Original Research

Placental *Neuropeptide Y* (*NPY*) and NPY receptors expressions and serum NPY levels in preeclampsia

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Impact statement

Neuropeptide Y (NPY) has been reported as a vasoconstrictive substance which might be associated with preeclampsia. The novel findings of this study were that *Y1R*, *Y2R*, and *Y5R* expressions were significantly lower in the PE than the NP group. Moreover, the NPY receptor expression ratio between the PE/NP groups was lowest for *Y2R* (0.27) compared to *Y1R* (0.42) and *Y5R* (0.40) suggestive of a reduction of this receptor in the preeclampsia group. Our results suggested that decreased *Y2R* mRNA in the PE group might be associated with abnormalities of placental angiogenesis which probably contributes to the pathophysiology of preeclampsia.

Abstract

Neuropeptide Y (NPY) has been reported as a vasoconstrictive substance that might be associated with preeclampsia. NPY mediates different effects via its specific NPY receptors. NPY action via Y1 receptor (Y1R) and/or Y5 receptor (Y5R) induces vascular smooth muscle cells proliferation while it is implicated in angiogenesis via Y2 receptor (Y2R) and/or Y5R. The objectives of this study were to (1) compare placental *NPY*, *Y1 receptor* (*Y1R*), *Y2 receptor* (*Y2R*), and *Y5 receptor* (*Y5R*) expressions between normal (NP) and preeclamptic (PE) pregnancies to determine whether gene expression of different NPY receptors are altered in the PE condition; (2) compare maternal serum NPY levels between NP and PE subjects; and (3) determine correlations between placental gene expressions as well as serum NPY levels with maternal and neonatal clinical parameters. There were 22 subjects each in the NP (gestational age 37–42 weeks) and PE (gestational age $\geq$34 weeks) groups. Clinical parameters and serum NPY levels were measured before delivery. *NPY* expression and serum NPY levels were comparable between NP and PE subjects. *Y1R*, *Y2R*, and *Y5R* expressions were significantly lower in PE than NP subjects. In all and NP subjects, placental *Y2R* showed the highest expression, tended to be higher than *Y5R*, and was significantly higher than *Y1R*. In PE subjects, placental *Y2R* was comparable to *Y5R* and both *Y2R* and *Y5R* were significantly higher than *Y1R*. The *NPY* receptor expression ratio between the PE/NP groups showed that it was lowest for *Y2R* (0.27) compared to *Y1R* (0.42) and *Y5R* (0.40) suggestive of decreased *Y2R* expression in PE subjects. In summary, a decrease in placental *Y2R* mRNA might be associated with abnormalities of placental angiogenesis which probably contributes to the pathophysiology of preeclampsia. The roles of NPY receptors mediating placental vascularization need to be further investigated.

Keywords: Preeclampsia, neuropeptide Y, NPY receptors, gene expression, blood pressure

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Introduction

Preeclampsia (PE) is a common hypertensive complication of pregnancy and is a leading cause of maternal and fetal morbidity and mortality, especially in developing countries.[1] There are many theories regarding pathogenesis of PE, among of these, placental vascular resistance and ischemia/reperfusion, caused by an imbalance of angiogenesis of the uteroplacental unit, are major theories in the pathogenesis of PE.[2] Certain predisposing factors, such as genetic and environmental factors, may reduce uteroplacental

perfusion pressure resulting in placental ischemia/hypoxia which leads to the release of the anti-angiogenic factors including soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng), cytokines, hypoxia-inducible factor, reactive oxygen species, and angiotensin II type 1 agonistic autoantibodies (AT1-AA).[2] The binding of excessive sFlt-1 with vascular endothelial growth factor (VEGF) and placental growth factor inhibits their angiogenic effects. sFlt-1 and sEng decrease the production of the endothelium-derived relaxing factors nitric oxide (NO) and other vasorelaxing factors.[2] Alterations of these bioactive factors cause endothelial dysfunction.[2] Elevated AT1-AA action and decreased NO bioavailability lead to increased vascular smooth muscle cells (VSMCs) contraction.[2] Diminished vascular relaxation and increased VSMC contraction cause hypertension in PE.[2,3] Furthermore, PE is associated with sympathetic hyperactivity[4] leading to vasoconstriction. Neuropeptide Y (NPY) is a candidate peptide involved in sympathetic activation and induced vasoconstriction.[5]

NPY, a 36 amino acid peptide, is highly expressed centrally in the brain and peripherally in the adrenal medulla,[6] sympathetic nerves, non-neuronal endothelial cells,[7] and adipose tissue.[8] Evidences suggest that NPY is released as a part of a sympathetic nervous system response.[9,10] In pregnancy, NPY is also produced by cytotrophoblastic cells, amnion, chorion, and deciduas[11] and it is abundant in both plasma and amniotic fluid throughout pregnancy.[12] The complete form of NPY is $NPY_{1–36}$, which is rapidly cleaved into three main truncated forms with the following order of efficacy: $NPY_{3–36} \gg NPY_{3–35} > NPY_{2–36}$.[13] NPY acts through its specific receptors (NPY receptor 1 (Y1R), 2 (Y2R), 3 (Y3R), 4 (Y4R), 5 (Y5R), and 6 (Y6R)).[14,15] $NPY_{1–36}$ is cleaved by NPY-converting enzyme dipeptidyl peptidase IV to the truncated form, $NPY_{3–36}$, which loses affinity of Y1R and turns to Y2R and Y5R agonists.[7] Moreover, the Y3 receptor has never been cloned, therefore it most likely does not exist.[16] The Y4 receptor has the highest affinity to pancreatic polypeptide,[17] while the Y6 is not functional in humans.[18] Therefore, we focus on Y1, Y2, and Y5 receptors.

NPY is the most potent orexigenic peptide in the brain, and it increases body weight and adiposity by increasing appetite.[19] Other important roles of NPY include cardiovascular homeostasis,[9] the increase in blood pressure,[20] vasoconstriction of VSMCs, and abnormal myocardial growth.[21] Y1R agonist induces VSMCs proliferation,[22] stimulates vasoconstriction,[22–25] and increases mean arterial pressure (MAP).[26] Y2R agonist has been shown to promote angiogenesis[27,28] and induces vasodilation.[29] Y5R agonist alone or in conjunction with Y1R agonist is implicated in VSMCs proliferation,[22,30] while Y5R agonist alone or together with Y2R agonist is involved in angiogenesis via VEGF/NO-dependent pathways.[27] NPY might participate in the pathogenesis of hypertension in preeclamptic women[31] by inducing vasoconstriction, as well as by regulating the proliferation of endothelial cells and VSMCs through its corresponding receptors.[7]

To summarize, NPY is an interesting peptide which is synthesized from adipose and placental tissues and is associated with the regulation of blood pressure. To the best of our knowledge, comparisons of different NPY receptor expressions between normal pregnancy (NP) and preeclampsia (PE) have not been studied. In addition, there is no evidence revealing an association between blood NPY levels with *NPY* and NPY receptor expressions in the placenta of pregnant women especially in the preeclamptic condition. Since different NPY receptors mediate various vasoconstriction/vasodilation/vascularization effects, we determined expressions of various NPY receptor isoforms in the placenta. This study aimed to (1) compare placental gene expressions of *NPY*, *Y1R*, *Y2R*, and *Y5R* between NP and PE pregnancies to determine whether gene expression of different NPY receptors is altered in the PE condition; (2) compare maternal serum levels of NPY between NP and PE pregnancies; (3) determine correlations between placental gene expressions of *NPY*, *Y1R*, *Y2R*, and *Y5R* as well as serum NPY levels with maternal and neonatal clinical parameters; and (4) determine the highest expressed gene among *Y1R*, *Y2R*, and *Y5R* in the placental tissue in all, NP, and PE subjects.

Materials and methods

Subjects

The study protocol was approved by the Siriraj Institutional Review Board of the Faculty of Medicine Siriraj Hospital, Mahidol University (Si545/2015). Pregnant women who underwent routine antenatal care and were in labor at the Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, were recruited for this study. All patients signed informed-consent forms prior to the study. The inclusion criteria included a maternal age of at least 18 years, undergoing cesarean section, and singleton pregnancy. Subjects were allocated into two groups: the NP and PE groups. There were a total of 44 subjects (all subjects) which included 22 subjects in the NP group and 22 subjects in the PE group. The gestational age was between 37 and 42 weeks for the NP group and was at least 34 weeks for the PE group. PE was diagnosed by SBP $\geq$ 140 mmHg, DBP $\geq$ 90 mmHg, and proteinuria $\geq$ 300 mg/dL in a 24-h urine collection or 1+ dipstick of the qualitative proteinuria reading.[32] The exclusion criteria for both the NP and PE groups included subjects with human immunodeficiency virus infection, diabetes mellitus, metabolic syndrome, hypertension, polycystic ovarian syndrome, other endocrine disorders, a previous history of chronic diseases, smoking habits, malignancies, pre-term membrane rupture, drug use that might affect blood glucose and insulin levels, drug administration for pre-term delivery risk, fetal malformations, and fetal distress during delivery.

Blood and tissue collection

For all subjects, maternal blood samples were collected in the fasting state before caesarean surgery and were centrifuged at 3634*g*, aliquoted, and stored at −70°C until analysis.

The placental tissues were collected immediately after delivery and their wet weights were recorded. Placentas had been washed repeatedly with 0.9% normal saline

until all residual blood was removed. The placentas were subsequently dissected into small pieces, snap frozen in liquid nitrogen, and stored at −70°C.

Demographic details and anthropometric measurements

Maternal age, prepregnancy body weight, height, predelivery body weight, body mass index (BMI), and waist and hip circumferences were collected. In accordance with the WHO guidelines, the waist circumference was obtained by measuring at midpoint between the lower margin of the last palpable rib and the top of the iliac crest, whereas the hip circumference was obtained by measuring around the widest portion of the buttocks.[33] SBP and DBP were measured by an automated sphygmomanometer. Neonatal clinical data, including gestational age, baby weight, neonatal length, and neonatal head circumference were obtained from medical records.

Analysis of *NPY*, *Y1R*, *Y2R*, and *Y5R* mRNA expressions in the placental tissue

NPY, *Y1R*, *Y2R*, and *Y5R* mRNA expressions were quantified as described previously.[34,35] Briefly, total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) in accordance with the manufacturer's instructions; 1 μg of RNA was reverse transcribed to complementary DNA (cDNA) using the iScript cDNA Synthesis Kit (Bio-RAD, Hercules, CA). A real-time polymerase chain reaction (Real-time PCR) was carried out using the reagents and protocol contained in the VeriQuest SYBR Green qPCR Master Mix (Affymetrix, Santa Clara, CA). In this study, $2^{-\Delta CT}$ method was applied to quantify the relative gene expressions using *TOP1* as the reference gene because it is the most stably expressed gene in the placenta, with unchanged expression across normal and preeclamptic pregnancies.[36] Primer sequences of *TOP1* were designed by the authors using published nucleotide sequences from PubMed database and were blasted to confirm primer specificity (Table 1). Primer sequences of *NPY*, *Y1R*, *Y2R*, and *Y5R* and their product sizes were obtained from previously published papers (Table 1).[34,35,37] PCR amplification was performed under the following conditions: Taq DNA polymerase activation at 95°C for 10 min; 40 cycles of DNA denaturing at 95°C for 15 s; and 60 s of annealing at 57°C for *NPY*, *Y1R*, *Y2R*, and *Y5R*, and 58°C for *TOP1*, with a final extension at 72°C for 30 s. For every real-time PCR reaction, no template control was performed as a negative control, and human brain and fat tissues were used as positive controls. The real-time PCR product sizes were proven by gel electrophoresis (Bio-Rad, Hercules, CA, USA).

Analysis of serum NPY levels

Serum NPY levels were measured by the enzyme immunoassay kit (Phoenix Pharmaceuticals, Burlingame, California, USA) as the manufacturer's protocol. The range of NPY detection was 0–100 ng/mL; the minimum detectable concentration was 0.11 ng/mL. The intra-assay and inter-assay coefficients of variances were 7.229% and 5.009%, respectively.

Table 1. Oligonucleotide primer sequences used for real-time PCR.

Primers	Nucleotide sequence (5′→3′)	Product size (base pairs)
NPY[34]	Forward-CCAGGCAGAGATATGGAAAACGA Reverse-GGTCTTCAAGCCGAGTTCTGGG	102
Y1R[34]	Forward-ATCATGCTGCTCTCCATTGTGGT Reverse-GTTGAAGAAGAACTGCAAGTCTCTCT	222
Y2R[35]	Forward-GGCCTACTGCTCCATCATCTTG Reverse-CCCTGGGCATAGGGCACC	228
Y5R[37]	Forward-CTGATAGCTACTGTCTGGACACT Reverse-AGAGTTAAGTTGATCATCTCATTTTCTTC	302
TOP1	Forward-TCCAAGCATAGCAACAGTGAACA Reverse-AATAGCCATCATCTTCAGGTTCATC	238

Statistical analysis

The Kolmogorov–Smirnov test was performed to test the normality of the data. For normally distributed data, comparisons between NP and PE groups were performed by the unpaired *t*-test. Comparisons of non-normally distributed data were performed with a non-parametric test. Correlation coefficients were calculated using 2-tailed Pearson's product-moment correlation for normally distributed data or Spearman's Rank Correlation Coefficient for ranked or non-normally distributed data. Data were presented as mean (±S.E.M.). All statistical analyses were performed with PASW statistics 18.0 (SPSS Inc., Chicago, IL, USA). A *P*-value less than 0.05 was considered as statistical significance.

Results

Comparisons between the NP and PE groups

Comparisons between the NP and PE groups are shown in Table 2. SBP, DBP, and heart rate were significantly higher in the PE group than the NP group ($P < 0.01$ all; Table 2). The prepregnancy body weight ($P = 0.056$), predelivery body weight ($P = 0.073$), and predelivery BMI ($P = 0.062$) tended to be higher but neonatal length tended to be lower ($P = 0.058$) in the PE group than the NP group (Table 2).

Maternal age, height, prepregnancy BMI, gestational age, waist circumference, hip circumference, neonatal head circumference, placental weight, *NPY* gene expression, and serum NPY levels were comparable between the NP and PE groups (Table 2). Neonatal weight, gene expressions of *Y1R*, *Y2R*, and *Y5R* were significantly lower in the PE group than the NP group ($P < 0.05$ all; Table 2).

Comparisons between gene expressions in placental tissue in all, normal pregnancy, and PE subjects

Comparisons between NPY receptor gene expressions, including *Y1R*, *Y2R*, and *Y5R*, in placental tissue in all (Panel A), NP, and PE subjects (Panels B) are shown in Figure 1. Among these genes, *Y2R* showed the highest expression in the placenta, and was significantly higher

Table 2. Comparisons between normal pregnancy and preeclampsia.

Parameters	Normal pregnancy	Preeclampsia	*P*
Maternal age (year)	29.50 ± 1.21	29.29 ± 1.57	0.917
Maternal height (m)	1.55 ± 0.01	1.56 ± 0.02	0.658
Prepregnancy BW (kg)	54.43 ± 1.92	62.87 ± 3.80	0.056
Prepregnancy BMI (kg/m^2)	22.52 ± 0.68	24.37 ± 1.59	0.292
Gestational age (weeks)	38.29 ± 0.18	37.58 ± 0.42	0.127
Predelivery BW (kg)	70.13 ± 2.19	77.93 ± 3.64	0.073
Predelivery BMI (kg/m^2)	29.03 ± 0.76	31.65 ± 1.14	0.062
Waist circumference (cm)	99.18 ± 2.39	100.52 ± 2.30	0.686
Hip circumference (cm)	102.00 ± 1.77	104.84 ± 2.31	0.342
SBP (mmHg)	118.33 ± 1.15	153.04 ± 2.39	<0.001***
DBP (mmHg)	77.08 ± 1.27	96.96 ± 1.71	<0.001***
Heart rate (bpm)	84.87 ± 1.38	92.52 ± 2.73	0.005**
Neonatal weight (g)	2998.33 ± 73.25	2674.17 ± 123.07	0.029*
Neonatal head circumference (cm)	33.00 ± 0.24	32.83 ± 0.33	0.667
Neonatal length (cm)	49.71 ± 0.40	48.30 ± 0.59	0.058
Placental weight (g)	633.75 ± 21.08	573.13 ± 30.46	0.108
NPY gene expression	0.007 ± 0.002	0.007 ± 0.002	0.725
Y1R gene expression	1.936 ± 0.709	0.778± 0.316	0.030*
Y2R gene expression	4.515 ± 1.375	1.221 ± 0.440	0.015*
Y5R gene expression	2.774± 0.894	1.156 ± 0.405	0.022*
Serum NPY levels (ng/mL)	0.50 ± 0.02	0.50 ± 0.03	0.589

Note: Values are expressed as mean (±S.E.M.).
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared between groups.
BW: body weight; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

than *Y1R* in all ($P < 0.01$; Figure 1(a)), NP ($P < 0.01$; Figure 1(b)), and PE subjects ($P < 0.05$; Figure 1(b)). Additionally, *Y2R* tended to be higher than *Y5R* in all ($P = 0.059$; Figure 1(a)) and NP subjects ($P = 0.066$; Figure 1(b)). For the PE group, *Y2R* expression was comparable to *Y5R* expression, and both *Y2R* and *Y5R* expressions were significantly higher than *Y1R* expression ($P < 0.05$ all; Figure 1(b)). The NPY receptor expression ratio between the PE/NP groups was lowest for *Y2R* (0.27) compared to *Y1R* (0.42) and *Y5R* (0.40) (Figure 1(c)).

Correlations between placental gene expressions and maternal and neonatal clinical parameters

Correlations between placental gene expressions and maternal and neonatal clinical parameters in all, NP, and PE subjects in aspects of SBP and DBP are shown in Table 3, and in aspects of gene expressions, serum levels, and placental weight are presented in Table 4. SBP had positive correlations with prepregnancy body weight, prepregnancy BMI, and predelivery body weight, but had negative correlations with expressions of *Y1R*, and *Y5R* ($P < 0.05$ all) only in the NP group (Table 3). SBP had a positive correlation with predelivery BMI in all and NP subjects ($P < 0.05$ all; Table 3). Furthermore, SBP had negative correlations with gestational age, neonatal weight, neonatal length, and placental weight in all subjects ($P < 0.05$ all), but with placental *NPY* gene expression ($P < 0.05$) only in the PE group (Table 3).

DBP was positively correlated with prepregnancy BMI only in the NP group ($P < 0.05$; Table 3). DBP had positive correlations with predelivery body weight and predelivery BMI, but it was negatively correlated with neonatal weight, neonatal length, and *Y5R* expression in all subjects ($P < 0.01$ all; Table 3). Moreover, DBP was negatively correlated with placental weight and *Y2R* expression in all and PE subjects ($P < 0.05$ all; Table 3).

NPY expression was positively correlated with *Y1R*, *Y2R*, and *Y5R* expressions in all and PE subjects ($P < 0.01$ all).

Y1R expression was positively correlated with *Y2R* and *Y5R* expressions in all, NP, and PE subjects ($P < 0.001$ all). *Y2R* expression was positively correlated with *Y5R* expression in all, NP, and PE subjects ($P < 0.001$ all; Table 4).

Serum NPY levels had a negative correlation with neonatal head circumference in all subjects (Table 4).

For all and PE subjects, placental weight was positively correlated with gestational age and neonatal length ($P < 0.001$ all; Table 4). Placental weight had positive correlations with neonatal weight and neonatal head circumference in all, NP, and PE subjects ($P < 0.05$ all; Table 4).

Discussion

This study investigated the expressions of *NPY* and its corresponding receptors (*Y1R*, *Y2R*, and *Y5R*) mRNA in placental tissue, as well as maternal serum NPY levels in the NP and PE groups. This is the first study which examines *Y1R*, *Y2R*, and *Y5R* expressions in the placenta and reveals alterations of these receptor expressions in the PE condition suggestive of the involvement of NPY receptors in the pathophysiology of PE. Furthermore, the current study also determined correlations between placental expressions of *NPY*, *Y1R*, *Y2R*, and *Y5R* as well as serum NPY levels with maternal and neonatal clinical parameters.

Gene expressions of *Y1R*, *Y2R*, and *Y5R* were significantly lower in the PE compared to the NP group. In all and NP subjects, *Y2R* showed the highest expression in placental tissue, was significantly higher than *Y1R*, and tended to be

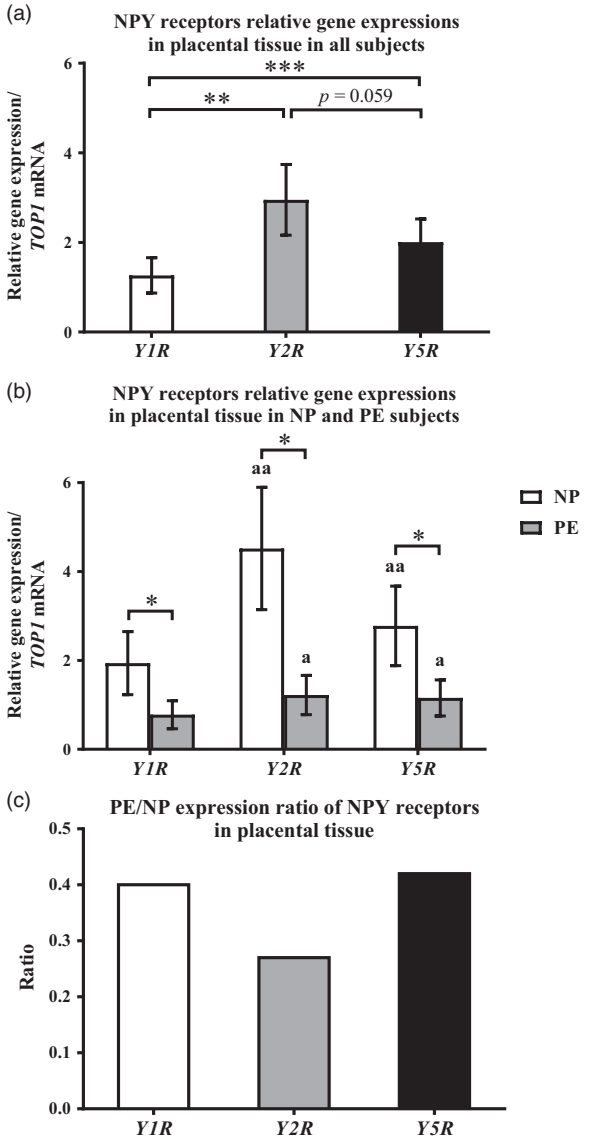

Figure 1. Comparisons between NPY receptor gene expressions including *Y1R*, *Y2R*, and *Y5R* in all (a), normal pregnancy (NP), and preeclampsia (PE) (b) subjects, and NPY receptor expression ratio between PE/NP subjects in the placenta (c) normalized to *TOP1* (reference gene). Data are presented as mean (±S.E.M.). $^{*}P < 0.05$, $^{**}P < 0.01$ compared between the NP and PE groups. $^{a}P < 0.05$, $^{aa}P < 0.01$ compared to *Y1R* in the same group.

higher than *Y5R*. In the PE group, placental *Y2R* expression was comparable to *Y5R* expressions and both *Y2R* and *Y5R* expressions were significantly higher than *Y1R* expression. This study found that *Y2R* was 2.33 times and 1.63 times higher than *Y1R* and *Y5R*, respectively, in NP subjects and 1.57 times higher than *Y1R* in PE subjects. In addition, in the PE group, there was no difference between mRNA levels of *Y2R* and *Y5R*. Furthermore, the NPY receptor expression ratio between the PE/NP groups showed that *Y2R*, which is the highest-expressed NPY receptor in the placenta and responsible for NPY-mediated angiogenesis[38] and vasodilation,[29] was the lowest among NPY receptors. These results indicate that *Y2R* expression was most decreased among these NPY receptors in subjects with PE. Y1R, either alone or together with Y5R, is responsible for vasoconstriction and atherosclerosis under stress conditions or ischemia.[38]

The role of NPY via Y2R is implicated in angiogenesis activating NO release either directly via endothelial NO synthase (eNOS), or indirectly via VEGF and fibroblast growth factor.[38] A decrease in *Y2R* expression probably leads to decreased angiogenesis and vasodilation property. Placental pathologies such as PE are described as the failure of placental angiogenesis, which is associated with dysregulation in angiogenic factors including VEGF and a diminished eNOS pathway.[2] NO plays important roles in mediating angiogenesis[39] and protection of human endothelial cells against apoptosis.[40] A previous study in mice found that eNOS deficiency led to decreased VEGF mRNA and protein expressions and reduced placental vascularization.[41] Thus, a reduction in *Y2R* mRNA in PE might be associated with decreased NO and may impair placental vascularization, which can contribute to PE.

Furthermore, a previous study in humans suggests that dysregulated levels of angiogenic factors at mid-pregnancy, including low circulating levels of pro-angiogenic VEGF and PGF, are associated with low placental weight at delivery.[42] Low placental weight was associated with an increased risk of multiple adverse perinatal outcomes.[42] In our study, the placental weight of the PE group tended to be lower than the NP group and tended to have a negative correlation with SBP and had a significant negative correlation with DBP. These results indicate that an imbalance of angiogenic factors is associated with abnormal placentation, reduced placental weight, and development of PE.

Y5R can induce either VSMCs proliferation or angiogenesis, depending on the NPY ligand.[38] Thus, a decrease in placental *Y5R* expression might lead to decreased VSMCs proliferation or angiogenesis. In this study, DBP had significant negative correlations with *Y2R* and *Y5R* expressions in all subjects, but had a significant negative correlation only with *Y2R* expression in PE subjects. These results suggest that a reduction in expression(s) of *Y2R* and/or *Y5R* in all subjects, and *Y2R* in PE subjects, might lead to elevated blood pressure. Thus, a decrease in placental *Y2R* mRNA expression might primarily contribute to the pathogenesis of PE. In the NP group, SBP had negative correlations with placental *Y1R* and *Y5R* expressions, which are receptors mediating VSMCs proliferation and a vasoconstrictive effect, indicating that an increase in blood pressure in normal pregnancy might be associated with a decrease in *Y1R* and *Y5R* expressions leading to a reduced vasoconstrictive effect of these receptors. However, these associations were not found in the PE group. Furthermore, a decrease in expression of *Y1R* in the PE group compared to the NP group, suggests that there might be a compensatory down-regulation of this receptor in order to prevent an adverse vasoconstrictive effect in PE.

This study found that placental *NPY* gene expression was comparable between NP and PE subjects. SBP was negatively correlated with *NPY* expression only in PE subjects suggesting that there was an inverse relationship between placental NPY expression and blood pressure only in the PE group. This result might be partly explained

Table 3. Correlations between placental gene expressions and maternal and neonatal clinical parameters in aspects of SBP and DBP in all, NP, and PE subjects.

	All subjects		NP		PE	
Factors	**R**	***P***	**R**	***P***	**R**	***P***
SBP						
- prepregnancy BW	0.261	0.079	0.490	0.015*	−0.154	0.495
- prepregnancy BMI	0.124	0.408	0.545	0.006**	−0.187	0.393
- predelivery BW	0.257	0.081	0.424	0.039*	−0.087	0.693
- predelivery BMI	0.297	0.042*	0.478	0.018*	−0.030	0.891
- gestational age	−0.290	0.048*	−0.075	0.729	−0.186	0.397
- neonatal weight	−0.396	0.006**	0.016	0.942	−0.327	0.127
- neonatal length	−0.412	0.004**	−0.163	0.446	−0.416	0.054
- placental weight	−0.325	0.026*	−0.011	0.959	−0.384	0.070
- *NPY* expression	−0.142	0.353	−0.248	0.243	−0.475	0.030*
- *Y1R* expression	−0.251	0.110	−0.499	0.021*	−0.356	0.113
- *Y2R* expression	−0.293	0.070	−0.342	0.140	−0.318	0.185
- *Y5R* expression	−0.282	0.074	−0.509	0.018*	−0.237	0.314
- serum NPY	0.113	0.450	0.014	0.946	0.352	0.100
DBP						
- prepregnancy BW	0.408	0.124	0.356	0.087	0.273	0.220
- prepregnancy BMI	0.254	0.085	0.445	0.029*	0.136	0.537
- predelivery BW	0.411	0.004**	0.302	0.152	0.358	0.094
- predelivery BMI	0.446	0.002**	0.404	0.050	0.373	0.080
- neonatal weight	−0.415	0.004**	−0.268	0.205	−0.279	0.197
- neonatal length	−0.390	0.007**	−0.356	0.088	−0.224	0.317
- neonatal HC	−0.237	0.113	−0.354	0.089	−0.321	0.145
- placental weight	−0.373	0.010**	−0.097	0.652	−0.464	0.026*
- *NPY* expression	−0.053	0.729	0.053	0.804	−0.275	0.227
- *Y1R* expression	−0.275	0.078	−0.416	0.060	−0.286	0.209
- *Y2R* expression	−0.383	0.016*	−0.348	0.133	−0.487	0.035*
- *Y5R* expression	−0.343	0.028*	−0.432	0.051	−0.316	0.175
- serum NPY	0.147	0.325	0.249	0.241	0.266	0.220

NP: normal pregnancy; PE: preeclampsia; SBP: systolic blood pressure; DBP: diastolic blood pressure; BW: body weight; BMI: body mass index; WC: waist circumference; HipC: hip circumference; HC: head circumference. *$P < 0.05$, **$P < 0.01$

Table 4. Correlations between placental gene expressions and maternal and neonatal clinical parameters in aspects of SBP and DBP in all, NP, and PE subjects.

	All subjects		NP		PE	
Factors	**R**	***P***	**R**	***P***	**R**	***P***
***NPY* expression**						
- *Y1R* expression	0.554	<0.001***	0.224	0.328	0.893	<0.001***
- *Y2R* expression	0.576	<0.001***	0.441	0.052	0.874	<0.001***
- *Y5R* expression	0.431	0.005**	0.275	0.227	0.748	<0.001***
***Y1R* expression**						
- *Y2R* expression	0.974	<0.001***	0.978	<0.001***	0.981	<0.001***
- *Y5R* expression	0.974	<0.001***	0.977	<0.001***	0.978	<0.001***
***Y2R* expression**						
- *Y5R* expression	0.963	<0.001***	0.972	<0.001***	0.952	<0.001***
Serum NPY						
- neonatal HC	−0.297	0.043*	−0.270	0.201	−0.313	0.146
Placental weight						
- gestational age	0.501	<0.001***	−0.003	0.989	0.625	<0.001***
- neonatal weight	0.826	<0.001***	0.711	<0.001***	0.861	<0.001***
- neonatal length	0.599	<0.001***	0.338	0.107	0.715	<0.001***
- neonatal HC	0.624	<0.001***	0.464	0.022*	0.724	<0.001***

NP: normal pregnancy; PE: preeclampsia; BW: body weight; BMI: body mass index; WC: waist circumference; HipC: hip circumference; HC: head circumference. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

by the evidence showing that high blood pressure in PE is associated with increased placental *angiotensin II* expression, which might lead to local inhibition of *NPY* expression.[43] The present result was inconsistent with a previous report showing that lower *NPY* placental expression was found in the preeclamptic pregnancy than in gestational age-matched controls.[44] The inconsistency of the result might be due to the different placental reference gene to normalize the expressions of genes of interest. *TOP1,* which is the most stably expressed gene, was used as the reference gene because it is unchanged across normal and preeclamptic pregnancies and was found to be the best reference gene for the TRIzol RNA isolation technique.[36] In the previous study, *β-actin* and *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) were used to normalize *NPY* mRNA expression. Both placental *β-actin* and *GADPH* expressions were found to be affected by hypoxia and gestational age.[45] Collectively, although no difference was detected in placental *NPY* mRNA expression, a decrease in downstream signaling of NPY via Y2R might lead to decreased angiogenesis and vasodilation property probably resulting in impaired placental vascularization, which can contribute to PE.

Serum NPY levels were comparable between the NP and PE groups, which had similar pattern to placental gene expression. Furthermore, we did not observe a correlation between blood pressure and serum NPY levels. As a result, NPY in serum might not primarily contribute to the pathophysiology of PE. Our result was consistent with a previous study revealing that concentrations of NPY were similar among healthy pregnant women and women with PE in plasma[31] and in platelet-poor plasma.[46] However, another study showed that higher NPY levels were found in preeclamptic subjects compared to controls which were observed only in blood fractions containing platelets (platelet rich plasma), suggestive of NPY accumulation in platelets.[46] This inconsistency might be because in the present study, NPY concentrations were determined in serum which contains lower amount of platelet-derived proteins, including NPY than in platelet-rich plasma which contains high platelet concentration in this fraction.

In this study, maternal anthropometric characteristics (maternal age, height, prepregnancy BMI, gestational age, waist circumference, and hip circumference) did not differ between the NP and PE groups, but prepregnancy body weight and predelivery body weight and BMI tended to be higher in the PE group compared to the NP group. Moreover, SBP had positive correlations with prepregnancy body weight, prepregnancy BMI, predelivery body weight, and predelivery BMI, while DBP had a positive correlation with prepregnancy BMI. These results suggest that increased body weight and BMI, especially before pregnancy, are associated with high blood pressure leading to an increased risk of PE as described by previous studies.[47–49]

Maternal SBP, DBP, and heart rate were significantly higher in the PE than the NP group. An increase in heart rate in the PE group might be due to an elevation of sympathetic activity.[4] There was no correlation between placental gene expressions of *NPY, Y1R*, *Y2R*, and *Y5R* with neonatal clinical parameters. For neonatal clinical parameters, neonatal weight was significantly lower and the neonatal length tended to be lower in the PE group than in the NP group. There were no statistically significant differences in gestational age or neonatal head circumference between the PE and NP groups indicative of preservation of fetal brain blood flow and development of fetal head growth.[50] Placental weight of the PE group had positive correlations with gestational age, neonatal weight, neonatal length, and neonatal head circumference. These results indicate that placenta plays a crucial role in fetal growth and inadequate placentation is assumed to be important for the fetal development.[51]

Conclusion

In conclusion, placental *NPY* expression and serum NPY levels were comparable between the NP and PE groups. Placental NPY receptor gene expressions including *Y1R*, *Y2R*, and *Y5R* were decreased in PE subjects compared to NP subjects. Among these receptors, *Y2R* expression was most decreased in PE subjects compared to other receptors indicating that a reduction in placental *Y2R* mRNA expression might be associated with abnormalities of placental angiogenesis which probably contributes to the pathophysiology of PE. The roles of NPY receptors mediating placental vascularization need to be further investigated. A reduction in *Y1R* and *Y5R* expressions in the PE group might be a compensatory mechanism to buffer hypertension in PE subjects. Further studies to investigate effects of blocking Y1R/Y5R or activating Y2R/Y5R in the placental tissue are required to confirm the involvement of these receptors in the pathophysiology of preeclampsia.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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