The expression of ATP-sensitive potassium channels in human umbilical arteries with severe pre-eclampsia

Benlan Yin¹,³, Yujiao Zhang¹,³, Xiaohong Wei¹, Chunrong Pang¹, Ting Hou², Chao Yang¹, Yuzhi Ning¹ & Xiaodong Fu¹

The aim of this study is to establish the expression of ATP-sensitive potassium channels (KATP) in human umbilical arteries with severe pre-eclampsia. Real-time quantitative PCR and western blotting were used to detect the mRNA and protein expression levels of KATP channel subunits Kir6.1 and SUR2B in human umbilical arteries from normal pregnant and those with severe pre-eclampsia, early onset severe pre-eclampsia and late onset severe pre-eclampsia. The mRNA and protein levels of SUR2B in the severe pre-eclampsia group were lower than those in the normal group (P < 0.001), and the expression of Kir6.1 was not statistically significant between the two groups (P > 0.05). The mRNA and protein levels of SUR2B in early onset severe pre-eclampsia group were lower than those in late onset severe pre-eclampsia group (P < 0.001). There was no significant difference in expression of Kir6.1 between the two groups (P > 0.05). The mRNA and protein expression levels of SUR2B in pregnant women with severe pre-eclampsia were lower than those in normal pregnant women, suggesting that the expression of the SUR2B of the KATP channel may be related to the occurrence and development of severe pre-eclampsia. Compared with late onset severe pre-eclampsia, the mRNA and protein expression levels of SUR2B were lower in the umbilical arteries of women with early onset severe pre-eclampsia, suggesting that the occurrence time of severe pre-eclampsia may be related to the extent reduced expression of the SUR2B of the KATP channel.

Abbreviations

KATP  ATP-sensitive potassium channel
SP   Severe pre-eclampsia
Kir  Inwardly rectifying potassium channel
SUR  Sulphonylurea receptor
QPCR  Real-time fluorescent quantitative PCR experiment
NP   Normal pregnant
LSP  Late onset severe pre-eclampsia
ESP  Early onset severe pre-eclampsia
cDNA Complementary DNA
SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis
GAPDH Glyceraldehyde phosphate dehydrogenase
PBST Phosphate buffered saline with Tween 20
ET-1  Endothelin-1
NO   Nitric oxide
BKca Large-conductance calcium-activated potassium channels

Severe pre-eclampsia (SP) is mainly characterized by hypertension, proteinuria and edema, including early onset (before 34 weeks of gestation) and late onset (after 34 weeks of gestation). The basic pathological changes

¹Department of Obstetrics, The Affiliated Hospital of Southwest Medical University, No.8 Kangcheng Road, Luzhou 646000, Sichuan Province, China. ²Department of Obstetrics, Guizhou Provincial People’s Hospital, No.83 Zhongshan East Road, Guiyang 550000, Guizhou Province, China. ³These authors contributed equally: Benlan Yin and Yujiao Zhang ⁴email: dongerfu@163.com
are small vasospasm, ischemia and endothelial cell damage, and various organ dysfunctions may occur, which may be accompanied by fetal growth restriction, resulting in increased maternal and perinatal mortality. Therefore, it is necessary to strengthen the etiology of the disease and explore its mechanism of development to raise awareness of SP.

The ATP-sensitive potassium channel (KATP) is composed of the structural subunit inward rectifier potassium channel (Kir) and the regulating subunit sulfonylurea receptor (SUR), which are composed of Kir6.1, Kir6.2, SUR1, SUR2A, SUR2B1–3. It is widely expressed in a variety of excitable cells, and the subunits expressed in different tissues are not identical. Kir6.1 and SUR2B are more widely distributed in vascular smooth muscle cells4. KATP can control membrane potential, couple cell metabolism and activity, regulate smooth muscle cell proliferation and apoptosis, modulate vascular remodeling and play an important role in controlling function of vascular smooth muscle5,6.

Previous ion channel studies of the chorionic plate arteries suggested a correlation between severe pre-eclampsia and ion channel gene expression7. As a major potassium channel, the role of KATP in the development of severe pre-eclampsia requires further study. The umbilical artery is an important hub connecting the fetus and the mother. Pregnant women with severe pre-eclampsia often experience intrauterine growth restriction or intrauterine distress. Therefore, this experiment aims to detect and compare the mRNA and protein expression levels of the KATP channel subunits Kir6.1 and SUR2B in normal and severe pre-eclampsia umbilical arteries by real-time quantitative PCR (QPCR) and western blotting. On this basis, the mRNA and protein expression levels of KATP subunits in human umbilical arteries in late onset severe pre-eclampsia and early onset severe pre-eclampsia were detected to explore the correlation between changes in this channel and severe pre-eclampsia. From the molecular level to the mechanism of severe pre-eclampsia, the results of this study may provide a basis for the further study of severe pre-eclampsia at the genetic level.

Results

Basic situation of the research objects. All pregnant women had a clear gestational age. There was no significant difference in age between the 40 normal pregnant women and 40 severe pre-eclampsia pregnant women (P > 0.05). There was no significant difference in gestational age (P > 0.05), but the blood pressure difference was statistically significant (P < 0.001). See Table 1 for details. There was no significant difference in age between the 20 pregnant women with late onset severe pre-eclampsia and the 20 pregnant women with early onset severe pre-eclampsia (P > 0.05). There was no significant difference in blood pressure between the two groups (P > 0.05). The difference in gestational age was statistically significant (P < 0.001), see Table 2.

Comparative analysis of the normal group and the severe pre-eclampsia group. The QPCR results of Kir6.1 and SUR2B subunits in umbilical arteries from the normal group and the severe pre-eclampsia group are shown in Table 3 and Fig. 1A. There was no significant difference in the expression of Kir6.1 subunit between the two groups (P = 0.234). The expression of SUR2B subunit was significantly different (P < 0.001). The

| Parameter  | NP group | SP group | P-value |
|------------|----------|----------|---------|
| Age        | 28.03 ± 3.98 | 29.08 ± 5.29 | 0.319   |
| Gestational age | 38.98 ± 0.96 | 38.50 ± 1.20 | 0.053   |
| Blood pressure | 117.50 ± 7.13/74.65 ± 6.54 | 169.88 ± 10.41/108.70 ± 10.74 | P < 0.001/P < 0.001 |

Table 1. The basic information of normal pregnancy patients and severe pre-eclampsia patients.

| Parameter  | LSP group | ESP group | P-value |
|------------|-----------|-----------|---------|
| Age        | 29.40 ± 6.43 | 28.15 ± 4.99 | 0.496   |
| Gestational age | 38.06 ± 1.64 | 32.20 ± 1.32 | P < 0.001 |
| Blood pressure | 167.05 ± 11.10/106.70 ± 10.04 | 175.85 ± 17.08/111.20 ± 13.82 | 0.061/0.246 |

Table 2. The basic information of late onset severe pre-eclampsia patients and early onset severe pre-eclampsia patients.

| Parameter  | n | NP group ΔCt | SP group ΔCt | P-value |
|------------|---|--------------|--------------|---------|
| Kir6.1     | 40 | 1.4297 ± 0.5665 | 1.6601 ± 0.7089 | 0.234   |
| SUR2B      | 40 | 1.6435 ± 0.6663 | 1.4066 ± 0.5452 | P < 0.001 |

Table 3. The mRNA expression levels of Kir6.1 and SUR2B in normal patients and severe pre-eclampsia patients.
relative expression value was calculated to be $0.5013 \pm 0.1809$, suggesting that SUR2B subunit mRNA expression is lower in umbilical arteries of the severe pre-eclampsia group than in the normal group.

Densitometry analysis of the western blot results of the Kir6.1 and SUR2B subunits in the two groups is shown in Table 4 and Figs. 1B and 2. The Kir6.1/GAPDH values were not significantly different between the two groups ($P=0.367$); however, the SUR2B/GAPDH values were extremely different ($P<0.001$). The SUR2B subunit protein level was lower in umbilical arteries of the severe pre-eclampsia group than the normal group.

### Comparative analysis of umbilical arteries in patients with late onset severe pre-eclampsia and early onset severe pre-eclampsia

The QPCR results of the LSP group and the ESP group are shown in Table 5 and Fig. 3A. There was no significant difference in the ΔCt values of the Kir6.1 subunit between the LSP and ESP groups ($P=0.333$). There was a statistically significant difference in the ΔCt values of the SUR2B subunit between two groups ($P<0.001$). The relative expression value was calculated to be $0.1176 \pm 0.0040$, suggesting that the SUR2B subunit mRNA level was lower in umbilical arteries of the ESP group than in LSP group.

Densitometry analysis of the western blot results of the LSP group and the ESP group are shown in Table 6 and Figs. 3B and 4. There was no significant difference in Kir6.1/GAPDH values between the two groups ($P=0.159$). The SUR2B/GAPDH values were significantly different between the two groups ($P<0.001$). The expression level of SUR2B subunit protein was lower in umbilical arteries of the ESP group than in LSP group.
Discussion

First, regarding the role of KATP in vascular-related diseases, studies have shown that the opening of KATP is an endogenous protective mechanism against ischemia, hypoxia, cardiac hypertrophy, etc. Moreover, KATP on the cell membrane and KATP on the mitochondrial membrane can protect against myocardial ischemia–reperfusion injury through different mechanisms. In response to this finding, a large number of studies have been conducted on KATP channel function in recent years, and it has been shown that KATP can play a cardioprotective role.

### Table 5. The mRNA expression levels of Kir6.1 and SUR2B in late onset severe pre-eclampsia patients and early onset severe pre-eclampsia patients.

| Parameter | n  | LSP group ΔCt             | ESP group ΔCt          | P-value |
|-----------|----|---------------------------|------------------------|---------|
| Kir6.1    | 20 | 1.5402 ± 0.5072           | 1.6921 ± 0.4710        | 0.333   |
| SUR2B     | 20 | 2.5685 ± 0.5705           | 5.6608 ± 0.5244        | P < 0.001 |

![Figure 3](image-url)  
**Figure 3.** Bar graph of the mRNA and protein expression levels of Kir6.1 and SUR2B in late onset severe pre-eclampsia patients and early onset severe pre-eclampsia patients.

### Table 6. The protein expression levels of Kir6.1 and SUR2B in late onset severe pre-eclampsia patients and early onset severe pre-eclampsia patients.

| Parameter | n  | LSP group ΔCt             | ESP group ΔCt          | P-value |
|-----------|----|---------------------------|------------------------|---------|
| Kir6.1    | 20 | 0.6436 ± 0.0799           | 0.6874 ± 0.1106        | 0.159   |
| SUR2B     | 20 | 0.4544 ± 0.0997           | 0.2047 ± 0.0344        | P < 0.001 |

![Figure 4](image-url)  
**Figure 4.** Imaging of the protein expression levels of Kir6.1 and SUR2B in late onset severe pre-eclampsia patients and early onset severe pre-eclampsia patients.
in many species, which are widely used to study myocardial ischemia and cardiac hypertrophy. Another study demonstrated that compared with rats with normal blood pressure, the expression levels of Kir6.1 subunit and SUR2B subunit in the aortic vascular smooth muscle cells of hypertensive rats were reduced. In the clinical treatment of hypertension, the main mechanism of action of K\textsuperscript{ATP} is to promote cell membrane superclosure to prevent L-type Ca\textsuperscript{2+} influx, inhibit the storage of Ca\textsuperscript{2+} in cells, reduce the amount of intracellular Ca\textsuperscript{2+}, relax the smooth muscle, and thus protect against hypertension-induced injury. When hypoxia pulmonary hypertension occurs, pulmonary vascular endothelial cells synthesize and secrete vasoactive substances such as endothelin-1 (ET-1) and nitric oxide (NO), which act on their corresponding receptors to induce intracellular signal transduction acting on the K\textsuperscript{ATP} channel of pulmonary artery smooth muscle cell membrane. This causes contraction or relaxation of pulmonary artery smooth muscle cells. There was no significant difference in the mRNA expression of Kir6.1 subunit between the pulmonary hypertension model group and the normal group. It is believed that the occurrence of pulmonary hypertension may be associated with inhibition of K\textsuperscript{ATP} channels and reduction of SUR2B subunits.

Second, regarding the expression and significance of potassium channels in severe pre-eclampsia, QPCR and western blotting experiment were used to confirm that down-regulated Kv7 channel function in tension regulation of chorionic plate arteries in women with pre-eclampsia could be associated with considerably altered expression profiles of Kv7 subunits. Studies have shown that the regulation of K⁺ channels is an important mechanism in the adaptation of uterine vascular tone to pregnancy. Our previous experiments showed that, compared to normal pregnant women, there was no significant change in the expression of large-conductance calcium-activated potassium channel (BKCa) a subunit mRNA or protein in the uterine arterioles and mesenteric arteries of patients with severe pre-eclampsia as determined by QPCR and western blotting, respectively. The mRNA and protein expression levels of the β1 subunit were significantly decreased, further suggesting that functional changes in the BKCa channel may be involved in the pathology of severe pre-eclampsia. In addition, the protein expression of the Kir6.1 subunit was lower in early onset severe pre-eclampsia. These data suggest that functional loss of K\textsuperscript{ATP} channel may involve in the development of severe pre-eclampsia. Therefore, we hypothesize that early versus late occurrence of severe pre-eclampsia may be related to the degree of K\textsuperscript{ATP} channel SUR2B subunit loss.

Conclusions
The decrease in K\textsuperscript{ATP} channel SUR2B subunit expression may be related to the occurrence and development of severe pre-eclampsia; however, this study did not address we its causal relationship. One possibility is that the reduction of SUR2B causes the potassium channel to be inhibited, which leads to vasoconstriction occurs, ultimately resulting in severe pre-eclampsia. Alternately, the occurrence of severe pre-eclampsia leads to changes in the SUR2B subunit of the K\textsuperscript{ATP} channel, or the interaction between SUR2B expression and pre-eclampsia causes persistent damage, ultimately leading to a reduction in SUR2B subunit expression and the development of severe pre-eclampsia. Such hypotheses need to be further studied at the genetic level.

K\textsuperscript{ATP} channel agonists such as ipatikalim, pinacidil and nicorandil are not currently used for the clinical treatment of hypertension, the main mechanism of action of K\textsuperscript{ATP} is to promote cell membrane superclosure to prevent L-type Ca\textsuperscript{2+} influx, inhibit the storage of Ca\textsuperscript{2+} in cells, reduce the amount of intracellular Ca\textsuperscript{2+}, relax the smooth muscle, and thus protect against hypertension-induced injury. When hypoxia pulmonary hypertension occurs, pulmonary vascular endothelial cells synthesize and secrete vasoactive substances such as endothelin-1 (ET-1) and nitric oxide (NO), which act on their corresponding receptors to induce intracellular signal transduction acting on the K\textsuperscript{ATP} channel of pulmonary artery smooth muscle cell membrane. This causes contraction or relaxation of pulmonary artery smooth muscle cells.

Materials and methods
Research object and experimental materials. Pregnant women with severe pre-eclampsia and normal pregnant women admitted to the Obstetrics Department of the Affiliated Hospital of Southwest Medical University at the same time were selected as the experimental subjects. The diagnostic criteria were based on current Chinese 9th edition of Obstetrics and Gynecology (edited by Xie Xing): pre-eclampsia accompanied by one of the following conditions: systolic blood pressure at ≥ 140 mmHg or diastolic blood pressure at ≥ 90 mmHg; platelet count < 100 000/μL; transaminases more than twice the normal value; creatinine ≥ 1.1 mg/dL; pulmonary edema; new neurological abnormalities. The exclusion criteria included the following: multiple pregnancy; single umbilical artery; comorbidity with endoscopic disease or infectious disease; comorbidity with other
transferred to a −80 °C ultra-low temperature freezer for storage prior to QPCR and western blotting.

Enzyme-free water and separated on ice. The umbilical artery was placed in an enzyme-free cryotube and rapidly

aseptic procedures) and transferred to the laboratory. The umbilical cord was submerged in the pre-cooled
terium was delivered, an umbilical cord specimen with a length of about 5 cm was taken immediately (following

Specimen collection, separation and cryopreservation. When a placenta meeting the inclusion criteria

was approved by the hospital's ethics committee. All pregnant women were informed about the postpartum

materials were umbilical artery specimens collected during vaginal delivery or cesarean section from pregnant

pregnancy-specific diseases or obstetric complications including gestational diabetes, intrahepatic cholestasis of

pregnancy, placenta previa, placental abruption, etc.; and history of smoking or alcohol abuse. The experimental

were umbilical artery specimens collected during vaginal delivery or cesarean section from pregnant women who met the inclusion criteria. The sample size of the study was as follows: a total of 120 cases including

40 cases of normal pregnant (NP) women of the childbearing age and 40 cases of severe pre-eclampsia (SP), 20

cases of late onset severe pre-eclampsia (LSP) and 20 cases of early onset severe pre-eclampsia (ESP). This study

was approved by the hospital’s ethics committee. All pregnant women were informed about the postpartum

specimens and provided written informed consent.

QPCR experiment. Total RNA extraction from umbilical arteries was performed according to the instructions

of a Tiangen Total RNA Extraction Kit (Tiangen Biotech, Beijing, China). The tissue was repeatedly ground to a fine powder form. Poured into liquid nitrogen continuously to avoid the increase of tissue temperature and degrading RNA. After grinding, quickly took about 80 mg of sample powder, added to 1 ml lysis buffer. Sufficiently lysed the mixture, added 200 μL chloroform, and centrifuged the mixture at 12,000 rpm for 10 min. Then collected 400μL supernatant , added 200 μL anhydrous ethanol , and transferred the mixture to an adsorption column, washed with 500 μL deproteinized solution, rinsed and dried, and then dissolved in 30μL enzyme-free water. The extracted RNA concentration and purity were measured using a nucleic acid concentration detector (Thermo Scientific, Waltham, MA, USA). Reverse transcriptase (Toyobo, Osaka, Japan) was used to reverse-transcribe total RNA into complementary DNA(cDNA), and QPCR was performed according to the instructions of a QuantiNova SYBR Green PCR Kit (QIAGEN, Hilden, Germany) to obtain a corresponding Ct value. The upstream and downstream primers of KIR6.1, SUR2B and GAPDH genes were designed and synthesized by Sangon Biotech (Shanghai, China). Each primer sequence is shown in Table 7. In Fig. 5, the melting curves of GAPDH, Kir6.1 subunit and SUR2B subunit are all single peaks, narrow and sharp, indicating that the designed primers are acceptable and no dimers are produced.

The QPCR results were the Ct value of each gene in each sample, and the ΔCt value was calculated as the Ct value of the target gene minus the Ct value of the reference gene. The larger the ΔCt value, the lower the substrate concentration. The ΔΔCt value was calculated as the experimental group gene ΔCt value minus the control group ΔCt value, and the 2^ΔΔCt (relative expression) value indicated that the experimental group gene was a multiple of the control group gene.

Western blotting experiment. Tissue samples were ground as described above, after grinding, quickly took about 80 mg of sample powder, then added to 800 μl lysis buffer and protease inhibitor (protease inhibitor/lysis buffer: 1:100). Samples were fully lysed and centrifuged at 12,000 rpm for 15 min. The supernatant was then collected and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The percentage of separating gel used for WB was 10% while the concentrated gel was 5%. Loaded 40ug protein into each well. Proteins were then transferred to PVDF membranes, blocked in 5% skim milk at room temperature for 2 h, and then incubated in primary antibody at 4 °C overnight. Primary antibodies used include glyceraldehyde phosphate dehydrogenase(GAPDH) (Biorwold, Irving, TX, USA) 1:10,000, Kir6.1 (Alomone, Jerusalem, Israel) 1:400, SUR2B (Abcam, Cambridge, UK) 1:500. Phosphate buffered saline with Tween 20 (PBST, Tween 20/PBS: 1:1000) was used to wash PVDF membranes, which were incubated in secondary antibody for 2 h at room temperature. Secondary antibodies used include Goat anti-Mouse IgG (H&L)-HRP (GAPDH secondary antibody, Biorwold, Irving, TX, USA) 1:10,000, Goat anti-Rabbit IgG Secondary Antibody (Kir6.1 and SUR2B secondary antibody, Millipore, Burlington, MA, USA) 1:10,000. The PVDF membrane again washed with PBS, then imaged by a chemiluminescence imaging system (Fusion Solo, Paris, France).

Densitometry analysis of the images obtained by western blotting was performed using Quantity-One (Bio-Rad, California, USA). The relative expression levels of the target proteins Kir6.1 and SUR2B were obtained as the ratio of band intensities to those of the internal reference protein GAPDH.

| Gene     | Primer sequence | Tm (°C) | PCR product size (bp) |
|----------|-----------------|--------|-----------------------|
| GAPDH F  | 5′-CCACTCTCCACCCCTTCTG-3′ | 52.7   | 106                   |
| GAPDH R  | 5′-CACCACTCCACCCCTTCTG-3′  | 55.7   | 106                   |
| Kir6.1 F | 5′-GATCACTCCTCCACCCCTTCTG-3′ | 58.0   | 150                   |
| Kir6.1 R | 5′-GATCACTCCTCCACCCCTTCTG-3′ | 60.0   | 150                   |
| SUR2B F  | 5′-CGGGACATATACCTTGAGATGG-3′ | 59.8   | 130                   |
| SUR2B R  | 5′-ATCAGGCTGGGATAAGAGG-3′  | 57.8   | 130                   |

Table 7.  Forward and reverse primer sequences. Tm: DNA melting temperature.
Statistical analysis of experimental results. Data were compared via Student’s t test using SPSS version 19.0 (IBM Corporation, Armonk, NY, USA). The results are expressed as the mean ± standard deviation (± s). If \( P < 0.05 \), then the difference was considered statistically significant.

Ethics approval and consent to participate. This study protocol was approved by The Ethics Committee of The Affiliated Hospital of Southwest Medical University. This study was performed according to the principles expressed in the Declaration of Helsinki and informed consent was obtained from the subjects.

Data availability The datasets that support the findings of this study are available from the corresponding author, Xiaodong Fu, upon reasonable request.

Received: 31 December 2020; Accepted: 22 March 2021
Published online: 12 April 2021

References
1. Geiseler, S. J., Ludvigsen, S. & Folkow, L. P. KATP-channels play a minor role in the protective hypoxic shut-down of cerebellar activity in eider ducks (Somateria mollissima). Neuroscience 284, 751–758. https://doi.org/10.1016/j.neuroscience.2014.10.046 (2015).
2. Yang, H. et al. The antiepileptic effect of the glycolytic inhibitor 2-deoxy-D-glucose is mediated by upregulation of K(ATP) channel subunits Kir6.1 and Kir6.2. Neurochem. Res. 38(4), 677–685. https://doi.org/10.1007/s11064-012-0958-z (2013).
3. Huang, Y., Hu, D., Huang, C. & Nichols, C. G. Genetic discovery of ATP-sensitive K+ channels in cardiovascular diseases. Circ. Arrhythm. Electrophysiol. 12(5), e007322. https://doi.org/10.1161/CIRCEP.119.007322 (2019).
4. Sun, Y. et al. Hydrogen sulfide upregulates KATP channel expression in vascular smooth muscle cells of spontaneously hypertensive rats. J. Mol. Med. (Berl). 93(4), 439–455. https://doi.org/10.1007/s00109-012-1227-1 (2015).
5. Foster, M. N. & Coetzee, W. A. KATP channels in the cardiovascular system. Physiol. Rev. 96(1), 177–252. https://doi.org/10.1152/physrev.00003.2015 (2016).
6. Wareing, M. Oxygen sensitivity, potassium channels, and regulation of placental vascular tone. Microcirculation 21(1), 58–66. https://doi.org/10.1111/micc.12069 (2014).
7. Wei, X. et al. The expression and function of KCNQ potassium channels in human chorionic plate arteries from women with normal pregnancies and pre-eclampsia. PLoS ONE 13(3), e0192122. https://doi.org/10.1371/journal.pone.0192122 (2018).
8. Cohen, M. V., Baines, C. P. & Downey, J. M. Ischemic preconditioning: from adenosine receptor to KATP channel. Annu. Rev. Physiol. 62, 79–109. https://doi.org/10.1146/annurev.physiol.62.1.79 (2000).
9. Lee, T. M., Lin, M. S. & Chang, N. C. Effect of ATP-sensitive potassium channel agonists on ventricular remodeling in healed rat infarcts. J. Am. Coll. Cardiol. 51(13), 1309–1318. https://doi.org/10.1016/j.jacc.2007.11.067 (2008).
10. Zhu, R. et al. Iptakalim attenuates hypoxia-induced pulmonary arterial hypertension in rats by endothelial function protection. Mol. Med. Rep. 12(2), 2945–2952. https://doi.org/10.3892/mmr.2015.3695 (2015).
11. Salamon, E., Mannhold, R., Weber, H., Lemoine, H. & Frank, W. 6-Sulfonylchromenes as highly potent K(ATP)-channel openers. J. Med. Chem. 45(5), 1086–1097. https://doi.org/10.1021/jm010999g (2002).
12. Pankey, E. A. et al. Imatinib attenuates monocrotaline pulmonary hypertension and has potent vasodilator activity in pulmonary and systemic vascular beds in the rat. Am. J. Physiol. Heart Circ. Physiol. 305(9), H1288–1296. https://doi.org/10.1152/ajpheart.00329.2013 (2013).
13. Zhu, R., Xiao, D. & Zhang, L. Potassium channels and uterine vascular adaptation to pregnancy and chronic hypoxia. Curr. Vasc. Pharmacol. 11(5), 737–747. https://doi.org/10.2174/1570161111311050011 (2013).
14. Nevalainen, J., Skarp, S., Savolainen, E. R., Ryynänen, M. & Järvenpää, J. Intrauterine growth restriction and placental gene expression in severe pre-eclampsia, comparing early-onset and late-onset forms. J. Perinat. Med. 45(7), 869–877. https://doi.org/10.1515/jpm-2016-0406 (2017).

15. Xing, X., Beihua, K. & Tao, D. Obstetrics and gynecology. Version 9 (People's Medical Publishing House, Beijing, 2018).

Acknowledgements
This study was supported by Sichuan Provincial Science and Technology Department (2012SZ0178).

Author contributions
B.Y. and Y.Z. contributed equally to the research and the writing of the manuscript. B.Y. and Y.Z. designed and performed the research, analyzed the data and wrote the paper. X.W., C.P., T.H., C.Y., Y.N., X.F. were involved in the editing of the manuscript. X.F finalized and revised the manuscript. All authors have read and approved the final version of the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-87146-6.

Correspondence and requests for materials should be addressed to X.F.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021