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Title: Effect of maternal and fetal β2-adrenoceptor and nitric oxide synthase genotype on vasopressor requirement and fetal acid-base status during spinal anesthesia for cesarean delivery

Short Title: β2AR genotype and fetal acid-base status

Article Type: Research Report

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Abstract: Background
Previous work demonstrated that maternal haplotypes of the beta2-adrenoceptor gene (ADRB2) influence ephedrine requirements during cesarean delivery. The use of ephedrine versus a pure alpha-adrenergic agonist such as phenylephrine has been associated with lower umbilical artery pH, thought to be secondary to increased fetal metabolism. There are no data evaluating the effect of fetal/neonatal genotypes on the metabolic response to maternally-administered vasopressors. We hypothesized that neonatal ADRB2 genotype would affect the extent of neonatal acidemia. We also examined the effect of maternal ADRB2 and the endothelial nitric oxide synthase gene (NOS3) on ephedrine and phenylephrine requirements for treatment of maternal hypotension.

Methods
The study was performed on 104 Chinese women scheduled for cesarean delivery under spinal anesthesia who were participating in a double-blinded randomized clinical trial evaluating the maternal and neonatal effects of ephedrine versus phenylephrine infusions. Blood samples were drawn from umbilical artery (UA), umbilical vein and maternal radial artery to measure blood gas values, lactate, ephedrine and phenylephrine, and determine maternal and neonatal genotype at non-synonymous SNP at codons 16 (rs1042713) and 27 (rs1042714) of ADRB2 and codon 298 (rs1799983) of NOS. Clinical variables (UA pH, UA lactate and dose of vasopressors) between genotypes were compared, and regression models were created to assess the effect of genotype on vasopressor doses and fetal acid-base status.

Results
Maternal ADRB2 genotype did not affect the ephedrine dose. Neonatal genotype at codon 16 influenced acid-base status. UA pH was higher in Arg16 homozygous neonates (7.31 ± 0.03 in p.16Arg/Arg vs 7.25 ± 0.11 in p.16 Arg/Gly and p.16 Gly/Gly; $p < 0.001$, 95% C.I 0.031 ~ 0.089) and UA lactate was lower (2.67 mmol/L ± 0.99 in p.16Arg/Arg vs 4.28 mmol/L ± 2.79 in p.16 Arg/Gly and p.16 Gly/Gly; $p < 0.001$, 95% C.I -2.398 ~ -0.822). In neonates born to mothers receiving ephedrine, the magnitude of the difference was even greater (pH 7.30 ± 0.02 in p.16Arg/Arg vs 7.19± 0.10 in p.16 Arg/Gly and p.16

Gly/Gly; p < 0.001, 95% C.I 0.068 ~ 0.143) and UA lactate was lower (3.66 mmol/L ± 1.30 in p.16Arg/Arg vs 5.79 mmol/L ± 2.88 in p.16 Arg/Gly and p.16 Gly/Gly; p = 0.003, 95% C.I -3.476 ~ -0.795). In a multiple linear regression model (R2 = 63.6%; P = 0.029), neonatal ADRB2 genotypes (p.16Arg/Arg and p.27Gln/Glu) and lower neonatal birth weight predicted lower UA lactate concentrations.
Phenylephrine dose was not affected by maternal ADRB2 or NOS3 genotypes, and neonatal NOS3 genotype did not affect UA pH or UA lactate.

Conclusion
In contrast to previous findings in a North American cohort, maternal ADRB2 genotype did not affect ephedrine requirements during elective cesarean delivery. However, our findings in this Chinese cohort suggest that neonatal p.Arg16 homozygosity of ADRB2 confers a protective effect against developing ephedrine-induced fetal acidemia.

Response to Reviewers: Journal: Anesthesia & Analgesia
Title: Effect of maternal and fetal β2-adrenoceptor and nitric oxide synthase genotype on vasopressor requirement and fetal acid-base status during spinal anesthesia for cesarean delivery
Manuscript AA-D-10-01472
Format: Full-Length Article
Authors: Ruth Landau, MD; Shih-Kai Liu, MD; Jean-Louis Blouin; Richard Smiley, MD, PhD; Warwick Ngan Kee. MBChB, MD

Response to Reviewers
* Reviewers comments are in a different font (Arial narrow bold) to facilitate the reading of the responses.
* Responses of the authors are indicated after each comment (in Times new roman).
* Changes in the manuscript are indicated in red

Reviewer #1:
1. P5L19 The R2 value is not interpretable in isolation without stating that the three independent variables listed are the sole independent variables. Add 1-2 words to make that clear. Add partial correlation coefficients, shortening Abstract Introduction as needed.
The following sentence has been simplified under the Abstract section:
In a multiple linear regression model (R2 = 63.6%; P = 0.029), neonatal ADRB2 genotypes (p.16Arg/Arg and p.27Gln/Glu) and lower neonatal birth weight predicted lower UA lactate concentrations.

2. From supplemental material, UA lactate is influenced by neonatal birth weight. Provide partial correlation coefficients (and P-values) for the two neonatal codons. P11L52 give partial correlation coefficients.
The following sentence has been added under the Results section.
In a multiple linear regression model (R2 = 63.6%; P = 0.029), neonatal genotypes at codon 16 (homozygous p.16Arg/Arg, partial correlation coefficient = - 0.536, P = 0.008) and 27 (heterozygous p.27Gln/Gln, partial correlation coefficient = - 0.502, P = 0.015) and lower neonatal birth weight (partial correlation coefficient = 0.523, P = 0.010) had significant effects on UA lactate (lower UA lactate concentrations).

3. Figure 1: What is the line for? Which genotype? The association for Gly16 seems weak. In caption, repeat using rank correlation coefficient and confirm statistical significance and estimate of strength of relationship.
The line represents the 'fit line' for Arg16Gly and Gly16 homozygous.
The Figure legend has been modified to:
Figure 1. Regression between UA pH and ephedrine dose (mg) according to neonatal ADRB2 genotype at codon 16.
X axis represents UA pH, Y axis represents total ephedrine dose (mg) given to the mothers from the time of the spinal injection until delivery of the baby.
The line represents the "fit line" for p.16Arg/Gly and p.16Gly/Gly.
Using Pearson's correlation rank, UA pH and ephedrine dose were correlated in p.16Arg/Gly and p.16Gly/Gly neonates (p = 0.002, r = -0.499) but not in p.16Arg/Arg neonates (p = 0.118, r = -0.559).
- p.16Arg/Arg (N = 9) (r2 = 31.2%, p = 0.118)
- p.16Arg/Gly and p.16Gly/Gly (N = 35) (r2 = 24.9%, p = 0.002)

UA pH = umbilical artery pH
There was no neonate Arg16 homozygous with a UA pH < 7.28.

4. MINOR changes (i.e., presentation purposes)
1.1 P5L13 Give the P-values themselves, and do the same for the other Abstract P-values.
P-values were added
1.2 P5L21 Shorten the Abstract Introduction and instead of stating that lactate concentration was significantly affected, give the magnitude of difference.
Abstract was edited and magnitude of difference was added. The results section in the Abstract reads as follows:
Maternal ADRB2 genotype did not affect the ephedrine dose. Neonatal genotype at codon 16 influenced acid-base status. UA pH was higher in Arg16 homozygous neonates (7.31 ± 0.03 in p.16Arg/Arg vs 7.25 ± 0.11 in p.16 Arg/Gly and p.16 Gly/Gly; p < 0.001, 95% C.I 0.031 ~ 0.089) and UA lactate was lower (2.67 mmol/L ± 0.99 in p.16Arg/Arg vs 4.28 mmol/L ± 2.79 in p.16 Arg/Gly and p.16 Gly/Gly; p < 0.001, 95% C.I -2.398 ~ -0.822). In neonates born to mothers receiving ephedrine, the magnitude of the difference was even greater (pH 7.30 ± 0.02 in p.16Arg/Arg vs 7.19± 0.10 in p.16 Arg/Gly and p.16 Gly/Gly; p < 0.001, 95% C.I 0.068 ~ 0.143) and UA lactate was lower (3.66 mmol/L ± 1.30 in p.16Arg/Arg vs 5.79 mmol/L ± 2.88 in p.16 Arg/Gly and p.16 Gly/Gly; p = 0.003, 95% C.I -3.476 ~ -0.795). In a multiple linear regression model (R2 = 63.6%; P = 0.029), neonatal ADRB2 genotypes (p.16Arg/Arg and p.27Gln/Glu) and lower neonatal birth weight predicted lower UA lactate concentrations.
Phenylephrine dose was not affected by maternal ADRB2 or NOS3 genotypes, and neonatal NOS3 genotype did not affect UA pH or UA lactate.

1.3 P5L21 is also confusing versus conclusion as the conclusion suggests protection, but P5L21 does not specify directionality. These changes can be made in 1-2 words.
Direction of change is now specified, and as reported above, the sentence of the results section in the Abstract is:
In a multiple linear regression model (R2 = 63.6%; P = 0.029), neonatal ADRB2 genotypes (p.16Arg/Arg and p.27Gln27Glu) and lower neonatal birth weight predicted lower UA lactate concentrations.
1.4 P8L51 With unequal or equal variances? Add and justify choice.
The following sentence was added:
Equal variances were assumed if p ≧ 0.05 with Levene's test; equal variances were not assumed if p < 0.05 with Levene's test. Fisher exact test was used for comparisons of fetal acidemia (defined as UA pH < 7.20) among genotypes.
1.5 P10L45 Give magnitudes, which is what matters.
Magnitude of difference was added and direction was also mentioned as follow:

Based on neonatal genotype at codon 16 of ADRB2, neonates homozygous for Arg16 had higher UA pH values (7.31 ± 0.11 vs 7.25 ± 0.11, p < 0.001, with a difference of 0.06 ± 0.15, 95% C.I 0.031 ~ 0.089) and lower UA lactate concentrations (2.67 mmol/L ± 0.99 vs 4.28 ± 2.79, with a difference of -1.61 ± 0.40, 95% C.I -2.398 ~ 0.822) compared to neonates carrying the two other genotypes (Table 3A). There was no difference between p.16Arg/Arg neonates and p.16Gly/Gly neonates (no dose-gene effect).

1.6 P11L18 Not "required," the authors mean "administered". Same P11L31.
'Required' was replaced with 'administered' whenever needed

1.7 P11L20 Give the actual P-value. More importantly, give the magnitude of difference.
P values and magnitude now indicated

1.8 Table 3A legend give the P-values themselves in legend. Same for Table 3B. For the significant values, consider giving the 95% CI for the differences.
Requested changes were made in the tables

Reviewer #2: This is a review of the article, "Effect of B2-adrenoceptor genotype on vasopressor requirement and fetal acid-base status during spinal anesthesia for cesarean section" by Landau et al. The manuscript is extremely well written and is quite clear. The study overall is quite interesting and has been performed in a rather unique population-newborns. The major shortcoming of this trial is its low number of subjects enrolled, especially in the rarer genotype groups. While this does appear to affect some of the findings I do not feel that it invalidates the trial. Please see additional comments.

Methods section:

1. While the section makes it clear that patients were randomized to each of the infusion groups there is no mention as to whether the staff administering the infusions were blinded or not. This point should be clarified either way.
Per clinical protocol, staff involved with vasopressor administration was blinded to infusion, and they were also blinded to genotypic group. This is now indicated under the Methods section:
To facilitate double-blinding, the drugs were prepared in identical syringes by one of the investigators who was not involved with subsequent patient management or collection.

2. Page 7, line 41. The comment is that blood was taken from the, "maternal artery, umbilical artery?". In table 3A there is reference to the UA for "maternal" and "neonatal" with different values. The method section fails to explain how and when the different blood samples were taken and why one is considered maternal while the other is neonatal.
The UA pH values presented in Table 3A are those from the umbilical artery; the difference in results under the Mother and Neonates is due to the fact that UA pH values were calculated in relation with either maternal genotype or neonatal genotype (hence different values). While UA pH was not affected by maternal genotype, it was affected by neonatal genotype.
Since this appears to be confusing/misleading, all UA pH values according to maternal genotypes have been removed from the various tables (the same as in table 3C, and UA pH values according to maternal genotype in phenylephrine group have also been removed), and are now indicated in the text (shown in red throughout the Result section).

Results section:

3. The data given with its subsequent tables requires some clarification. While it is true that the UA pH is higher in the Arg/Arg group compared to Arg/Gly it is not statistically higher than the homozygous Gly/Gly. While this is implied in the text and tables I could not find where it is explicitly stated. I feel this may be misleading a bit to the reader.
The results section was substantially modified to improve clarity. Information from Table 3A was deleted and is now presented under the text in the section.

Overall, induction-to delivery-time was similar between maternal genotypic groups for codon 16 of ADRB2 (28.2 minutes ± 7.9 in p.16Arg/Arg, 30.4 minutes ± 10.9 in p.16Arg/Gly, 30.2 minutes ± 8.7 in p.16Gly/Gly). Maternal genotype at codon 16 of ADRB2 did not influence UA pH (7.27 ± 0.09 in p.16Arg/Arg, 7.29 ± 0.07 in p.16Arg/Gly, 7.26 ± 0.10 in p.16Gly/Gly).
Based on neonatal genotype at codon 16 of ADRB2, neonates homozygous for Arg16 had higher UA pH values (7.31 ± 0.11 vs 7.25 ± 0.11, p = 0, 95% C.I 0.031 ~ 0.089) and lower UA lactate concentrations (2.67 mmol/L ± 0.99 vs 4.28 ± 2.79, p = 0, 95% C.I -2.398 ~ 0.822) compared to neonates carrying the two other genotypes (Table 3A). There was no difference between p.16Arg/Arg neonates and p.16Gly/Gly neonates (no dose-gene effect).
In the ephedrine group (N = 45), maternal genotype of ADRB2 at codon 16 did not affect UA pH (7.23 ± 0.11 in p.16Arg/Arg, 7.23 ± 0.08 in p.16Arg/Gly, 7.18 ± 0.09 in p.16Gly/Gly). Maternal genotype of ADRB2 at codon 16 did not affect the dose of administered ephedrine (Table 3B) and neither maternal nor neonatal genotype affected the concentration of ephedrine in the UA, UV, or MA, or the UA/UV or UV/MA ratios of ephedrine concentration (Table 3B).

4. The tables relevant to the pH and lactate data (3A and 3B) do demonstrate statistical significance for the Arg/Arg compared to a combination of Arg/Gly + Gly/Gly but never states that Arg/Arg compared to Gly/Gly was not statistically significant as mentioned above. This does create a problem since it would be expected that if this effect is real, a gene dose effect should possibly be noted. If a gene dose effect is not seen than it should be expect that the hetero and homo Gly groups should at least be equivalent and that is not really seen, in fact the opposite. No doubt these result are due to the fact that the number of Gly/Gly subjects was limted. These points need to be clarified in the results and in the discussion section. Once again while they are implied they are not clearly stated.
Indeed, there does not seem to be a gene-dose effect and the maim difference is attributed to the larger clinical difference noted between the Arg16 homozygotes and Arg/Gly16 heterozygotes.
The following sentence was added in the Results section:
There was no difference between Arg16 homozygote neonates and Gly16 homozygote neonates (no dose-gene effect).

5. Page 11, line 41. The authors make the statement that, "? babies in the ephedrine group had overall lower UA pH and higher UA lactate measures than babies in the phenylephrine group" (Table 4B and 4C). However those tables have the subjects results partially broken down by genotype and while the tables have the comment "Different from the ephedrine group" I am just not clear what was being compared. Was it all the data combined for the ephedrine receiving subjects compared to the phenylephrine group? This needs to be clarified.
The reviewer is correct in that we compared the clinical outcome irrespective of genotype (as per original data already published by Ngan Kee in 2009) where the acid-base status was different between the ephedrine group and phenylephrine group irrespective of neonatal genotype, and therefore this information need not be under Table 4. We removed this data altogether, to void duplicate information and confusion.

Once again the major shortcoming of the trial is the low number of patients in rarer genotype groups. The interpretation and statistics of the data need to be clarified as mentioned above. With these revisions I believe that this is an important and useful trial.

Effect of maternal and fetal β_2-adrenoceptor and nitric oxide synthase genotype on vasopressor requirement and fetal acid-base status during spinal anesthesia for cesarean delivery

Short: β_2AR genotype and fetal acid-base status

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Conflict of Interest:

No

Abstract

Background

Previous work demonstrated that maternal haplotypes of the β_2-adrenoceptor gene (*ADRB2*) influence ephedrine requirements during cesarean delivery. The use of ephedrine versus a pure α-adrenergic agonist such as phenylephrine has been associated with lower umbilical artery pH, thought to be secondary to increased fetal metabolism. There are no data evaluating the effect of fetal/neonatal genotypes on the metabolic response to maternally-administered vasopressors. We hypothesized that neonatal *ADRB2* genotype would affect the extent of neonatal acidemia. We also examined the effect of maternal *ADRB2* and the endothelial nitric oxide synthase gene (*NOS3*) on ephedrine and phenylephrine requirements for treatment of maternal hypotension.

Methods

The study was performed on 104 Chinese women scheduled for cesarean delivery under spinal anesthesia who were participating in a double-blinded randomized clinical trial evaluating the maternal and neonatal effects of ephedrine versus phenylephrine infusions. Blood samples were drawn from umbilical artery (UA), umbilical vein and maternal radial artery to measure blood gas values, lactate, ephedrine and phenylephrine, and determine maternal and neonatal genotype at non-synonymous SNP at codons 16 (rs1042713) and 27 (rs1042714) of *ADRB2* and codon 298 (rs1799983) of *NOS*. Clinical variables (UA pH, UA lactate and dose of vasopressors) between genotypes were compared, and regression models were created to assess the effect of genotype on vasopressor doses and fetal acid-base status.

Results

Maternal *ADRB2* genotype did not affect the ephedrine dose. Neonatal genotype at codon 16 influenced acid-base status. UA pH was higher in Arg16 homozygous neonates (7.31

± 0.03 in p.16Arg/Arg vs 7.25 ± 0.11 in p.16 Arg/Gly and p.16 Gly/Gly; p < 0.001, 95% C.I 0.031 ~ 0.089) and UA lactate was lower (2.67 mmol/L ± 0.99 in p.16Arg/Arg vs 4.28 mmol/L ± 2.79 in p.16 Arg/Gly and p.16 Gly/Gly; p < 0.001, 95% C.I -2.398 ~ -0.822). In neonates born to mothers receiving ephedrine, the magnitude of the difference was even greater (pH 7.30 ± 0.02 in p.16Arg/Arg vs 7.19± 0.10 in p.16 Arg/Gly and p.16 Gly/Gly; p < 0.001, 95% C.I 0.068 ~ 0.143) and UA lactate was lower (3.66 mmol/L ± 1.30 in p.16Arg/Arg vs 5.79 mmol/L ± 2.88 in p.16 Arg/Gly and p.16 Gly/Gly; p = 0.003, 95% C.I -3.476 ~ -0.795). In a multiple linear regression model (R^2 = 63.6%; *P* = 0.029), neonatal *ADRB2* genotypes (p.16Arg/Arg and p.27Gln/Glu) and lower neonatal birth weight predicted lower UA lactate concentrations.

Phenylephrine dose was not affected by maternal *ADRB2* or *NOS3* genotypes, and neonatal *NOS3* genotype did not affect UA pH or UA lactate.

Conclusion

In contrast to previous findings in a North American cohort, maternal *ADRB2* genotype did not affect ephedrine requirements during elective cesarean delivery. However, our findings in this Chinese cohort suggest that neonatal p.Arg16 homozygosity of *ADRB2* confers a protective effect against developing ephedrine-induced fetal acidemia.

Introduction

Spinal anesthesia-induced hypotension during cesarean delivery has been the focus of numerous clinical studies searching for the most effective and safe vasopressor to maintain maternal blood pressure and avoid adverse maternal and neonatal outcomes.[1,2] It has been established that ephedrine increases the risk for neonatal acidemia due to stimulation of fetal metabolism prior to delivery[3]; however, it is unclear whether the degree of neonatal acidemia is proportionate to the dose of ephedrine given to the mother. Recently-gathered evidence shows that transplacental transfer of ephedrine exceeds that of phenylephrine and that ephedrine is associated with greater umbilical arterial (UA) and umbilical venous (UV) plasma concentrations of lactate, glucose, epinephrine, and norepinephrine and greater UV P_{CO2} compared with phenylephrine.[4] These findings are consistent with the hypothesis that the underlying mechanism by which ephedrine causes neonatal acidemia is transfer of ephedrine across the placenta and stimulation of metabolic processes in the fetus.

We have previously demonstrated that genetic variability (sequence variability) of *ADRB2* influences the dose of ephedrine administered to treat hypotension during elective cesarean delivery under spinal anesthesia.[5] Women carrying two common haplotypes that were present in 20% of a North-American cohort were found to require substantially lower doses of ephedrine.

We hypothesized that while maternal genetic variability will influence ephedrine requirement, neonatal *ADRB2* genotype will directly influence the degree of neonatal acidemia in response to ephedrine given to the mother prior to delivery. We present here the results of the genetic analysis of mothers and neonates participating in the randomized controlled trial of placental transfer and fetal metabolic effects of phenylephrine and ephedrine during spinal anesthesia for cesarean delivery.[4]

Methods

Ethics committee approval was obtained from the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee, Shatin, Hong Kong, China for the previously-reported aspects of this work[4] and the genetic analysis. Ethics committee approval was obtained from the University Hospitals of Geneva, Geneva, Switzerland for genetic analysis of de-identified samples. Written informed consent was obtained from all women for participation in the randomized clinical trial and genetic analysis. One hundred and four women with term singleton pregnancies who were scheduled for elective cesarean delivery under spinal anesthesia were enrolled. As previously reported[4], clinical management included spinal anesthesia with hyperbaric 0.5% bupivacaine (10 mg) and fentany15 µg. Women were then placed in the left-tilt supine position, and blood pressure was recorded every 1-min beginning 1 min after spinal injection. Women were randomly allocated to infusions of ephedrine (8 mg/ml) or phenylephrine (100 µg/ml) according to sequentially numbered sealed envelopes that each contained a computer-generated randomization code (Statview for Windows 5.0.1;SAS Institute Inc, Cary, NC). To facilitate double-blinding, the drugs were prepared in identical syringes by one of the investigators who was not involved with subsequent patient management or collection. The vasopressors were administered by infusion using a syringe pump (Graseby 3500 Anaesthesia Pump; Graseby Medical Ltd., Watford, Herts, United Kingdom) connected via fine-bore tubing to the IV cannula by a 3-way stopcock, aiming to maintain blood pressure near the baseline value.[4] Maternal radial artery (MA), umbilical arterial (UA) and umbilical venous (UV) blood samples were drawn at the time of delivery, for assays including blood gas analysis and plasma concentrations of lactate, ephedrine and phenylephrine, as described previously.[4] As initially planned at the time of study design, an aliquot of maternal arterial blood and umbilical

cord blood was obtained and sent to Geneva, Switzerland for maternal and neonatal DNA isolation and analysis.

DNA Collection and Purification, and Genotyping

Maternal arterial (3 ml, EDTA tubes) and umbilical cord (1 ml, EDTA tubes) blood samples underwent DNA purification and genotyping of *ADRB2* and *NOS3* at the University Hospitals of Geneva, Switzerland. DNA was purified by a Puregene extraction Kit (Gentra, Minneapolis, MN) and tested for quantity, purity, and quality by optical densitometry (ratio, 260/280 nm) and gel electrophoresis. For the identification of the polymorphisms of the *ADRB2* gene, 60 ng DNA was amplified by polymerase chain reaction (PCR) (96-well microtiter plate block thermocycler; Biometra, Göttingen, Germany) using specific primers. Primers were chosen in single-copy DNA regions surrounding polymorphisms p.16Arg/Gly and p.27Gln/Glu located in the single exon of *ADRB2* using Oligo6-primer designing software (Molecular Biology Insight, Cascade, CO) with specificity checking by sequence comparison as previously described.[5] Each assay was tested for specificity and reliability by sequencing before its use for the entire cohort. Polymorphism genotypes were determined by Sanger sequencing reaction and electrophoresis on a fluorescent DNA fragment analyzer apparatus. For p.298Glu/Asp of *NOS3* gene, the PCR–pyrosequencing analysis was used, as we have previously reported.[6]

Statistical analysis

The study was powered to detect differences in clinical outcomes. Based on potential differences in UA pH, a sample size of 38 patients per vasopressor allocation group would be required to have 90% power with a two-sided value of 0.05 in neonatal acid-base status between fetuses

exposed to ephedrine versus phenylephrine. However, in anticipation that obtaining sufficient maternal arterial and umbilical cord blood could be difficult in a proportion of cases, the sample size was increased to 52 women per group. [4] Based on previous genetic studies examining the frequency of the p.16Arg/Gly and p.27Gln/Glu in various cohorts, we hypothesized that 20% of mothers/neonates would carry one of two haplotype that were previously shown to influence the response to ephedrine. Therefore we anticipated at least 10 mothers/babies out of 52 receiving ephedrine to be either heterozygous p.27Gln/Glu or homozygous p.27Glu/Glu.

Dose of vasopressor (ephedrine or phenylephrine), time interval from induction to delivery, UA pH, and UA lactate were compared among genotypes using one-way ANOVA analysis. The paired t-test was used for comparing UA pH between genotypes (homozygous p.16Arg/Arg versus homozygotes p.16Gly/Gly - and heterozygous p.16Arg/Gly) and equal variances were assumed if $p \geqq 0.05$ with Levene's test; equal variances were not assumed if $p < 0.05$ with Levene's test. Fisher exact test was used for comparisons of neonatal acidemia (defined as UA pH < 7.20) among genotypes. Regression models were constructed to adjust for possible confounders affecting neonatal outcomes (UA pH and UA lactate). Dummy variables were created for *ADRB2* genotype, setting p.16Arg/Gly as reference for codon 16 and p.27Gln/Gln as reference for codon 27. Dependent variables were dose of vasopressor, UA pH and UA lactate. Independent variables included maternal genotypes, neonatal genotypes, neonatal birth weight, maternal weight, maternal height, body mass index, baseline diastolic blood pressure, baseline systolic blood pressure, and baseline heart rate. In addition, dose of ephedrine was used as independent variable when UA pH and UA lactate were used as dependent variables. Data were analyzed using SPSS 18.0.0 (SPSS Chicago IL).

Results

One hundred and four mothers/baby pairs participated in this study. Technical problems occurred in 10 cases during *ADRB2* genotyping and in 8 mothers and 3 neonates during *NOS3* sequencing. Haplotypes for *ADRB2* at codon 16 and 27 are presented in Table 1A for both mothers and neonates. Due to known linkage disequilibrium between codon 16 and 27 (Glu27 almost never occurs in the presence of Arg16), only six genotype combinations (instead of the theoretical 9) were found in this cohort. Only one neonate was found to be homozygote for Glu27 (p.16Gly/Gly/p.27Glu/Glu) and he was born to a mother p.16Arg/Gly/p.27Gln/Glu. Genetic distribution in mother and neonates at codon 16 of *ADRB2* is presented in Table 1B. Due to the overall rare occurrence of Glu at codon 27 in both mothers and neonates, all clinical data were analyzed according to codon 16 only and not per haplotype of codons 16 and 27 of *ADRB2*. Genetic distribution in mothers and neonates at codon 298 of *NOS3* gene is presented in Table 2. Genotype distribution for both mothers and neonates for both *ADRB2* and *NOS3* appeared to be in Hardy-Weinberg equilibrium.

Overall, induction-to delivery-time was similar between maternal genotypic groups for codon 16 of *ADRB2* (28.2 minutes ± 7.9 in p.16Arg/Arg, 30.4 minutes ± 10.9 in p.16Arg/Gly, 30.2 minutes ± 8.7 in p.16Gly/Gly). Maternal genotype at codon 16 of *ADRB2* did not influence UA pH (7.27 ± 0.09 in p.16Arg/Arg, 7.29 ± 0.07 in p.16Arg/Gly, 7.26 ± 0.10 in p.16Gly/Gly).

Based on neonatal genotype at codon 16 of *ADRB2*, neonates homozygous for Arg16 had higher UA pH values (7.31 ± 0.11 vs 7.25 ± 0.11, $p < 0.001$, with a difference of 0.06 ± 0.15, 95%

C.I 0.031 ~ 0.089) and lower UA lactate concentrations with an order of magnitude of 30% (2.67 mmol/L ± 0.99 vs 4.28 ± 2.79, with a difference of -1.61 ± 0.40, 95% C.I -2.398 ~ 0.822) compared to neonates carrying the two other genotypes (Table 3A). There was no difference between p.16Arg/Arg neonates and p.16Gly/Gly neonates (no dose-gene effect).

In the ephedrine group (N = 45), maternal genotype of *ADRB2* at codon 16 did not affect UA pH (7.23 ± 0.11 in p.16Arg/Arg, 7.23 ± 0.08 in p.16Arg/Gly, 7.18 ± 0.09 in p.16Gly/Gly). Maternal genotype of *ADRB2* at codon 16 did not affect the dose of administered ephedrine (Table 3B) and neither maternal nor neonatal genotype affected the concentration of ephedrine in the UA, UV, or MA, or the UA/UV or UV/MA ratios of ephedrine concentration (Table 3B). Ten neonates were Arg16 homozygotes; pH values at birth were available for 9 of these (Table 3B). Out of these 9 Arg16 homozygous neonates, none had a pH lower than 7.28. In the 35 neonates carrying the Gly16 allele, 17 (49%) where found to have a pH ≤ 7.20. In comparing Arg16 homozyous neonates and neonates with the two other genotypes, neonatal acidosis defined as pH<7.20 was significantly less frequent with Arg16 homozygosity (p = 0.008, Fishers Exact). In the linear regression model, the dose of ephedrine administered to the mother was associated with a significantly greater degree of acidemia (lower UA pH) in neonates carrying one or two Gly16 alleles (p = 0.002, r = - 0.499); however, this association was not present in neonates who were Arg16 homozygous (p = 0.118, r = - 0.559) (Figure 1).

In a multiple linear regression model in the ephedrine group (R^2 = 63.6%; *P* = 0.029), only neonatal genotypes at codon 16 (p.16Arg/Arg, partial correlation coefficient = - 0.536, *P* = 0.008) and 27 (heterozygous p.27Gln/Gln, partial correlation coefficient = - 0.502, *P* = 0.015) and lower neonatal birth weight (partial correlation coefficient = 0.523, *P* = 0.010) had significant effects

on UA lactate (lower UA lactate concentrations). Other factors (maternal genotype at codon 16 and 27 of *ADRB2*, dose of ephedrine, weight, height, body mass index (BMI), baseline systolic or diastolic blood pressure, and heart rate) were not significant predictors of UA pH (R^2 = 53.5%; *P* = 0.15). (Supplemental Digital Content 1, illustrating the regression models with *ADRB2* and *NOS3*).

In the phenylephrine group (N = 49), the dose of phenylephrine administered to maintain baseline maternal blood pressure was not affected by maternal genotype at codon 16 of *ADRB2* (Table 3C). Maternal *ADRB2* genotype at codon 16 did not predict UA pH (7.32 ± 0.04 in p.16Arg/Arg, 7.33 ± 0.03 in p.16Arg/Gly, 7.32 ± 0.05 in p.16Gly/Gly). Neonatal *ADRB2* genotype at codon 16 did not affect UA pH or UA lactate concentrations (Table 3C).

Overall, UA pH was neither predict by maternal *NOS3* genotype (7.30 ± 0.02 in p.298Glu/Glu, 7.27 ± 0.10 in p.298Glu/Asp, 7.27 ± 0.10 in p.298Asp/Asp), nor neonatal *NOS3* genotype (7.27 ± 0.09 in p.298Glu/Glu, 7.27 ± 0.11in p.298Glu/Asp, 7.26 ± 0.05 in p.298Asp/Asp).

Ephedrine dose was not predicted by maternal *NOS3* genotype, nor were UA pH and UA lactate predicted by neonatal NOS3 genotype (Table 4A). Phenylephrine dose was not predicted by maternal *NOS3* genotype, nor were UA pH and UA lactate predicted by neonatal NOS3 genotype (Table 4B).

Discussion

This Chinese cohort of healthy women scheduled for cesarean delivery had a distribution of *ADRB2* genotypes and allele (genotype) combination at codon 16 and 27 that was significantly different from that described in other obstetrical cohorts.[5,7,8] In particular, in our previous work in a North-American cohort of women scheduled for cesarean delivery also assessing spinal hypotension and vasopressor requirement, [5] 20% of women carried at least one Glu27 allele (heterozygous p.27Gln/Glu or homozygous p.27Glu/Glu). In this current cohort of Chinese women, only 7% of women were found to be heterozygous at codon 27 and no mother was Glu27 homozygous. A comparison between these two different cohorts reveals an overall haplotype distribution with a significant difference ($p < 0.001$). This relatively low occurrence of Glu27 homozygosity among Chinese cohorts has been previously reported.[9,10]

Another significant finding, also in contrast with our findings in the North-American cohort, is that genotype of *ADRB2* did not influence the dose of ephedrine administered to maintain maternal blood pressure during spinal anesthesia for cesarean delivery. Our previous work had described a presumed pharmacogenetic effect of *ADRB2*, with Glu27 carriers requiring lower doses of ephedrine to treat spinal hypotension.[5] There are several possible explanations for these discrepant findings. One is the difference of genotype distribution according to ethnic background; since the 2 combinations that were found to reduce the ephedrine requirement were 'under-represented' in the current study compared to the North American cohort, this could explain why we could not find a pharmacogenetic effect in this Chinese group. Alternatively, the dose response to adrenoceptor-agonists could be attenuated in Asians.[11]

Secondly, ephedrine was not given in a similar manner in both studies (bolus in the North-American cohort versus continuous infusion in the current study) and the criteria applied for treatment of hypotension, and therefore targeted systolic blood pressure, were different (systolic blood pressure decrease greater than 20% or to less than 90mmHg in North-American study versus near baseline values in the current study). As a consequence, the total ephedrine dose was significantly higher in the current study as compared to the doses used in our previous work, and this strategy might overwhelm differences between genotype groups. It is also of course possible that our previous finding was a Type 1 error (false positive) and there is no effect of *ARDB2* genotype on ephedrine requirements.

The most clinically relevant and intriguing finding was that UA pH was overall higher and UA lactate was lower in neonates that were Arg16 homozygous as compared to neonates with the two other genotypes of *ADRB2*. Furthermore, among babies born to mothers receiving ephedrine, ephedrine dose was associated with neonatal acidemia (decreased UA pH) only in neonates carrying a Gly16 allele, but not in neonates that were Arg16 homozygous. Since there was no significant difference in ephedrine concentration as determined by maternal and umbilical assays (MA, UA, UV or ratio of UA/UV and UV/MA) between genetic groups, any difference in metabolic markers are unlikely to have resulted from differential transplacental transfer of drug or a pharmacokinetic effect. Arg16 homozygous neonates seem to be protected from the risk of developing acidemia when exposed to ephedrine, irrespective of the dose given to the mother. Previous studies have demonstrated differential metabolic responses based on *ADRB2* genotype,[12-14] so it is reasonable to postulate that such genetic variants in the fetus could lead to altered responses to a given dose of a cardiac and vascular stimulant such as ephedrine.

One potential mechanistic explanation for the apparent protective effect of Arg16 homozygosity against neonatal acidemia could be increased desensitization of β-adrenergic receptor in response to ephedrine. Arg16 homozygous individuals have been shown to undergo rapid desensitization in response to β-agonists,[15] therefore, it is possible that the continuous infusion of ephedrine could have resulted in a greater degree of desensitization, i.e. tachyphylaxis, in p.16Arg/Arg neonates. If that is indeed the case, this could provide an explanation of the observed effect in Arg16 homozygous neonates who seem to have been protected from developing the metabolic effects associated with ephedrine, irrespective of the dose. It should be noted that there is considerable controversy in the literature regarding the assumption of increased desensitization of Arg16 homozygotes in vivo,[12,15] and the time course of desensitization *in vivo* is unclear, so conclusions about mechanism must be viewed as preliminary.

Genetic distribution of *NOS3* p.298Glu/Asp was similar to previous reports in a Chinese population.[16] A pharmacogenetic effect of *NOS3* with an enhanced response to phenylephrine in subjects carrying the Asp298 allele has been shown in a study in Caucasian patients undergoing cardiopulmonary bypass.[17] We did not find a difference in phenylephrine dose according to *NOS3* genotype in this study, although our study was underpowered for this clinical outcome due to the low prevalence on the rare variant in this ethnic group. Other considerations include the very different study population and conditions (healthy pregnant women undergoing cesarean delivery rather than a cardiac population undergoing cardiopulmonary bypass), ethnicity (Chinese rather than Caucasian), and different mode of phenylephrine administration (infusion in our study rather than increasing bolus dosing) and the targeted blood pressure. Neonatal

acidemia and other markers of fetal metabolism were also not associated with any specific genotype of *NOS3* in either the phenylephrine or ephedrine groups. Based on our findings, *NOS3* genotype may not play an important role in determining the response to phenylephrine given as an infusion to maintain baseline systolic blood pressure in pregnant Chinese women under spinal anesthesia.

Obvious limitations of this study relate to the overall small sample of patients. In addition, the possible effect of p.27Gln/Glu on maternal ephedrine response could not be examined because contrary to our expectations, no mother was found to be homozygous for Glu at codon 27 (only 5/9 possible combined genotypes were found in this cohort instead of the expected 6/9). Furthermore, due to the study design, only half of the neonates were exposed to ephedrine, and a smaller proportion of neonates exposed to ephedrine were found to be Arg16 homozygotes (10/45) as compared to the proportion of Arg16 homozygous neonates in the phenylephrine group (22/49). Thus, although we found that Arg16 homozygous neonates had higher pH values, and none had a pH below 7.28 we must acknowledge that this neonatal cohort (Arg16 neonates receiving ephedrine) only consisted of 9 babies. The proportion of Arg16 homozygous women was similar in the ephedrine (18/45) and phenylephrine groups (16/49), which was expected since women were randomly assigned to one treatment group or the other, so this uneven distribution of neonatal genotype is almost certainly a chance occurrence.

In conclusion, maternal genotypes of *ADRB2* and *NOS3* did not impact on the total dose of ephedrine or phenylephrine infusions administered to maintain maternal systolic blood pressure close to baseline during spinal anesthesia for cesarean delivery in a healthy cohort of Chinese

women. Neonatal parameters and not maternal genotypes or ephedrine dose were found to predict acid-base status and neonatal acidemia. Neonatal homozygosity for Arg16 of *ADRB2*, which was found to occur in more than 30% of babies in this Chinese cohort, seemed to protect from the risk of developing neonatal acidemia when mothers were treated with ephedrine. Whether this finding is specific to this Chinese cohort and/or can be replicated in this or other ethnic groups remains to be determined.

Tables

Table 1A. Overall distribution of *ADRB2* haplotypes (codons 16 and 27)

Genotypic combination	Mothers (N = 94)	Neonates (N = 94)
p.16Arg/Arg-p.27Gln/Gln	34 (36.2%)	32 (34.0%)
p.16Arg/Arg-p.27Gln/Glu	0	0
p.16Arg/Arg-p.27GluGlu	0	0
p.16ArgGly-p.27Gln/Gln	33 (35.1%)	40 (42.6%)
p.16Arg/Gly-p.27Gln/Glu	10 (10.6%)	6 (6.4%)
p.16ArgGly-p.27Glu/Glu	0	0
p.16Gly/Gly-p.27Gln/Gln	10 (10.6%)	10 (10.6%)
p.16Gly/Gly-p.27Gln/Glu	7 (7.4%)	5 (5.3%)
p.16Gly/Gly-p.27Glu/Glu	0	1 (1.1%)

Table 1B. Overall distribution of p.16Arg/Gly genotype

Genotype	Mothers (N = 94)	Neonates (N = 94)
p.16Arg/Arg	34 (36.2%)	32 (34.0%)
p.16Arg/Gly	43 (45.7%)	46 (48.9%)
p.16Gly/Gly	17 (18.1%)	16 (17.0%)

Values presented are N = number of subjects, and percentage

Table 2. Overall distribution of *NOS3* genotype (p.298Glu/Asp)

Genotype	Mothers (N = 96)	Neonates (N = 101)
p.298Glu/Glu	69 (71.9%)	75 (74.3%)
p.298Glu/Asp	24 (25.0%)	22 (21.8%)
p.298Asp/Asp	3 (3.1%)	4 (4.0%)

Values presented are N = number of subjects, and percentage.

NOS3 = endothelial nitric oxide synthase.

Table 3A. Acid-base status according to neonatal *ADRB2* codon 16 genotype

	p.16Arg/Arg	**p.16Arg/Gly**	**p.16Gly/Gly**
All Neonates	N = 32	N = 46	N = 16
UA pH	7.31 ± 0.03§	7.24 ± 0.12	7.27 ± 0.07
UA lactate (mmol/L)	2.67 ± 0.99§§	4.55 ± 3.05	3.51 ± 1.71

Values are mean ± standard deviation.

One-way ANOVA, post hoc test: Bonferroni, and paired t-test.

§ p.16Arg/Arg different from p.16Arg/Gly, p = 0.001 (95% C.I 0.030 ~ 0.103);

p.16Arg/Arg different from p.16Arg/Gly and p.16Gly/Gly (7.25± 0.11; p < 0.001, 95% C.I 0.031 ~ 0.089)

§§ p.16Arg/Arg different from p.16Arg/Gly, p < 0.001 (95% C.I -2.845 ~ -0.911);

p.16Arg/Arg different from p.16Arg/Gly and p.16Gly/Gly (4.28± 2.79; p < 0.001, 95% C.I -2.398 ~ -0.822)

UA pH = umbilical artery pH;

UA lactate = umbilical artery lactate;

Table 3B. Ephedrine group

	p.16Arg/Arg	p.16Arg/Gly	p.16Gly/Gly
Mothers	N = 18	N = 19	N = 8
Dose of ephedrine (mg)	61 ± 26	72 ± 20	60 ± 29
MA ephedrine concentration (ng/ml)	368.3 ± 186.4	449.2 ± 144.9	428.0 ± 243.2
UV ephedrine concentration (ng/ml)	449.9 ± 250.9	488.2 ± 156.2	442.3 ± 122.2
UA/UV ratio	0.83 ± 0.17	0.83 ± 0.12	0.77 ± 0.11
UV/MA ratio	1.22 ± 0.27	1.09 ± 0.16	1.26 ± 0.24
Neonates	N = 10	N = 28	N = 7
UA ephedrine concentration (ng/ml)	374.0 ± 204.4	402.8 ± 211.0	314.5 ± 101.0
UV ephedrine concentration (ng/ml)	406.7 ± 191.2	481.3 ± 223.9	384.7 ± 163.6
UA/UV ratio	0.90 ± 0.15	0.86 ± 0.20	0.83 ± 0.13
UV/MA ratio	1.06 ± 0.21	1.19 ± 0.26	1.13 ± 0.14
	N = 9	N = 28	N = 7
UA pH	7.30 ± 0.02[§]	7.18 ± 0.11	7.22 ± 0.07
UA lactate (mmol/L)	3.66 ± 1.30[§§]	6.10 ± 3.04	4.60 ± 1.86

Values are mean ± standard deviation.

One-way ANOVA, post hoc test: Bonferroni, and paired t-test.

[§] p.16Arg/Arg different from p.16Arg/Gly $p < 0.001$ (95% C.I 0.069 ~ 0.158);

p.16Arg/Arg different from p.16Arg/Gly and p.16Gly/Gly (7.19± 0.10; $p < 0.001$, 95% C.I 0.068 ~ 0.143)

§§ p.16Arg/Arg different from p.16Arg/Gly, p = 0.002 (95% C.I -3.923 ~ -0.962);

p.16Arg/Arg different from p.16Arg/Gly and p.16Gly/Gly (5.79 ± 2.88; p = 0.003, 95% C.I -3.476 ~ -0.795)

UA pH = umbilical artery pH

UA lactate = umbilical artery lactate

MA = maternal artery

UA = umbilical artery

UV = umbilical vein

Table 3C. Phenylephrine Group

	p.16Arg/Arg	p.16Arg/Gly	p.16Gly/Gly
Mothers	N = 16	N = 24	N = 9
Dose of phenylephrine (µg)	1342 ± 436	1403 ± 494	1260 ± 691
Neonates	N = 22	N = 18	N = 9
UA pH	7.32 ± 0.03	7.34 ± 0.03	7.31 ± 0.05
UA lactate (mmol/L)	2.27 ± 0.43	2.12 ± 0.40	2.67 ± 1.02

Values are mean ± standard deviation.

One-way ANOVA, post hoc test: Bonferroni, and paired t-test.

UA pH = umbilical artery pH

UA lactate = umbilical artery lactate.

Table 4. Outcomes according to *NOS3* genotype

Table 4A. Ephedrine Group

	p.298Glu/Glu	**p.298Glu/Asp and p.298Asp/Asp**
Mothers	N = 34	N = 13
Dose of ephedrine (mg)	64.1 ± 24.8	66.8 ± 32.4
Neonates	N = 37	N = 12
UA pH	7.21 ± 0.10	7.22 ± 0.12
UA lactate (mmol/l)	5.40 ± 2.71	4.77 ± 2.64

Table 4B. Phenylephrine Group

Mothers	N = 35	N = 14
Dose of phenylephrine (µg)	1356.6 ± 496.4	1252.9 ± 566.2
Neonates	N = 38	N = 12
UA pH	7.33 ± 0.04	7.32 ± 0.04
UA lactate (mmol/l)	2.25 ± 0.61	2.37 ± 0.50

Values are mean ± standard deviation.

Paired t-test.

UA pH = umbilical arterial pH

UA lactate = umbilical arterial lactate.

Figures and Illustrations

Figure Legends

Figure 1. Regression between UA pH and ephedrine dose (mg) according to neonatal *ADRB2* genotype at codon 16.

X axis represents UA pH, Y axis represents total ephedrine dose (mg) given to the mothers from the time of the spinal injection until delivery of the baby.

The line represents the "fit line" for p.16Arg/Gly and p.16Gly/Gly.

Using Pearson's correlation rank, UA pH and ephedrine dose were correlated in p.16Arg/Gly and p.16Gly/Gly neonates (p = 0.002, r = -0.499) but not in p.16Arg/Arg neonates (p = 0.118, r = -0.559).

◆ p.16Arg/Arg (N = 9) (r^2 = 31.2%, p = 0.118)

○ p.16Arg/Gly and p.16Gly/Gly (N = 35) (r^2 = 24.9%, p = 0.002)

UA pH = umbilical artery pH

There was no neonate Arg16 homozygous with a UA pH < 7.28.

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