Association between the Presence of Autoantibodies against Adrenoreceptors and Severe Pre-Eclampsia: A Pilot Study

Guiling Ma1*, Yanfang Li2*, Juan Zhang1*, Hao Liu2, Dongyan Hou1, Lei Zhu2, Zhenyu Zhang2*, Lin Zhang1*

1 Heart Centre, Capital Medical University, Chao-Yang Hospital, Beijing, China, 2 Gynaecology and Obstetrics Department, Capital Medical University, Chao-Yang Hospital, Beijing, China

Abstract

**Background:** Pre-eclampsia is the leading cause of maternal and neonatal morbidity and mortality with incompletely understood etiopathogenesis. The purpose of the current study is to determine whether there is a relationship between the presence of autoantibodies against \( \beta_1, \beta_2 \), and \( \alpha_1 \) adrenoreceptors and severe pre-eclampsia.

**Methodology/Principal Findings:** Synthetic peptides corresponding to amino acid sequences of the second extracellular loops of \( \beta_1, \beta_2 \), and \( \alpha_1 \) adrenoreceptors were synthesized as antigens to test 34 patients with severe pre-eclampsia, 36 normal pregnancy women and 40 non-pregnant controls for the presence of autoantibodies using enzyme-linked immunosorbent assay. The respective frequencies of autoantibodies against \( \beta_1, \beta_2 \), and \( \alpha_1 \) adrenoreceptors were 50.0% (17/34), 52.9% (18/34) and 55.9% (19/34) in patients with severe pre-eclampsia, 19.4% (7/36) \((p=0.011)\), 19.4% (7/36) \((p=0.006)\), and 17.6% (6/36) \((p=0.001)\) in normal pregnancy women and 10% (4/40), 7.5% (3/40) and 10% (4/40) \((p=0.001)\) in non-pregnant controls. Titters of these autoantibodies were also significantly increased in patients with severe pre-eclampsia. By logistic regression analysis, the presence of these three autoantibodies significantly increased the risk of neonatal death (odds ratio, 13.5; 95% confidence interval, 1.3–141.3; \( p=0.030 \)) and long-term neonatal hospitalization (odds ratio, 5.0; 95% confidence interval, 1.3–19.1; \( p=0.018 \)). The risk of hypertension and fetal distress were also associated with the presence of these three autoantibodies.

**Conclusions/Significance:** This novel pilot study demonstrated for the first time that the presence of autoantibodies against \( \beta_1, \beta_2 \), and \( \alpha_1 \) adrenoreceptors are increased in patients with severe pre-eclampsia. Pregnant women who are positive for the three autoantibodies are at increased risks of neonatal mortality and morbidity. We posit that these autoantibodies may be involved in the pathogenesis of severe pre-eclampsia.

Citation: Ma G, Li Y, Zhang J, Liu H, Hou D, et al. (2013) Association between the Presence of Autoantibodies against Adrenoreceptors and Severe Pre-Eclampsia: A Pilot Study. PLoS ONE 8(3): e57983. doi:10.1371/journal.pone.0057983

Editor: T. Mark Doherty, Glaxo Smith Kline, Denmark

Received October 7, 2012; Accepted January 30, 2013; Published March 4, 2013

Copyright: © 2013 Ma et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was funded by the Natural Science Foundation of China (30971236, 81250011) and Beijing Natural Science Foundation project (7102058). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: cyzhangzhenyu@sina.com (ZZ); linzhangpeking@hotmail.com (L. Zhang)

† These authors contributed equally to this work.

Introduction

Pre-eclampsia is a serious hypertensive disorder during pregnancy that affects 3%-5% of pregnancies, and remains the leading cause of maternal and neonatal mortalities and morbidities in the world [1,2]. It is a multi-systemic disease with features such as hypertension and proteinuria [3]. In serious cases, termination of pregnancy is the only available option to prevent further health deterioration of the fetus and mother [4]. To date, the factors and mechanisms involved in the pathogenesis of pre-eclampsia remain poorly understood.

Studies have described the role of autoantibodies against \( \alpha_1 \) adrenoreceptor (anti-\( \alpha_1 \)-AR) in primary and malignant hypertension [5,6]. Previously, we demonstrated that the presence of autoantibodies against \( \beta_1, \beta_2, \) and \( \alpha_1 \) adrenoreceptors (anti-\( \beta_1, \beta_2, \) and \( \alpha_1 \)-ARs), which bind to the second extracellular loop of the receptors, are highly prevalent in hypertensive heart disease and may participate in its pathogenesis [7,8]. In recent years, evidence has accumulated that suggests that autoimmunity participates in the pathogenesis of pre-eclampsia. Recently, numerous studies have shown that pre-eclamptic women possess autoantibodies against angiotension II type 1 receptor, which bind to and activate the receptor, consequently provoking biological responses relevant to the pathogenesis of pre-eclampsia [9-13]. The aim of this study was to investigate differences in the frequencies of anti-\( \beta_1, \beta_2, \) and \( \alpha_1 \)-ARs among patients with severe pre-eclampsia, compared to normal pregnancy women and non-pregnant controls. The second aim was to investigate the relationship between the presence of anti-\( \beta_1, \beta_2, \) and \( \alpha_1 \)-ARs and perinatal mortality and morbidity.

We used synthetic peptides corresponding to amino acid sequences...
of the second extracellular loop of the human $\beta_1$, $\beta_2$, and $\alpha_1$ ARs, to test sera from patients with severe pre-eclampsia, normal pregnancy women, and non-pregnant controls.

**Results**

Study subjects were enrolled from May 2011 to November 2011. Clinical characteristic of women from three study groups are shown in Table 1.

**Maternal Clinical Characteristics**

Maternal hospital stay was significantly longer in the severe pre-eclampsia group than in the normal pregnancy group (3.9±1.1 days versus 4.5±0.5 days, p<0.001). Complications of pregnancy, such as placental abruption, placenta remnants and postpartum hemorrhage, were significantly higher in the severe pre-eclampsia group than in the normal pregnancy group (7/34 versus 0/36, p = 0.004).

**Perinatal Clinical Characteristics**

The proportion of fetuses in the severe pre-eclampsia group that suffered from fetal growth restriction (58.8% (20/34)) and fetal distress (29.4% (10/34)), respectively, was significantly higher than 2.5% (1/36) and 8.33% (3/36) in the normal pregnancy group (p both <0.05). Preterm birth and low birth weight were significantly increased in the severe pre-eclampsia group compared with the normal pregnancy group (64.7% versus 11.1%, 76.5% versus 5.6%, p<0.001, respectively). Perinatal death was also increased in the severe pre-eclampsia group (11.8% versus 0%, p = 0.021) (Table 2).

Birth weight in the severe pre-eclampsia group was significantly lower than in the normal pregnancy group (1807.3±137.7 g versus 3279.2±65.9 g, p<0.001). Similarly, placental weight was also reduced in the severe pre-eclampsia group (507.9±39.6 g versus 649.6±20.1 g, p = 0.002, Figure 1).

Neonatal Apgar score (includes assessment of the appearance of skin color, pulse, grimace, activity, and respiration) was used to classify or grade newborn infants [14]. The Apgar scores were shown in Table 1.

**ELISA Results**

Sera positive for anti-$\beta_1$-AR was found in 50.0% (17/34) of the severe pre-eclampsia group, 19.4% (7/36) (p = 0.011) of the normal pregnancy group, and 10.0% (4/40) (p<0.001) of non-pregnant controls. Sera positive for anti-$\beta_2$-AR was found in 52.9% (18/34) of the severe pre-eclampsia group, 19.4% (7/36) (p = 0.006) of the normal pregnancy group, and 7.5% (3/40) (p<0.001) of non-pregnant controls. Positive sera for anti-$\alpha_1$-AR was found in 55.9% (19/34) of the severe pre-eclampsia group, 16.7% (6/36) (p = 0.001) in the normal pregnancy group, and 10.0% (4/40) (p<0.001) of non-pregnant controls. Tiers of the anti-$\beta_1$, $\beta_2$, and $\alpha_1$-ARs were significantly higher in the severe pre-eclampsia group than in the other two groups (p<0.001), Figure 3.

Positive sera from the severe pre-eclampsia group contained different kinds of autoantibodies. Twelve patients were positive for a single autoantibody, four patients were positive for two kinds of autoantibody, and 11 patients were positive for all the three kinds of autoantibody. A significant correlation was found between anti-$\beta_1$-AR and anti-$\alpha_1$-AR (r = 0.4, p = 0.02), anti-$\beta_1$-AR and anti-$\alpha_2$-AR (r = 0.6, p<0.001). In the severe pre-eclampsia group, 76.5% (13/17) of the 17 women with anti-$\beta_1$-AR, were also positive for anti-$\alpha_1$-AR, and 82.4% (14/17) had anti-$\beta_2$-AR.

**The Association of Autoantibody and Clinical Outcomes**

Univariate logistic regression assessed the association of autoantibody with hypertension (≥160 mmHg systolic or ≥110 mmHg diastolic), pregnancy complications, fetal growth restriction, fetal distress, preterm birth, low birth weight, neonatal long-term hospitalization (more than two weeks), and perinatal death. The presence of one or two kinds of autoantibody against $\beta_1$, $\beta_2$, and $\alpha_1$ ARs had no statistical relationship with any complication. In contrast, the risks for both maternal and perinatal complications were closely associated with the presence of the three autoantibodies. Positivity for anti-$\beta_1$, $\beta_2$, and $\alpha_1$-ARs was associated with hypertension (OR, 6.5; 95% CI, 1.6–25.7; p = 0.001), pregnancy complication (OR, 4.1; 95% CI, 1.0–16.0; p = 0.008).

**Table 1. Clinical characteristic of women from three groups in the present study.**

|                      | Non-pregnant (n = 40) | Normal pregnancy (n = 36) | Severe pre-eclampsia (n = 34) |
|----------------------|----------------------|---------------------------|-------------------------------|
| Age (years)          | 30.4±3.0             | 29.6±3.3                  | 31.3±4.7                      |
| Gestational age      | NA                   | 38.4±1.7                  | 33.1±3.4*                     |
| Systolic blood pressure (mmHg) | 118.7±6.8           | 115.2±6.9                | 166.5±17.2*                   |
| Diastolic blood pressure (mmHg) | 74.7±6.3            | 73.0±5.7                 | 105.0±11.4*                   |
| Urinary protein (mg/24 h) | Nd                  | Nd*                      | 3062.7±2538.5                |

Mean ± SD are shown. Student’s unpaired two-tailed t-test was made between non-pregnant group versus normal pregnancy group and normal pregnancy group versus severe pre-eclampsia group, significant differences (p<0.001) are indicated by*. Nd: not determined; NA: not applicable. 

doi:10.1371/journal.pone.0057983.t001
p = 0.044), fetal growth restriction (OR, 3.7; 95%CI, 1.1–12.0; p = 0.032), fetal distress (OR, 6.0; 95% CI, 1.7–21.7; p = 0.006), preterm birth (OR, 4.5; 95%CI, 1.3–15.1; p = 0.016), perinatal death (OR, 13.5; 95% CI, 1.3–141.3; p = 0.030), and long-term neonatal hospitalization (OR, 5.0; 95% CI, 1.3–19.1; p = 0.018).

Discussion

Main Findings

In this study, we demonstrated for the first time that positivity for anti-β1, β2, and α1-ARs was associated with severe pre-eclampsia. The frequencies and titers of anti-β1, β2, and α1-ARs were significantly higher in women with severe pre-eclampsic, when compared to normal pregnancy women and non-pregnant healthy controls. The presence of the three autoantibodies was associated with both adverse maternal and perinatal clinical outcomes including hypertension, pregnancy complications, fetal growth restriction, fetal distress, preterm birth, low birth weight, perinatal death, and long-term hospitalization of neonates.

Immune Mechanisms in Pre-eclampsia

The pathogenesis of pre-eclampsia remains obscure, but is likely multifactorial involving abnormal placentation, reduced placental perfusion, endothelial cell dysfunction, and systemic vasospasm [15]. An immune mechanism has long been postulated in the pathogenesis of pre-eclampsia. Immune maladaptation may impair invasion of spiral arteries by endovascular cytotrophoblast cells [16]. Studies have suggested that repeated exposure to sperm from a particular male partner prior to pregnancy promotes immune tolerance and reduces the risk of defective trophoblast invasion [17]. Autoantibodies, such as anticardiolipin and anti-β2-glycoprotein-1 antibody, have been detected in pre-eclampsia patients [18]. From the first report that described the presence of autoantibody against angiotensin II type 1 receptor in pre-eclampsia patients [9], researchers have gained a greater understanding on the pathogenic mechanisms underlying pre-eclampsia which implicate the immune system.

Autoantibodies and Pre-eclampsia

While our results need to be confirmed by larger studies, there are biologically plausible mechanisms by which anti-β1, β2, and α1-ARs may lead to severe pre-eclampsia. The β1 and β2 adrenoreceptors in the human heart couple to the G protein Gs, which activates adenyl cyclase. Stimulation of both receptor subtypes increases the intracellular level of cAMP, which leads to phosphorylation of target proteins [19]. Autoantibodies, such as anticardiolipin and anti-β2-glycoprotein-1 antibody, have been detected in pre-eclampsia patients [18]. From the first report that described the presence of autoantibody against angiotensin II type 1 receptor in pre-eclampsia patients [9], researchers have gained a greater understanding on the pathogenic mechanisms underlying pre-eclampsia which implicate the immune system.

Table 2. Perinatal complications.

| Complications                  | Severe pre-eclampsia (%) | Normal pregnancy (%) | p value   |
|-------------------------------|--------------------------|----------------------|-----------|
| Fetal growth restriction      | 20(58.8)                 | 1(2.8)               | <0.001**  |
| Fetal distress                | 10(29.4)                 | 3(8.3)               | 0.032*    |
| Low birth weight              | 26(76.5)                 | 2(5.6)               | <0.001**  |
| <1500 g                       | 15(44.1)                 | 0                    |           |
| <1000 g                       | 4(11.8)                  | 0                    | 0.021*    |
| Preterm                       | 22(64.7)                 | 4(11.1)              | <0.001**  |
| <34 weeks                     | 19(55.9)                 | 0                    | <0.001**  |
| Neonatal asphyxia             | 7(17.7)                  | 0                    | 0.001*    |
| Mild                          | 5(14.7)                  | 0                    |           |
| Severe                        | 2(5.9)                   | 0                    | 0.150     |
| Perinatal death               | 4(11.8)                  | 0                    | 0.021*    |
| Intrauterine death            | 3(8.8)                   | 0                    | 0.056     |
| Neonatal death                | 1(2.9)                   | 0                    | 0.391     |

*p<0.05; **p<0.001.

doi:10.1371/journal.pone.0057983.t002

Figure 1. Birth weight and placenta weight. The birth weight of neonates in severe pre-eclamptic women was significantly lower than that in normal pregnancy women (1807.3±137.7 g versus 3279.2±65.9 g, p=0.001). Placenta weight was significantly reduced in severe pre-eclamptic women (507.9±39.6 g versus 649.6±20.1 g, p=0.002).

doi:10.1371/journal.pone.0057983.g001
that severe pre-eclampsia triggers the production of anti-$\beta_1$, $\beta_2$, and $\alpha_1$-ARs. Further studies are needed to delineate these two possible pathways.

Studies have shown that the risk of long-term sequelae such as chronic hypertension, ischemic heart disease, stroke, and venous thromboembolism are significantly increased in pre-eclamptic women [21,22]. In this study, we were able to collect blood samples from six of the 34 patients with severe pre-eclampsia at the end of puerperium without scheduled follow-up. Three of the six samples were positive for anti-$\beta_1$, $\beta_2$, and $\alpha_1$-ARs at similar titers to levels at recruitment. This is but distinct from what has been observed for autoantibodies against angiotension II type 1 receptor which have been shown to subside after pregnancy termination [9]. We infer that the presence of autoantibody might

**Figure 2. Apgar scores of neonates.** The Apgar scores were significantly lower in severe pre-eclamptic women than in normal pregnancy women. The scores were: at one minute (7.7±0.5 versus 9.5±0.1, p<0.0001), five minutes (8.4±0.5 versus 9.9±0.06, p<0.0001) and ten minutes (8.4±0.6 versus 10.0±0.03, p<0.0001). doi:10.1371/journal.pone.0057983.g002

**Figure 3. Frequencies and titers of autoantibodies among the three groups.** Frequencies and geometric mean titers of anti-$\beta_1$, $\beta_2$, and $\alpha_1$-ARs were significantly higher in the severe pre-eclamptic women than in the normal pregnancy women and non-pregnant controls. #: p<0.05 severe pre-eclamptic women compared to normal pregnancy women; *: p<0.001 severe pre-eclamptic women compared to non-pregnant controls. doi:10.1371/journal.pone.0057983.g003
be related to high risk for cardiovascular sequelae, but this hypothesis remains to be further explored.

Three kinds of autoantibodies closely related to each other were detected in the severe pre-eclampsia group. Approximately 32.4% (11/34) of severe pre-eclamptic women had all three kinds of autoantibodies. This indicates that the autoimmune response in severe pre-eclamptic patients is multi-faceted.

Conclusion
This pilot study has demonstrated for the first time that the presence of anti-β1, β2, and α1-ARs were prevalent in a cohort of severe pre-eclamptic women. Risks of both maternal and perinatal complications were significantly increased when all the three kinds of autoantibody studied were present. We posit that autoantibodies against adrenoreceptors may be involved in the pathogenesis of severe pre-eclampsia. Further studies are needed to confirm these findings and dissect the underlying mechanisms for this novel observation.

Materials and Methods

Ethics Statement
The research protocol was conducted in accordance with the guidelines of the World Medical Association’s Declaration of Helsinki and was performed following approval from the Medical Ethics Committee (12-S-63) of Capital Medical University Beijing Chao-Yang Hospital. All pregnant women were included in the study during the prepartum or early intrapartum period and provided written informed consent before inclusion in the study.

Subjects
Patients who were admitted to Beijing Chao-Yang Hospital were managed by the obstetrics faculty of the Capital Medical University. Thirty-four patients were diagnosed with severe pre-eclampsia based on the criteria set by the National High Blood Pressure Education Program Working Group report [23]. The criteria include increased blood pressure (≥160 mmHg systolic or ≥110 mmHg diastolic on two occasions at least 6 hours apart) after 20 weeks of gestation in women with previously normal blood pressure or proteinuria of ≥2 g/24 h. Patients with severe pre-eclampsia and normal pregnancy women were approached for the study during the antepartum or early intrapartum period. We selected two comparison groups: 36 apparently healthy pregnant women (pregnancy group) and 40 healthy non-pregnant women (non-pregnant group). Exclusion criteria for all groups were diabetes mellitus, vasculitis, renal disease and autoimmune disease. Blood samples were collected from antecubital vein at recruitment using tubes containing EDTA, and centrifuged at 2000xg for 10 minutes at 4°C within 2 h of the collection. Serum samples were stored at −70°C. We were able to collect blood samples from six patients with severe pre-eclampsia at the end of puerperium. Placentas were collected from study subjects and weighed.

Table 3. Amino acid sequences of human β1, β2, and α1-adrenoreceptors.

| Adrenoreceptor | Position | Sequence |
|---------------|----------|----------|
| β1            | 197-222  | H-W-W-R-A-E-S-D-E-A-R-R-C-Y-N-D-P-K-C-C-D-F-V-T-N-R |
| β2            | 172-197  | H-W-Y-R-A-T-H-Q-E-A-I-N-C-Y-A-N-E-T-C-C-D-F-F-T-N-Q |
| α1            | 192-218  | G-W-K-E-P-V-P-P-D-E-R-F-C-G-I-T-E-E-A-G-Y-A-V-F-S-S-V |

We collected clinical data from mother and infants/neonates. In this study, low birth weight was defined as body weight less than 2500 g at birth. Gestational age less than 37 weeks was considered as preterm birth. Fetal growth restriction was defined as the failure to reach the predetermined growth potential. This was operationally defined as sonographic estimated fetal weight below the 10th percentile for their gestational age. A fetal heart rate ≥160 bpm or ≤110 bpm, evaluated by electronic fetal monitoring, or the third degree of meconium-stained amniotic fluid was considered evidence of fetal distress.

Materials
Three peptides corresponding to the amino acid sequence of the second extracellular loop of human β1, β2, and α1 ARs were synthesized by Genomed (Genomed Synthesis, Inc., CA, USA) and the sequences are shown in Table 3 [24–26]. The purity of the peptides, determined by high performance liquid chromatography (HPLC) using a Vydac C-18 column, was above 95% as shown in Figure S1. The molecular weight of peptides was analyzed by mass spectrometry as shown in Figure S2. Nunc microtiter plates were purchased from Kastrup, Denmark. Tween-20, thimerosal, and ABTS were obtained from Sigma, St. Louis, MO, USA. Fetal bovine serum, biotinylated goat anti-human IgG (H+L), and horseradish peroxidase-streptavidin were bought from Zhongshan Golden Bridge Biotech, Beijing, China. The microplate reader was purchased from Molecular Devices Corp, Menlo Park, CA.

ELISA Protocol
Samples were classified as positive or negative based upon the presence or absence of anti-β1-AR, anti-β2-AR, and anti-α1-AR. An ELISA protocol, previously described by Fu et al [27], was used to screen for the presence of the autoantibodies. Briefly, 50 μL of peptide (5 mg/L) in 100 mmol Na2CO3 solution (pH = 11) was coated on microtiter plates overnight at 4°C. The wells were saturated with PAT (1 × PBS, 1 mL/L Tween-20, and 0.1 g/L thimerosal (PBS-T)) supplemented with 100 mL/L fetal bovine serum for 1 hour at 37°C. Then 50 μL of serum diluted from 1:20 to 1:160, positive control and negative control were added to the wells for 1 hour at 37°C. After washing the wells with PBS-T for three times, affinity-purified biotinylated goat anti-human IgG (H+L) (1:500 dilution in PBS-T) was added for 1 hour at 37°C. Following another round of washing three times, the bound biotinylated antibody was detected by incubating the microtiter plate for 1 hour at 37°C with horseradish peroxidase-streptavidin (1:500 dilution in PAT). This was followed by three times washing in PBS, the addition of 2.5 mmol/L H2O2 and then of 2 mmol/L ABTS in citrate buffer solution. Absorbance (A) was measured after 30 minutes at 405 nm in a microplate reader.

Data Analysis
Quantitative data were expressed as the mean ± SD. Positivity was defined as the ratio of [sample A - blank A]/negative control A - blank A] ≥2.1. Antibody titer was reported as geometric mean.
Data were analyzed using SPSS 16.0. (SPSS, Chicago, Illinois, USA) Fisher’s exact test and unpaired t tests were used to determine the significance in differences between groups. The correlation with autoantibodies was tested using the Spearman correlation coefficient. Association between the presence of autoantibodies and categorical outcomes was assessed using univariable logistic regression analysis. Tests with p<0.05 were considered statistical significant.

Supporting Information

**Figure S1** Purity of the peptides. The purity of the synthesized peptides corresponding to the amino acid sequence of the second extracellular loop of human β1, β2, and α1 adrenoreceptors, determined by HPLC, was 96.66%, 96.21% and 95.34%.

**References**

1. Roberts JM, Pearson G, Cutler F, Lindheimer M (2003) Summary of the NHLBI Working Group on Research on Hypertension During Pregnancy. Hypertension 41(3): 437–445.
2. Hutcheon JA, Lisonkova S, Joseph KS (2011) Epidemiology of preeclampsia and the other hypertensive disorders of pregnancy. Best Pract Res Clin Obstet Gynaecol 25(4): 391–403.
3. Redman CW, Sargent IL (2005) Latest advances in understanding preeclampsia. Science 308(5726): 1592–1594.
4. Roberts JM, Lain KY (1998) Obstetrics. Preterm birth and preeclampsia bad news and good news. Lancet 352: SIV22.
5. Fu MLX, Herlitz H, Wallukat G, Hilme E, Hedner T, et al. (1994) Functional autoimmune epitope on α1-adrenergic receptors in patients with malignant hypertension. Lancet 343(8903): 1660–1663.
6. Luther HP, Homuth V, Wallukat G (1997) α1-Adrenergic receptor antibodies in patients with primary hypertension. Hypertension 29(2): 678–682.
7. Lin Z, Yuan Z, Zhenyin T, Yani L, Rutai H, et al. (1998) Study of Auto-antibodies Against G-protein Coupled β1 and M3 Receptors in Patients with Hypertensive Heart Diseases. Chin J Hypertens 6(1): 5–8.
8. Lei Z, Shuyan W, Guobin M, Xiulan L, Lin Z (2003) The correlation of autoantibodies against G-protein-coupled β2 and α1-adrenergic and angiotensin II receptors in patients with primary hypertension. Chin J Clin Rehabil 7(15): 2160–2161.
9. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, et al. (1994) Patients with preeclampsia develop antigenic autoantibodies against the angiotensin AT1 receptor. J Clin Invest 103(7): 945–952.
10. Dechend R, Homuth V, Wallukat G, Kreuzer J, Park JK, et al. (2000) AT1 receptor agonistic antibodies from preeclamptic patients cause vascular cells to express tissue factor. Circulation 101(20): 2302–2307.
11. Dechend R, Viedt C, Muller DN, Ugle B, Brandes RP, et al. (2003) AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. Circulation 107(12): 1632–1639.
12. Bobst SM, Day MC, Gilstrap LC, Xia Y, Kellens RE (2005) Maternal autoantibodies from preeclamptic patients activate angiotensin receptors on human mesangial cells and induce interleukin-6 and plasminogen activator inhibitor-1 secretion. Am J Hypertens 18(3): 330–336.
13. Zhou CC, Ahmad S, Mi T, Abbasi S, Xia L, et al. (2006) Autoantibody from women with preeclampsia induces soluble fms-like tyrosine kinase-1 production via angiotensin type I receptor and calcineurin/nuclear factor of activated t-cells signaling. Hypertension 51(4): 1010–1019.
14. Appgar Virginia (1955) A proposal for a new method of evaluation of the newborn infant. Curr Res Anesth Analg 32(4): 260–267.
15. Detil J (2000) The pathogenesis of preeclampsia: new aspects. J perinat Med 28(6): 464–471.
16. Redman CW, Sargent IL (2003) Preeclampsia, the placenta and the maternal systemic inflammatory response—a review. Placenta 24(Suppl.A): S81–S82.
17. Dekker GA, Sibai BM (1999) The immunology of preeclampsia. Semin perinatol 23(1): 24–33.
18. Lee RM, Brown MA, Branch DW, Ward K, Silver RM (2003) Anticardiolipin and anti-beta2-glycoprotein-I antibodies in preeclampsia. Obstet Gynecol 102(2): 294–300.
19. Walsh DA, Van Patten SM (1994) Multiple pathway signal transduction by the cAMP-dependent protein kinase. FASEB 8(13): 1227–1236.
20. Graham RM, Perez DM, Hwa J, Piascik MT (1996) α1-Adrenergic receptor subtypes. Molecular structure, function, and signaling. Circ Res 78(5): 737–749.
21. Bellamy L, Canas JP, Hingorani AD, Williams DJ (2007) Preeclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. BMJ 335(7527): 974–985.
22. McDonald SD, Malinowski A, Zhou Q, Yusuf S, Devereaux PJ (2008) Cardiovascular sequelae of preeclampsia/eclampsia: a systematic review and meta-analyses. Am Heart J 156(3): 918–930.
23. (2000) Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 183(1): 1–22.
24. Frielie T, Collins S, Daniel KW, Carson MG, LeKowsit RJ, et al. (1987) Cloning of the cDNA for the human β1-adrenergic receptor. Proc Natl Acad Sci USA 84(2): 7920–7924.
25. Kobikka BK, Dixon RA, Frielie T, Dohman HG, Bolanowski MA, et al. (1987) cDNA for the human β2-adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. Proc Natl Acad Sci USA 84(1): 46–50.
26. Lomasney JW, Coteccion S, Lorenz W, Leung WY, Schwinn DA, et al. (1991) Molecular cloning and expression of the cDNA for the α1A-adrenergic receptor. J Biol Chem 266(6): 6362–6369.
27. Fu ML, Hooebeke J, Matsui S, Matoba M, Magnsson Y, et al. (1994) Autoantibodies against cardiac G-protein-coupled receptors define different populations with cardiomypathies but not with hypertension. Clin Immunol Immunopathol 72(1): 15–20.

**Figure S2** Molecular weight of peptides. Molecular weight of peptides corresponding to the amino acid sequence of the second extracellular loop of human β1, β2, and α1 adrenoreceptors was analyzed by mass spectrometry and the molecular weight was 3484.9, 3237.5 and 2872.2.

**Author Contributions**

Conceived and designed the experiments: L. Zhang. Performed the experiments: GLM YFL JZ HL DYH. Analyzed the data: GLM ZYZ L. Zhang L. Zhu. Contributed reagents/materials/analysis tools: GLM DYH ZYZ. Wrote the paper: GLM L. Zhang.