Abnormal expression of the costimulatory molecule B7-H4 in placental chorionic villous and decidual basalis tissues of patients with preeclampsia and HELLP syndrome

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Abstract

Background: B7-H4, a checkpoint molecule of the B7 family, regulates a broad spectrum such as T-cell activation, cytokine secretion, tumour progression, and invasion capacities. Our previous data revealed that soluble B7-H4 (sB7-H4) blood serum levels are elevated in women at high risk for the hypertensive pregnancy disorder preeclampsia (PE) in the first trimester, as well as in patients with confirmed early/late-onset PE.

Aim: We here aim to investigate the expression pattern of B7-H4 in placental tissues of PE and HELLP Syndrome versus control group.

Methods: B7-H4 protein expression and localization were investigated by immunoblotting and co-immunohistochemistry in placental chorionic villous and decidual basalis tissues.

Results: B7-H4 protein was prominently expressed at the cell membrane, in the cytoplasm of the syncytiotrophoblast (STB) and interstitial extravillous trophoblast (EVT). B7-H4 protein levels in placental chorionic villous tissue were significantly higher in women with early-onset/late-onset PE and HELLP, while it was decreased in decidual basalis tissues of early-onset PE and HELLP compared with controls.

Conclusion: B7-H4 was inversely expressed in placental chorionic villous and decidual basalis tissues of PE and HELLP patients. The increase in B7-H4 in the STB in PE and HELLP may lead to excessive apical expression and release of soluble B7-H4 in the maternal circulation. In contrast, the decrease in B7-H4 in decidual basalis tissues could be related to the decrease in invasion ability of the EVT in PE. Thus, the current results strongly suggest that B7-H4 is involved in the pathogenesis of PE and HELLP.

Keywords
B7-H4, decidua basalis tissue, HELLP, immune tolerance, placenta chorionic villi, preeclampsia
1 | INTRODUCTION

During pregnancy, the maternal immune system plays an essential role. On one hand, numerous immunosuppressive processes can protect the "semi-allogeneic" fetus from damage to the maternal immune system. On the other hand, maternal immune activation supports the increase in placental hormones and metabolites secretion to meet the increased nutrient demands of the fetus. Disbalance between immune tolerance and activation is associated with pregnancy complications such as pregnancy loss, fetal growth restriction (FGR), preeclampsia (PE), and HELLP syndrome.

PE is one of the most important causes of maternal and fetal morbidity and mortality, and the estimated rate of this disease is between 2% and 8% of all pregnancies worldwide. PE has short- and long-term consequences for fetal and maternal health. Short-term effects on mother and fetus include hypertension, proteinuria and FGR and preterm birth. Long-term consequences on maternal health increased the risk of hypertension, ischemic heart disease, and stroke. There are two distinct subtypes according to different gestational weeks at disease manifestation: the early-onset form (<34 + 0 weeks of gestation) is often associated with a high rate of FGR, while the late-onset form (≥34 weeks' gestation) is usually not associated with FGR complication. Although many efforts have been made, there is still a gap in understanding its clear pathogenesis of this multifactorial disease. Because PE is life-threatening and effective treatments are lacking, it is urgent to explore the key pathogenesis of PE and find effective treatments to protect mothers and their offspring.

HELLP syndrome, a highly severe form of PE, occurs in 0.2–0.8% of pregnancies and in 70–80% of cases coexists with PE. However, the pathogenesis of HELLP syndrome is not completely understood and of the delivery is still the only therapy.

Therefore, additional and more specific/sensitive detection criteria are needed for improving PE and HELLP screening, diagnosis and treatment. In recent years, various studies have shown that insufficient adaptations of the maternal immune system are implicated in the pathophysiology of PE and HELLP, including alterations in levels and function of regulatory T cells. The B7 homolog 4, B7-H4, is a member of the B7 family that was independently discovered by a number of scientists in 2003. The currently known biological functions of B7-H4 mainly include as follows: (1) negative regulation of the T-cell immune response, inhibition of the proliferation of T lymphocytes and development of cytotoxicity; (2) and reduction in cytokine levels. Thus, we explore whether B7-H4 is linked to disturbed immune tolerance in pathological pregnancies such as PE and HELLP.

B7-H4 has two functional isoforms: the soluble form (sB7-H4) and the membrane-bound form (B7-H4). sB7-H4 acts as a decoy molecule to block B7-H4 signal transmission, inhibiting effects of B7-H4 in immune activation. However, the mechanism for the generation of sB7-H4 is still unknown. One possibility is that an enzyme cleaves the entire extracellular part of B7-H4 and another possibility is that sB7-H4 is generated by different splicing forms. In a previous study by us, we revealed an expression of B7-H4 mRNA in placenta chorionic villous tissue of third-trimester control, early-onset PE, and late-onset PE; here, the B7-H4 mRNA expression in placenta reflected the levels of sB7-H4 in blood serum. Galazka et al. found that B7-H4 has been detected on macrophages of the placenta and the decidua basalis tissue. In 2013, Dorota et al. also found the expression of the B7-H4 molecule on CD1c+ myeloid dendritic cells with no significant difference in the peripheral blood of patients with PE compared with controls.

Both the syncytiotrophoblast (STB) and extravillous trophoblast (EVT) are related to maternal-fetal interface immunity. It has been found that STB secretes extracellular vesicles into the maternal circulation as transmission factors, which interact with T cells, monocytes, granulocytes, and natural killer cells (uNK) and influence the function of these cells. EVT cells invade the uterine stroma and interact with decidual stromal cells, macrophages and uNK cells to regulate immunological acceptance of the placenta and fetus. Therefore, in the present study we investigated the expression and localization pattern of B7-H4 protein in normal and pathological placental chorionic villous tissue and decidua basalis tissue, in detail in STB and EVT cells in PE and HELLP patients.

2 | MATERIALS AND METHODS

2.1 | Study population—Human placental samples

The clinical study was approved by the ethics committee at the University Hospital of Essen, Germany (No.: 12–5212-BO). For this study we performed blinded experiments and the sample size was determined by PASS11 (NCSS Statistical software). Placental tissues were obtained at the time of vaginal delivery or caesarian section from the Department of Gynecology and Obstetrics at the University Hospital Essen, Germany, between 2014 and 2020. The following groups were analyzed: pregnancies complicated by early-onset PE (23 + 4 – 33 + 4 weeks, N = 13), late-onset PE (34 – 37 + 1 weeks, N = 10), and HELLP syndrome (23 + 4 – 33 + 2 weeks, N = 12), and a gestational-matched control group (control: 25 + 4 – 40 + 4 weeks, N = 17).

PE was diagnosed according to the American College of Obstetricians and Gynecologists (ACOG) (2019) of Hypertensive Disorders in Pregnancy. At the time of tissue collection, PE was defined as an occurrence of hypertension after 20 weeks of gestation with a blood pressure of at least 140/90 mm Hg or blood pressure of at least 160/110 mm Hg or proteinuria ≥300 mg in 24 h according to the guidelines of the International Society for the Study of Hypertension in Pregnancy and accompanied by a sFLT-1 (soluble fms-like tyrosine kinase-1)/PLGF (placental growth factor) blood serum level of >85 in early-PE or >110 in late PE. sFLT-1/PLGF ratio serves as a clinical biomarker for PE.

HELLP syndrome is characterized by hemolysis, elevated liver enzymes, and low platelet counts. At present, the HELLP diagnosis...
is characterized by blood levels with an increase in aspartate- and alanine-aminotransferase (AST and/or ALT ≥ 40 IU/L) and a decrease in platelet counts (platelets ≤150,000/mm³) to assess the liver damage and thrombocytopenia, respectively.

For the control group women who were chosen randomly and delivered a healthy child either by vaginally or by cesarean section in the second and third trimester of pregnancy. Patients in the pathological groups who had other pregnancy complications such as gestational and pre-gestational diabetes, chorioamnionitis, chronic villitis, or underlying autoimmune problems, were excluded from this study.

2.2 Tissue preparation—Placental dissection

For immunofluorescence, placental chorionic villous tissue was cut from the maternal side of the placenta between the umbilical cord and the outer border of the placenta, including the decidua basalis tissue, and washed twice briefly by sterile phosphate buffer solution (PBS) to avoid excessive blood contamination. All tissue samples were fixed in 4% paraformaldehyde overnight before standard processing to obtain paraffin-embedded sections. For protein isolation, samples from two parts of the placenta and decidua were collected: (1) Only placental chorionic villous tissue without decidua from the maternal side of the placenta between the umbilical cord and the outer border of the placenta and (2) only the decidua basalis tissue, located in the basal plate of the placenta where it is invaded by interstitial trophoblasts.

For the decidual basalis tissue and chorionic villous tissue dissection, the following reference was used. The tissue obtained according to the scheme in Figure 1. Briefly, one cotyledon between the umbilical cord and the edge was chosen, then a piece of the basal plate was dissected (Figure 1A, B). Using sharp fine scissors and forceps villi and blood vessels were removed (Figure 1C). The decidual basalis tissue is smooth and pale red in color (Figure 1D), and placental chorionic villous tissue is under the decidual basalis tissue (Figure 1A, B, E). Both decidual basalis and chorionic villous tissues were stored at −80°C until the extraction of protein samples.

2.3 Co-Immunofluorescence staining

7-μm placental/decidual sections were deparaffinized, and antigen retrieval was performed in a citrate buffer at 100°C for 40 min followed by permeabilization with 0.3% Triton X-100 in PBS for 20 min. Non-specific sites were blocked by incubation in 0.5% BSA in PBS for 20 min and autofluorescence was blocked by Sudan Black (Dianova). The following primary antibodies were used overnight at 4°C: mouse monoclonal anti-cytokeratin 7 (Novus biologicals, NBP1-22539; 1:100) or mouse monoclonal anti-HLA-G (Novus biologicals, NB500-302; 1:75), rabbit monoclonal IgG anti-B7-H4 (R&D Systems, 2318A; 1:30). The following appropriate secondary antibodies were used for one hour at room temperature: Cy3-conjugated anti-mouse IgG (Life Technologies, A10521; 1:100) and anti-rabbit Alexa Fluor 488 (Life Technologies, A10521; 1:200). All samples were counterstained with the DNA-specific dye 4′, 6- diamidin-2-phenylindol dihydrochloride (DAPI, 1 µg/mL, Sigma Aldrich), which was used to counterstain the nuclei for 15 min at room temperature. Controls were performed by omitting the primary antibody. Slides were covered with Mowiol and examined using a confocal fluorescence microscope (Leica SP5) and the software analysis program LAS AF (Leica). A minimum of three placental tissue samples for each condition was investigated (control N = 5; early-onset PE N = 4; late-onset PE N = 3; HELLP N = 4).

FIGURE 1 Placenta fetus scheme (A). The scheme of preparation of the placental villous tissue and the decidua basalis tissue (B). Trim-off the placental villi and blood vessels from the basal plate including the decidua basalis tissue (C), decidua basalis tissue after washing by 1× PBS (D), chorionic villous tissue (E)
2.4 | Immunoblotting

Protein extracts were prepared as described previously.26 Protein samples (20 µg) were separated on a 4%–15% polyacrylamide gel (BioRad). Next, proteins were transferred onto nitrocellulose membranes and incubated at 4°C overnight with the primary antibodies. Non-specific binding was blocked by incubation in 5% nonfat dry milk in 1× TBST for 1 h. The polyclonal rabbit anti-B7-H4 (R&D Systems, #2318A; 1:1000) and mouse monoclonal-β-Actin Peroxidase (Sigma A3845; 1:200,000) were used for incubating the membranes. The secondary antibody was incubated for 1 hour at room temperature (goat anti-rabbit Ig G(H+L), G21234; 1:7500). Detection was achieved with the SuperSignal West Dura Extended Duration Substrate Kit (Thermo Fisher Scientific) according to the protocol and analyzed using the Chemidoc XRS+ imaging system (BioRad). Densitometric analysis of single protein bands was performed by Image J2 × (Rawak Software Inc.), and then the protein expression levels were normalized to Actin expression. For normalization purposes, each signal's value was normalized to a same "internal control" sample, which was run on each blot.

2.5 | Statistical analysis

Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc.). We used Shapiro-Wilk normality test method to calculate whether the data in the single experimental group are normally distributed. Thus, the independent t test was used to compare two groups confirming normal distribution, and the Mann-Whitney test was used for non-parametric independent two-group comparisons to compare the results of the control group and PE as well as HELLP cases. Data are either presented in mean ± standard error. For all statistical tests, a probability value (p-value) of 0.05 or less was indicated with *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

3 | RESULTS

3.1 | Clinical features of pregnant women

Table 1 shows the clinical features of the participating pregnant women. Placental tissues from 52 pregnant women, early-onset PE (N = 13), late-onset PE (N = 10), and HELLP syndrome (N = 12) as well

| TABLE 1 | Characteristics of pregnant women participated in this study |
|----------|-------------------------------------------------------------|
|          | Controls N = 17                                            | Early-onset PE N = 13 | Late-onset PE N = 10 | HELLP N = 12 |
| Nulliparous, no. (%) | 4 (24)                                                     | 6 (46)                | 9 (90)                | 8 (67)        |
| Maternal age at delivery, years, median (IQR) | 29.0 (27.5–32.5)                                          | 33.0 (26.5–37.0)      | 32.5 (29.5–35.75)     | 31.5 (25.75–33.0) |
| Gestational age at delivery, weeks, mean (min and max), | 35±3 *(25+4–40+4)                                         | 29±2 **(23+4–33+4)    | 35±5 ***(34+0–37+1)   | 28±3 **(23+4–33+2) |
| Cigarette smoking, no. (%) | 1 (6)                                                      | 1 (8)                 | 0 (0)                 | 0 (0)         |
| Caucasian ethnicity, no. (%) | 16 (94)                                                   | 11 (85)               | 10 (100)              | 12 (100)      |
| Systolic Blood pressure, mmHg, median (IQR) | 125.5 (114.3–135.5)                                      | 151.0 *** (141.5–169.0) | 154.5 *** (136.5–176.5) | 153.0 *** (147.5–171.0) |
| Diastolic blood pressure, mmHg, median (IQR) | 70.00 (57.0–80.25)                                        | 94.0 *** (87.0–100.0) | 94.5 *** (82.5–99.25) | 100.0 *** (90.0–109.5) |
| Proteinuria, mg/24 hour, median (IQR) | nm | 1170 (470–5060)                                          | 440 (330–980)         | 285 (142.5–817.5)   |
| sFLT−1/PlGF ratio, median (IQR) | 3.33 (1.96–24.39)                                         | 942.7 *** (446.7–4350) | 160.4 * (105.4–639.7) | 992.0 * (311.1–1584) |
| Platelet count (cells/mm3, mean ± SD) | 276,000 (±78,035)                                        | 202,154 * (±81,710)   | 200,400 * (±78,736)   | 125,000 * * * (±44,241) |
| AST (GOT) (IU/L, mean ± SD) | 18.33 (±4.33)                                             | 24.62 (±9.042)        | 27.7 (±17.24)         | 64.75 ** *(±41.79) |
| ALT (GPT) (IU/L, mean ± SD) | 16.4 (±11.16)                                             | 23.1 (±13.57)         | 64.42 ***(±35.27)     |
| LDH (IU/L, mean ± SD) | 217.6 (±83.3)                                             | 216.9 (±59.14)        | 345.3 *(±125.5)       |
| Creatinine (mg/dl, mean ± SD) | 0.68 (±0.13)                                              | 0.75 *(±0.09)         | 0.82 *(±0.09)         | 0.70 (±0.09)  |
| Birth weight, g, median (IQR) | 2720.0 (2230.0–3286.0)                                    | 1030.0 *** (710.0–1565.0) | 2705.0 (2266.0–3303.0) | 905.0 *** (400.0–1603.0) |
| Birth weight, percentile, median (IQR) | 60.0 (30.0–81.0)                                         | 13.5 *(5.33–36.25)    | 50.0 (9.75–84.75)     | 3.3 ** *(3.0–20.0) |
| Cesarean section, no. (%) | 13 (76)                                                   | 13 (100)              | 10 (100)              | 12 (100)      |
| Pregnancy BMI before birth median (IQR) | 30.5 (27.0–36.5)                                         | 28.0 (25.5–38.45)     | 31.0 (29.0–38.5)      | 26.5 (22.5–33.0) |

Abbreviations: ALT(GPT), alanine aminotransferase; AST(GOT), aspartate aminotransferase; LDH, Lactate dehydrogenase; nm, not measured.

*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.
as the control group (N = 17) were analysed. The results showed no significant difference in the maternal age, ethnicity, cesarean section rate, and maternal BMI (p > 0.05) among the groups.

The PE and HELLP patients had significantly higher systolic and diastolic blood pressure compared with the control group (p < 0.0001). The level of the sFLT-1/PIGF ratio was significantly increased in early- and late-onset PE and HELLP compared with controls (p < 0.001, p < 0.01, p < 0.01, respectively). The laboratory indicators ALT and AST were significantly increased in HELLP group compared with controls (p < 0.001), while there was a non-significant
difference between the early- and late-onset PE group compared with the control group. Total serum lactate dehydrogenase (LDH) (evidence suggestive of hemolysis) was significantly elevated in HELLP group compared with controls (p < 0.05). The platelet count decreased significantly in the HELLP group compared with the control group (p < 0.0001). Although the platelet count was only slightly decreased in early and late PE (p < 0.05), it was within the normal range. The birth weight and birth weight percentile are significantly lower in early-onset PE and HELLP, while there is no difference in the late PE group compared with the control group.

### 3.2 Immunolocalization of B7-H4 in villous STB of PE and HELLP placentas

At the investigated pregnancy stages, the villous trophoblast consists nearly completely of STB by fusion of the CTBs. Immunolabeling of B7-H4 showed an intense staining at the STB membranes and in its cytoplasm (Figure 2A–E). B7-H4 was also expressed in mesenchymal stromal cells (Figure 2A,C-E), however, in a different spatial distribution and intensity. Interestingly, in controls staining of B7-H4 was predominantly at the basal membranes of the STB (Figure 2A) and

![Figure 4](image-url)

**Figure 4** Protein expression of B7-H4 in chorionic villi tissues of preeclamptic and HELLP placentas (A–C). Representative Western blot of B7-H4 protein expression in the control group (N = 17), early-onset PE group (N = 13), HELLP (N = 12), and late-onset PE (N = 10). Data represent means ± SD. *p < 0.05, **p < 0.001 significantly up-/down-regulated compared with the control after normalization to the β-actin expression.
in the cytoplasm, whereas the control tissue (Figure 2B) revealed less staining and predominantly distributed apically at the STB membranes and in the cytoplasm as marked by arrows (compare Figure 2A with B).

Compared with the control, in early-onset PE strong staining was detected apically in the STB and less at the basal side (Figure 2C). In the villous trophoblast of HELLP placentas, strong cytoplasmic staining was seen again with an enhanced intensity at the apical membranes (Figure 2E). Both early-onset PE and HELLP villi revealed an enhanced staining in the mesenchymal stroma compared with matched controls (Figure 2C, E).

In late-onset PE, an increased immunolabeling in the STB is obvious compared with matched controls. In contrast to the controls, B7-H4 is apically and basally expressed in STB and is furthermore predominantly found in the mesenchymal stroma (Figure 2D).

3.3 | Immunolocalization of B7-H4 in EVT cells of PE and HELLP placentas

HLA-G is a ligand for NK cell inhibitory receptor. It plays a role in immune tolerance in pregnancy and is expressed in the placenta by

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**FIGURE 5** Protein expression of B7-H4 in decidual basalis tissues in preeclamptic and HELLP placentas. (A–D) Representative western blot of B7-H4 protein expression in the control group (N = 17), early-onset PE group (N = 13), HELLP (N = 12), and late-onset PE (N = 10). Data represent means ± SD. *p < 0.05 significantly up-/down-regulated compared with the control after normalization to the β-actin expression, +p ≥ 0.05 indicates that there is no significant difference between the groups.
extravillous trophoblast cells (EVT), which appears to be a key component in trophoblast invasion.38 Here, we use HLA-G as a marker to locate EVT cells.

B7-H4 showed a diffuse punctate membrane and cytoplasmic staining in EVT cells of control placentas (Figure 3A,B). In contrast, only few B7-H4-positive EVT cells were detected in placental tissue from early-onset PE (Figure 3C) and late-onset PE patients (Figure 3D). B7-H4 was hardly detectable in HLA-G positive EVTs of the HELLP group as seen in Figure 3E, while B7-H4 was clearly expressed in the STB (refer to Figure 2E). In contrast, in HELLP placentas B7-H4 was strongly expressed in HLAG-negative cells in the decidua basalis tissue (Figure 3E).

### 3.4 | Protein expression of B7-H4 in the placental villous tissues of preeclamptic and HELLP placentas

B7-H4 protein levels were measured in placental villous tissues of women suffered from early-onset PE (N = 13; 23 ± 4 – 33 ± 4 weeks), late-onset PE (N = 10; 34 – 37 + 1 weeks), HELLP (N = 12; 23 + 4 – 33 + 2 weeks) and control group (N = 17; 25 ± 4 – 40 ± 4 weeks). B7-H4 protein expression was significantly higher in women with early-onset PE, late-onset PE (Figure 4A–C) and HELLP (Figure 4A, D) compared with normal third trimester controls \( p = 0.0196, p = 0.0011 \) and \( p = 0.0053 \), respectively.

### 3.5 | Protein expression of B7-H4 in decidua basalis tissues of preeclamptic and HELLP placentas

B7-H4 protein levels were measured in placental decidual tissue in women with early-onset PE, late-onset PE, HELLP, and control group, respectively, see above. Compared with the control group, B7-H4 protein expression was significantly lower in decidua basalis tissues of women with early-onset PE \( p = 0.0195 \) (Figure 5A, B). However, there is no significant difference between the late-onset PE and control group \( p = 0.615 \) (Figure 5A, C). B7-H4 protein was also significantly decreased in the HELLP group \( p = 0.0107 \).

Thus, the protein expression profile followed and confirmed the results obtained by immunohistochemistry.

### 4 | DISCUSSION

Normal pregnancy is considered to be a T-helper 2 (Th2)-type immunological state which is conductive to immune tolerance to prevent fetal rejection.39 In contrast, PE is hypothesized to be related to the induction failure of proper immune tolerance with a predominance of T-helper 1 (Th1)-type immunity.40,41 This leads to the question of whether there is some kind of trade-off between the responses to paternal alloantigens and those of microbes. B7-H4 acts as an immune checkpoint molecule that negatively regulates the immune responses. Compared with other B7-family members, the expression of B7-H4 in peripheral tissues is strictly controlled at the transcriptional level, that is B7-H4 mRNA is widely expressed, while the presence of B7-H4 protein on the surface of normal cells is still limited.21,43

In a previous study, we found that soluble B7-H4 levels in maternal blood serum were higher in the PE group compared with the control group in the third trimester of pregnancy. The B7-H4 mRNA levels in placental villous tissue were significantly higher in women with early-onset and late-onset PE compared with healthy controls, but not in fetal growth-restricted (FGR) placentas.27 Therefore, we designed this study to explore whether there is B7-H4 protein expression in placenta villous tissues and decidua basalis tissue in PE compared with control pregnancy. Also, the reason for the increase in sB7H4 levels in maternal blood serum of PE women should be investigated. This is the first study to analyze B7-H4 protein expression in the different parts of the fetomaternal compartment of the placenta—the fetal chorionic villous tissue and the maternal decidual tissue of women diagnosed with a placental dysfunction based on PE and HELLP. The main finding of our study was that B7-H4 was inversely expressed in chorionic villous tissue and decidua basalis tissues of normal versus PE and HELLP placentas.

B7-H4 expression showed significantly increased expression level in the placenta chorionic villous tissue of women suffering from early-/late-onset PE and HELLP compared with gestational-matched controls. Recently, we found that in first trimester serum samples, sB7-H4 was significantly higher expressed in women at elevated risk for PE compared with women without risk for PE.27 Here, B7-H4 protein, located at the membrane, is increased in the chorionic villous tissue, and in the apical membrane of the STB, of PE patients which correlated with increased levels of sB7H4 in the sera and B7-H4 mRNA in placental villous tissue of early-/late-onset preeclamptic patients, which opens the prospect of testing this factor as a prognostic clinical immunological marker. Therefore, we assume that B7-H4 is mainly expressed apically in the STB of PE and HELLP placentas compared with a more basically membrane expression in the STB of controls and may be thus increasingly released into the maternal circulation. This could explain the elevated serum levels of sB7-H4 as a result of its increased expression in chorionic villous tissue of PE patients. These results point to a possible speculation that the increase in B7-H4 in STB might be the source of excessive sB7H4 in the circulation, thereby affecting the regulation of T cells and the secretion of cytokines, which might have relationship with PE and HELLP. Similarly, in 2019, Feng Qiu et al. also found that sB7-H4 in blood serum was positively associated with B7-H4 expression in cervical tissue.44 Previous studies have reported that B7-H4 is also expressed in macrophages in the decidua28 and in dendritic cells29 in peripheral blood of pregnant women, which might be an additional source for sB7-H4. However, we need further study to verify that B7-H4 in STB is the only source of the excessive increase in sB7H4 in the maternal circulation in preeclampsia.

In 2009, Azuma et al. reported that sB7-H4 acts as a decoy molecule to block B7-H4 signal transmission, inhibiting effects
of B7-H4 in immune activation. This study showed that transgenic expression of sB7-H4 or genetic deletion of B7-H4 in mice accelerated the progression of collagen-induced arthritis, accompanied by enhanced T and B cell-mediated autoimmune responses. Our results showed that the B7-H4 protein expression in early-onset PE and HELLP placent al decidual basalis tissues is decreased, which is contrary to the level of sB7H4 found in blood serum of PE patients. Therefore, the increased B7-H4 levels in the chorionic villous in PE and the decrease in B7-H4 in decidual basalis tissues might enhance the Th1 responses, which may contribute to PE and HELLP. This possibility has to be investigated in detail in the future. Our previous study by Mach et al. strengthened our findings because second and third trimester preterm premature rupture of amniotic membranes (pPROM) also seems to be associated with elevated sB7-H4 in the first trimester. As we know, the rupture of the amniotic membrane promotes the transformation of the immune response to Th1 type. B7-H4 inhibits the Th1 immune response. Therefore, the increased expression of B7-H4 in placental villi may result in increased sB7H4 levels in serum enhancing the Th1 type immunity.

In our study, immunolocalization of B7-H4 in EVTs demonstrated that sparsely distributed B7-H4-positive EVT cells appeared in placental tissue from early-onset and late-onset PE patients, which was lower expressed compared with the normal control placentas. Moreover, B7-H4 was barely detectable in EVTs of the HELLP group. EVTs represent the source of trophoblast cells responsible for invasion into the decidua to invade maternal vessels. Therefore, B7-H4 may have also a second functional property in the feto-maternal compartment, a relationship with invasion properties of EVT cells for invading into the maternal compartment and vessels. If reduced decidual B7-H4 in early-onset PE and HELLP may have contributed to disturbed EVT invasion due to T-cell-mediated cytotoxicity has to be elucidated in future experiments.

However, there was no significant difference between late-onset PE samples and controls in protein expression levels. This may be due to the following reason: some literatures reported that early-onset PE is caused by poor placentation with minor invasion during early pregnancy, whereas late-onset PE is considered a consequence of exposure to pre-existing maternal risk factors. Our findings, therefore, are strongly corresponding to this hypothesis of a different pathogenesis of early- and late-onset PE.

Limitations of our study are the following: The analysis is retrospectively done but a supplement to our recent study of Mach et al. 2018. Despite the limitation of the sample size, this is the first study to analyze B7-H4 protein expression in the different parts of the fetomaternal compartment of the placenta, the fetal chorionic villous tissue and the maternal decidual basalis tissue. For future, prospective studies also evaluating FGR placentas and distinguishing between early- and late-onset HELLP placentas are recommended.

5 | CONCLUSION

To sum up, imbalance in B7-H4 expression between villous trophoblast and decidual basalis tissue suggests an immune dysregulation that may contribute to PE and HELLP. This study opens up new research ideas for the future investigation of the immune tolerance mediated by the costimulatory molecule B7-H4 in preeclampsia and HELLP and revealed sB7H4 as a new immunological predictive marker with potential new treatment options.

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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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