A Functional Fetal HSD11B2[CA]n Microsatellite Polymorphism is Associated with Maternal Serum Cortisol Concentrations in Pregnant Women

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Key Words
Pregnancy • Placenta • Cortisol I metabolism • 11 beta-hydroxysteroid dehydrogenase 2 • HSD11B2[CA]n polymorphism

Abstract
Background/Aims: Cortisol plays an important role during pregnancy. It controls maternal glucose metabolism and fetal development. Cortisol metabolism is partially controlled by the 11b-HSD2. This enzyme is expressed in the kidney and human placenta. The activity of the enzyme is partially controlled by functional polymorphisms: the HSD11B2[CA]n microsatellite polymorphism. The impact of this functional gene polymorphism on cortisol metabolism and potential effects on the newborn’s is unknown so far. Methods: In the current prospective birth cohort study in southern Asia, we analyzed the association of the HSD11B2[CA]n microsatellite polymorphisms in 187 mothers and their newborn’s on maternal and newborn’s serum cortisol concentrations. Results: Using multivariable regression analyses considering known confounding (gestational age, newborn’s gender, the labor uterine contraction states and the timing during the day of blood taking), we showed that the fetal HSD11B2[CA]n microsatellite polymorphisms in the first intron was related to maternal cortisol concentration (R²=0.26, B=96.27, p=0.007), whereas as the newborn’s cortisol concentrations were independent of fetal and maternal HSD11B2[CA]n microsatellite polymorphism. Conclusions: Our study showed for the first time that the fetal HSD11B2[CA]n microsatellite polymorphism of the HSD11B2 gene in healthy uncomplicated human pregnancy is associated with maternal
cortisol concentration. This indicates that fetal genes controlling cortisol metabolism may affect maternal cortisol concentration and hence physiology in healthy pregnant women.

Introduction

During mammalian pregnancy, the circulating concentration of cortisol (in rodents, corticosterone) in the mother is much higher than that in the fetus [1]. Since the placenta is the only barrier between the mother and her fetus, this gradient in cortisol concentrations suggests that there is a placental barrier preventing maternal cortisol from crossing into the fetus. Indeed, the intracellular enzyme 11 beta-hydroxysteroid dehydrogenase 2 (11b-HSD2) [2], because of converting hormonally active cortisol and corticosterone to inactive cortisone and 11-dehydrocorticosterone [2], and P-glycoprotein [3], which are expressed in the placental syncytiotrophoblast of humans and a range of other animal species, serve as the maternofetal interface glucocorticoids barrier. Substantial variability in 11b-HSD2 (encoded by the HSD11B2 gene) expression has recently been observed among placentas in humans. Although the variable expression may be caused by different physiopathologic factors [4, 5], genetic polymorphisms of the HSD11B2 gene are believed to represent a source of the 11b-HSD2 expression and activity variability. In our previous study, we had confirmed the fetal P-glycoprotein’s encoding gene ABCB1 subunit C3435T was related to the cortisol concentration in maternofetal interface during normal pregnancy [6]. Now, we analyzed the impact of functional polymorphisms of the human 11b-HSD2 gene (HSD11B2[CA]n microsatellite polymorphisms in the first intron) on maternal and fetal cortisol concentrations at delivery.

Materials and Methods

Clinic data collection

The study was approved by the ethics committee of the First Affiliated Hospital of Jinan University, Guangzhou, China. We invited a total of 400 Chinese women who delivered their babies at the Obstetric Department of the First Affiliated Hospital of Jinan University from March 2010 to October 2010 to participate in the study. Inclusion criteria were as follows: (1) the newborn was born without structural anomalies; (2) singleton pregnancy; (3) no HIV and no syphilis infection; (4) no drug abuse; (5) no history of smoking or alcohol consumption during pregnancy; (6) pregnancies complicated by pregnancy induced hypertension, diabetes mellitus, impaired glucose tolerance and stillborns were excluded from the study; (7) no steroid medication during pregnancy. After exclusion of cases that did not fulfill the inclusion criteria or were not willing to participate, we finally included 187 mother and fetus pair cases. After obtaining written consent, a structured medical history was taken. Chinese guidelines for medical follow-up in pregnancy comprise the perinatal health manual which contains essential data about the pregnancy. The data in the 'Perinatal health manual' were also used to judge whether the women fulfilled all inclusion. The following data were extracted into our database: nationality, age, body height, body weight before and during pregnancy, gravidity, parity, gestational age at delivery, smoking before/during pregnancy, and blood pressure readings at all follow-up visits.

The labor uterine contraction states when taking blood were noted. We used the definition of different labor-uterine contractions states according to a recent publication [7].

Category 1: No labor: normal pregnancy and no uterine contractions;
Category 2: The criteria of the threatened labor requires any one of the following: a) bloody "show", b) lightening, c) false labor: d) contractions occur at irregular intervals, e) contractions duration not exceeded 30 seconds, f) contractions intervals remain long, g) contractions intensity remains unchanged, h) discomfort is chiefly in the lower abdomen, j) cervix does not dilate, i) discomfort usually is relieved by sedation;
Category 3: In labor: a) contractions occur at regular intervals, b) contractions intervals gradually shorten, c) contractions duration exceed 30 seconds and 5-6 minutes intervals, d) contractions intensity gradually increases, e) discomfort is chiefly in the back and abdomen, f) cervix dilates, g) discomfort usually is not stopped by sedation;

Category 4: Oxytocin induction of labor: Oxytocin was used to induce or augment uterine contraction.

Category 5: Normal vaginal delivery.

All maternal blood samples were collected when pregnant women went to the hospital and waited for the delivery, so the maternal blood was taken before the usage of oxytocin or any other analgesics and before the delivery of the newborn in the delivery room or on the ward, so the uterine contractions states for maternal blood sample collection includes only three pathophysiological groups: a)Women without labor: group 1 b) Women with threatened labor: group 2 c) Women with fully active labor: group 3

Newborn's blood was taken right after delivery. Thus there were also cases of category 4 and 5.

Sample collection and blood cortisol assay

Newborn’s blood was collected from the umbilical cord within 10 minutes after delivery. Midwives collected maternal blood from a cubital vein in the delivery room or on the ward before delivery. We took only one blood sample from each mother and each newborn. Time of taking blood from both the mother and the newborn and the different labor-uterine contractions states of the pregnant women were documented. Samples were processed immediately and were storage at -20°C until analysis. Blood cortisol concentrations were measured by fluorescence polarization immunoassay (FPIA) (Abbott, USA, Catalog number 2G98 217-232 6/05) on the AxSYM System (Abbott, USA). All the blood samples were measured by experienced technologists in a certified laboratory of the hospital. Intra-assay variation was 5.9 ± 2.1 %, inter-assay variation was 9.1 ± 3.1 %, Cross reactivity to cortisone was less than 10%, Cross-reactivity to aldosterone was less 5 %.

Genetic analyses

DNA was extracted from peripheral whole blood of each subject using a DNA extraction kit (Simgen, Hangzhou, China). The PCR assay used Cy5-labelled sense primer: 5’-GGTGTTGCA and antisense primer: 5’-CTCTGAGGAGGAGGTACTTC-3’. PCR reaction System (15ul): the reaction mixture contained 1ul of genomic DNA, 1ul of each primer, 1ul dNTP, 1.5ul pfu buffer, 0.2ul of pfu Taq polymerase (Takara Biotech, Dalian, China) and double distilled water to a volume of 15 ul. The cycling conditions were as follows: After the initial denaturation at 95°C for 5 minutes, then 22 amplification cycles were carried out according to the following temperature profile: 30s at 95°C, 30s at 62°C, 20s at 72°C. The final extension lasted for 30 minutes at 72°C. PCR products were then sized by capillary electrophoresis on Beckman GeXP Genetic Analysis System (Beckman Coulter, Inc. Kraemer Boulevard Brea, CA, USA).

Statistical analysis

Data were analyzed with SPSS version 19.0. The difference in allele or genotype frequency was determined using the chi-square test. Hardy-Weinberg genotype frequencies were assessed using the chi-square test. 95% confidence intervals were calculated for all observed allele frequencies. Results are presented as mean±standard deviation (χ ±S). One-way ANOVA was used for comparison of continuous variables in different genotype group. Student's unpaired t-test was used for comparison of continuous variables between combined genotype groups. Multiple linear regression analysis was performed to analyze the impact of the HSD11B2 microsatellite polymorphism on newborn’s and maternal cortisol concentrations. We included the following known independent factors into the model: gestational age at delivery, newborn's gender, the labor uterine contraction states when taking blood and time (day-time) when taking blood (cortisol secretion is time dependent). Pearson's correlation coefficient and the regression coefficient B were used to estimate the strength of a correlation. A p-value of less than 0.05 was considered significant.

Results

Description of the cohort

The mean maternal age at delivery was 27.81±3.27 years. The mean maternal body mass
index (BMI) before pregnancy was 20.05±2.51 kg/m$^2$. The mean gestational age at delivery was 276.16±7.53 days. The mean birth weight was 3327.33±447.35 g. The maternal cortisol was 918.32±370.98 nmol/L, the newborn’s cortisol was 234.82±139.84 nmol/L and the ratio between maternal blood and newborn’s blood was 4.79±2.72 (for more details see table 1).

The cortisol diurnal rhythm and stress hormone trait

Based on the dynamic pattern of the cortisol diurnal rhythm [8, 9], we established the following groups to check in our cohort whether the cortisol secretions follows the physiological circadian rhythm. Cortisol values were separated by blood taking time points:

- group 1, range: 7:00-9:00,
- group 2, range: 9:00-15:00) and
- group 3, range: 15:00- the next day 7:00)

The newborn’s cortisol was 191.55±135.28 nmol/L (n=14) in group 1, 202.63±107.3 nmol/L (n=86) in group 2 and 273.59±159.2 nmol/L (n=87) in group 3. There is no difference between any groups. The maternal cortisol was 1314.59±674.79 nmol/L (n=9) in group 1, 914.57±274.78 nmol/L (n=82) in group 2 and 886.34±392.82 nmol/L (n=96) in group 3. The maternal cortisol in group 1 was significantly different from the other two groups.

Regarding to the physiological trait of glucocorticoid, it is easily influenced by the stress such as the uterine contractions before delivery [10]. Labor was thus divided in different pathophysiological stages (see Method section).

We analyzed the maternal and newborn’s cortisol in different uterine contractions states: Maternal blood samples were collected before the usage of oxytocin and the delivery:

- Maternal group 1 (no labor; n=74, cortisol=840.95±283.4 nmol/L),
- Maternal group 2 (threatened labor; n=68, cortisol=859.87±290.37 nmol/L),
- Maternal group 3 (in labor; n=45, cortisol=1117.8±500.47 nmol/L) for maternal blood collection state.

Maternal cortisol in group 3 (ladies in full labor) was significantly higher as compared to group 2 (threatened labor) (p<0.05) and group 1 (no labor) (p<0.05). Group 1 and 2 were statistically not different.

We also analyzed newborn’s cortisol in relationship to the uterine contractions: the labor-uterine contractions states for newborn’s blood sample collection includes 5 types. Blood was taken right after delivery (see Method section).
Newborn group 1 (no labor and then baby delivered by cesarean section, n=45, cortisol=141.55±30.87 nmol/L).

Newborn group 2 (threatened labor and then baby delivered by cesarean section, n=33, cortisol=150.53±51.35 nmol/L).

Newborn group 3 (in labor and then baby delivered by cesarean section, n=37, cortisol=260.33±140.72 nmol/L).

Newborn group 4 (oxytocin induction of labor and then baby delivered by cesarean section, n=13, cortisol=194.93±108.23 nmol/L)

Newborn group 5 (baby delivered by vaginal delivery, n=59, cortisol=352.45±144.87 nmol/L).

Newborn's cortisol in group 3 and group 5 were significantly different from other groups (p<0.05).

HSD11B2 allele distribution

The allele distribution in our population according to the CA repeat length is summarized in table 2. We confirmed 8 alleles (15-22 CA repeats, 235 bp to 249 bp of PCR product), yielding a heterozygosity of 49.20% in maternal group and 50.80% in fetal group. There was no statistic difference between the two groups (P>0.05).

Table 2. Allele distribution in our population according to the CA repeat length

<table>
<thead>
<tr>
<th>Allele</th>
<th>Maternal group (n=374)</th>
<th>Fetal group (n=374)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>4 (1.07)</td>
<td>2 (0.53)</td>
</tr>
<tr>
<td>16</td>
<td>30 (8.02)</td>
<td>21 (5.61)</td>
</tr>
<tr>
<td>17</td>
<td>10 (2.67)</td>
<td>12 (3.21)</td>
</tr>
<tr>
<td>18</td>
<td>18 (4.81)</td>
<td>19 (5.08)</td>
</tr>
<tr>
<td>19</td>
<td>252 (67.38)</td>
<td>252 (67.38)</td>
</tr>
<tr>
<td>20</td>
<td>46 (12.30)</td>
<td>49 (13.1)</td>
</tr>
<tr>
<td>21</td>
<td>13 (3.48)</td>
<td>16 (4.28)</td>
</tr>
<tr>
<td>22</td>
<td>1 (0.27)</td>
<td>3 (0.8)</td>
</tr>
</tbody>
</table>

Table 3. Clinical and biochemical parameters according to the maternal CA repeat allele length (n=187)

<table>
<thead>
<tr>
<th></th>
<th>SS (n=4)</th>
<th>SL(n=36)</th>
<th>LL(n=147)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal cortisol, nmol/L</td>
<td>813.18±403.73</td>
<td>946.79±380.75</td>
<td>913.87±369.63</td>
<td>0.689</td>
</tr>
<tr>
<td>Newborn’s cortisol, nmol/L</td>
<td>306.68±224.94</td>
<td>241.2±133.36</td>
<td>231.15±139.00</td>
<td>0.445</td>
</tr>
<tr>
<td>Maternal/newborn’s cortisol ratio</td>
<td>3.36±2.48</td>
<td>4.54±2.5</td>
<td>4.9±2.78</td>
<td>0.408</td>
</tr>
<tr>
<td>Gestational age at delivery (day)</td>
<td>277.25±5.91</td>
<td>278.11±6.71</td>
<td>275.65±7.71</td>
<td>0.731</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3500±752.77</td>
<td>3324.44±415.45</td>
<td>3323.33±448.44</td>
<td>0.639</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>2/2</td>
<td>24/12</td>
<td>76/71</td>
<td></td>
</tr>
<tr>
<td>Maternal age, years</td>
<td>29.25±3.77</td>
<td>27.89±3.13</td>
<td>27.75±3.3</td>
<td>0.546</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1.75±0.96</td>
<td>1.86±1.29</td>
<td>1.59±0.99</td>
<td>0.820</td>
</tr>
<tr>
<td>Parity</td>
<td>1.25±0.5</td>
<td>1.31±0.67</td>
<td>1.10±0.3</td>
<td>0.479</td>
</tr>
<tr>
<td>Maternal height ,cm</td>
<td>160±8.52</td>
<td>160.08±3.48</td>
<td>160.24±4.49</td>
<td>0.991</td>
</tr>
<tr>
<td>BMI before pregnancy, kg/m²</td>
<td>22.16±2.34</td>
<td>20.3±2.8</td>
<td>19.93±2.42</td>
<td>0.103</td>
</tr>
<tr>
<td>Maternal weight before pregnancy, kg</td>
<td>57.25±12.09</td>
<td>52.08±7.65</td>
<td>51.28±7.37</td>
<td>0.165</td>
</tr>
<tr>
<td>Maternal weight at delivery, kg</td>
<td>72.88±13.68</td>
<td>69.06±9.01</td>
<td>67.28±8.46</td>
<td>0.303</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD

HSD11B2 genotype and cortisol

Regarding to the HSD11B2[CA]n genotype, there were 24 genotypes of microsatellite polymorphisms in the first intron of the HSD11B2 gene on this study population. According to the previous studies [11, 12], we classified the CA repeats less than 17 as short allele(S), the CA repeat times more than 17 as long allele(L), so the 24 genotypes were divided into three genotype groups: SS, SL and LL group. Descriptive data of our population were not significantly different based on maternal or fetal SS, SL and LL group (see table 3, 4). However, there was a trend indicating that maternal cortisol concentrations might be different when considering fetal SS+SL and fetal LL groups (1067.68±538.74 vs 886.38±317.73, P=0.079) (see table 5). More over, this difference become significant (R²=0.26, B=96.27, p=0.007)
When considering confounding factors: gestational age at delivery, newborn's gender, the labor uterine contraction states and the cortisol diurnal rhythm, when the same analysis method were adopted, newborn's cortisol and newborn's cortisol/maternal cortisol-ratio were not significantly correlated with any of maternal HSD11B2 genotype group (data not shown). In this study cohort, 4 women are not of HAN Chinese ethnic background. All the results keep same when the four cases are deleted in the statistic analysis (data not shown).
Discussion

In the current prospective birth cohort study in southern Asian, we analyzed the association of a functional polymorphisms of the human 11b-HSD2 gene, the HSD11B2[CA]n microsatellite polymorphisms of mothers and their newborns and measured maternal and newborn's cortisol concentrations. We could demonstrate that the fetal HSD11B2[CA]n microsatellite polymorphism was associated with maternal cortisol concentrations, whereas newborn's cortisol concentrations was independent of the HSD11B2[CA]n microsatellite polymorphism.

The concentration of cortisol in maternal circulation is 5-10 times higher as compared to the fetal circulation, the placenta plays a barrier role between them [1]. Our data are in agreement with this concept. Cortisol secretion is affected by pathophysiological condition such as preeclampsia [13] and is associated with the glycemic control during pregnancy [14]. In order to avoid such confounding, we excluded cases of gestational diabetes and gestational hypertension/preeclampsia. Since we took blood, when the women came to the delivery room, maternal cortisol secretion might be modulated by stress such as labor and the cortisol diurnal rhythm. We thus considered labor status and the cortisol diurnal rhythm as confounding factor in our multivariate regression models analyzing the association between the HSD11B2[CA]n microsatellite polymorphism and cortisol concentrations in the mothers and newborns.

The HSD11B2 gene is located in the human chromosome 16q22, the gene consists of 5 exons. Single nucleic polymorphisms in the exons have been identified in many patients with the syndrome of apparent mineralocorticoid excess (AME). Offspring of these patients have a high mortality and low birth weight [15]. These mutations, however, are too rare to be relevant in our non-selected general cohort of healthy pregnant women. We thus decided to use the HSD11B2[CA]n microsatellite polymorphism. This polymorphism is much more frequent in the general population and was shown to affect cortisol plasma concentrations [11, 16]. The HSD11B2[CA]n microsatellite polymorphism in the first intron is associated with sodium sensitive essential hypertension [11, 17], impaired glucose tolerance and type 2 diabetes [16] and birth weight–adolescent blood pressure associations [18]. But polymorphisms in the gene have not been consistently associated with hypertension [11, 17, 19, 20]. In vitro gene transfer in human or rabbit kidney cortical collecting duct cells studies showed that longer CA repeats resulted in lower HSD11B2 expression [11]. Data in humans trying to associate HSD11B2 CA

![Image of graph showing maternal cortisol according to fetal HSD11B2 (LL versus SL+SS). Shown are means and 95% confidence interval of the mean. The correlation coefficient and probability value from multivariable regression analyses are given, see also table 6.](image-url)
repeats/genotypes with surrogates of the renal HSD11B2 activity (urinary excretion of cortisol and cortisone or the ratio of urinary cortisol to cortisone) did not confirm the in vitro data so far [11].

Beside the kidney, the HSD11B2 gene is also expressed in the human placenta [2]. Our study is the first to analyze the potential association between plasma cortisol of mothers and their newborns where the placental 11β-HSD2 might contribute to the regulation of plasma cortisol concentrations. We could demonstrate that the fetal HSD11B2[CA]n microsatellite polymorphism is associated with maternal cortisol concentrations. Based on our data, we suggest that the shorter fetal CA-repeat length results in decreased 11β-HSD2 expression or activity leading to slower cortisol degradation in the maternal circulation. The 11β-HSD2 is expressed in the syncytiotrophoblast layer as shown by immunohistochemistry. The syncytiotrophoblast layer is of fetal origin and has contact to maternal blood in the placenta. Thus our data fits very well with our knowledge of the anatomy of the human placenta [21]. Our study showed that fetal genes affect maternal physiology. The concept that genes of one organism influence the physiology of another organism originated from bacterial and viral infections. It was shown that bacteria/viruses may alter the host metabolism; for example, to stimulate nutrient supply to the parasite [22, 23]. Interaction of one organism with the metabolism of another organism of the same species is seen in mammals mainly during pregnancy; Tamimi et al. studied maternal dietary intake during the second trimester of pregnancy and suggested that the fetus may be able to modulate its mother's nutritional input, because women pregnant with a male fetus had a higher energy intake compared with women pregnant with a female fetus. After adjustment for confounding factors, this related to an extra 796 kJ/d contributed by 8% higher protein, 9.2% higher carbohydrates, and more than 10% higher lipid intake in women pregnant with a male fetus compared with women pregnant with a female fetus [24]. This finding is in line with previous reports from our group. We had shown that fetal sex may substantially affect maternal blood pressure and glycemic control during pregnancy in relation to the maternal PROGINS progesterone receptor gene polymorphism [25], the maternal peroxisome proliferator-activated receptor gamma2 (PPARc2) Pro12Ala polymorphism [26] and maternal ACE I/D polymorphism [27].

Based on these phenomenon, prenatal glucocorticoid therapy reduces birthweight, and steroids are known to exert long-term effects during specific “windows” of development. In rats, birthweight is reduced following prenatal exposure to the synthetic glucocorticoid dexamethasone, which readily crosses the placenta, or to carbenoxolone, an inhibitor of the 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). The placenta is the physiological fetoplacental “barrier” to endogenous glucocorticoids. Later in life these animals exhibit permanent hypertension, hyperglycemia, and increased hypothalamic-pituitary-adrenal axis activity, and gene mutations result and other reports, Seckl [28] had initially suggested that glucocorticoid programming may explain, in part, the association between fetal events and subsequent disorders in adult life. However, in our cohort, only the fetal HSD11B2[CA]n microsatellite polymorphism is associated with maternal cortisol concentrations, but not related to fetal growth (fetal birth weight). There is no contradiction to Seckl because neither the maternal or fetal HSD11B2 n microsatellite polymorphism is associated with fetal cortisol concentration. More over, this is line with the Dwyer et al. ‘s study [18] which showed also that the fetal HSD11B2[CA]n microsatellite polymorphism is not related to the birth weight in Caucasian population.

Study limitations are the fact that we did not measure concentrations of the inactive metabolite cortisone. The cortisol to cortisone ratio would provide more message of 11 β-HSD2 activity. In addition, a replication in an independent Asian birth cohort is missing. Thus, our data needs to be confirmed in independent Asian birth cohorts in the future.
Conclusion

We showed for the first time that the fetal HSD11B2[CA]n microsatellite polymorphism in healthy normal pregnancy is associated with maternal cortisol concentrations. Together with our previous work on the ABCB1 polymorphism, we suggest that the placental glucocorticoid barrier genes may play an important physiological regulation function on cortisol concentration in the materno-fetal interface during pregnancy.

Conflict of Interests

The authors declared no conflicting interests.

Acknowledgment

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