8,11].

The present study observed an increase in plasma BMPR-II levels in patients with PH, both with and without CHD. The elevation of plasma BMPR-II levels in patients with PH without CHD was more homogeneous and statistically significant ($p$<0.001 with a mean of 24.245 pg/mL), whereas the increase in patients with CHD and PH exhibited greater heterogeneity ($p$<0.001 with a mean of 14.15 pg/mL). These findings carried several important implications: first, elevated plasma BMPR-II levels were associated with the occurrence of PH; second, increased plasma BMPR-II levels were consistently observed in cases of PH in both patient groups; and third, variability in plasma BMPR-II levels was more pronounced among PH patients with CHD.

The most common genetic cause of PH involves mutations in the *BMPR-II* gene (including nonsense, missense, and frameshift mutations), which lead to endothelial dysfunction [18-20]. In the present study, the elevated plasma BMPR-II levels may represent soluble BMPR-II, an ectodomain that competes with BMPR-II ligands circulating in the bloodstream, indicating a reduced likelihood of surface expression on endothelial cells [21]. The primary mechanism driving this increase in plasma levels is hypothesized to be the cleavage of BMPR-II, resulting from enhanced activity of A disintegrin and metalloprotease 10 (ADAM10) and ADAM17 enzymes, mediated by TNF-α [22]. This process can lead to a loss of BMPR-II transmembrane expression, thereby disrupting BMPR-II signaling pathways [18,19].

Caspase-3 protein plays a critical role in the final stages of apoptosis, a process that may exacerbate the pathogenesis of PH [23,24]. In the present study, the highest plasma levels of caspase-3 were observed in patients with PH without CHD (21.74 ng/mL), indicating an increase in apoptotic activity among this group. Previous studies have identified caspase-3 as a marker of apoptotic activity in patients with atherosclerosis and sepsis [24]. Although patients with CHD and PH exhibited elevated levels of caspase-3 (5.12 ng/mL) compared to CHD patients without PH (0.98 ng/mL), these differences did not reach statistical significance ($p$=0.145).

Several hypotheses may explain these findings. First, PH in patients with CHD may be a compensatory response to the presence of a shunt, with ASD identified as a common condition in this study [25]. Second, the chronic elevation of pressure has direct implications for vascular remodeling [24]. Evidence suggests that PH can be reversible in patients with CHD who undergo shunt correction before the development of plexiform lesions and intimal fibrosis [26,27]. Finally, the apoptotic process may be inhibited by the hyperproliferation of endothelial cells, which exhibit antiapoptotic properties, resulting in lower caspase-3 levels in patients with CHD compared to those without CHD and with PH [23,24].

In the present study, elevated levels of HIF-1α were identified in patients with PH, regardless of the presence of CHD (3.86 ng/mL), with the highest levels observed in patients with CHD (5.89 ng/mL). Increased HIF-1α levels in pulmonary hypertensive patients without CHD may be attributed to alternative mechanisms. Comorbidities, such as upper respiratory tract obstruction commonly found in patients with Down syndrome, can induce hypoxic conditions that activate the p38 MAPK pathway, HIF-1α, and ROS [28,29]. In patients without CHD, other comorbidities, such as chronic airway obstruction, may provoke a pulmonary vascular vasoconstrictive response due to diminished lung elasticity [5-7]. This phenomenon is further supported by the observation of elevated HIF-1α levels in certain Down syndrome patients who present with comorbidities PH without associated CHD [9]. Notably, in the present study, HIF-1α levels did not increase in patients with CHD who did not have PH, enhancing the specificity of HIF-1α's role in the pathogenesis of PH due to non-cardiogenic hypoxic conditions.

The binding of BMPR-II receptors with their ligands triggers phosphorylation and the formation of SMAD4, which subsequently activates VE-cadherin on the surface of endothelial cells, playing a central role in maintaining the integrity of the endothelial monolayer [30]. In the present study, plasma levels of VE-cadherin in pulmonary hypertensive patients without CHD were found to be relatively high, comparable to those in CHD patients without PH. This increase in plasma levels suggests that VE-cadherin is being degraded from the surface of endothelial cells, potentially reducing its abundance on the cell surface [30]. Additionally, plasma levels of VE-cadherin in CHD patients without PH or in normal patients did not exhibit significant increases ($p$=0.626). This finding further supports the involvement of endothelial dysfunction in the pathogenesis of PH [30].

The present study provided an updated perspective on the role of endothelial dysfunction in the pathogenesis of PH in children with Down syndrome. Elevated levels of BMPR-II, caspase-3, HIF-1α, and VE-cadherin were associated with the incidence of PH in this population. The application of endothelial dysfunction markers presents an alternative approach to enhancing the diagnostic accuracy of PH and holds potential for development as biomarkers and therapeutic targets to prevent the condition [25-27]. Targeted therapies aimed at regulatory markers need to be established to mitigate the progression of PH and improve the quality of life for children with Down syndrome [28,29]. However, this study had some limitations. The cross-sectional design restricted the ability to assess disease progression, as measurements of PH and biomarkers were conducted at a single time point. Biomarker measurements were performed using the patient's plasma, which may reflect contributions from cell types or sources other than endothelial cells. The use of ELISA techniques for biomarker measurement remains relatively expensive and may not be practical for routine clinical application. Future research should focus on utilizing endothelial cell biopsy to establish biomarker cut-off levels, as the pathogenesis of vascular remodeling in PH primarily occurs in the pulmonary artery endothelium.

# Conclusion

Elevated levels of BMPR-II, caspase-3, HIF-1α, and VE-cadherin were associated with the incidence of PH in children with Down syndrome. The incorporation of endothelial dysfunction markers may serve as an alternative approach to enhance the diagnostic accuracy of PH and could be further developed as biomarkers and therapeutic targets for its prevention.

### Ethics approval

Protocol of the present study was reviewed and approved by the Ethical Clearance Committee for Health Research, Dr. Moewardi Hospital, Surakarta, Indonesia (Approval number:202/I/HREC/2023). The study followed the ethical principles established in the "Declaration of Helsinki".

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### Competing interests

All the authors declare that there are no conflicts of interest.

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### Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

### Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

# How to cite

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