Promoter Methylation Changes in the Placental DIO3 and CRH Genes Are Involved in the Second Trimester Maternal Depression Induced Preterm Birth and Small for Gestational Age Birth

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Abstract

Background

Recent studies suggest that the incidence of preterm birth and SGA birth related to maternal depression, but the mechanism is unclear. The aim of this study was to explore the placental epigenetic changes involved in maternal depression induced preterm birth and small for gestational age birth.

Methods

Three hundred forty-five pregnant women were enrolled in this cohort study. Maternal depression in the second and third trimesters was assessed using a self-rating depression scale (SDS). We selected placental samples from pregnant women with depression and an equivalent number for samples from pregnant women without depression. Methylation of the promoter regions of the placental DIO3 and CRH genes was determined using next generation sequencing based on bisulfite sequencing PCR (NGS-BSP).

Results

There were 97 (28.1%) and 95 (27.5%) pregnant women who had depression in the second trimester and third trimester, respectively. The risk factors of preterm birth were older maternal age (RR = 1.43, 95%CI = 1.01–2.03), uterine infection (RR = 129.31, 95%CI = 2.16-7725.55), and maternal depression in the second trimester (RR = 79.97, 95%CI = 3.57-1792.56). The risk factors of SGA birth were low maternal BMI (RR = 0.71, 95%CI = 0.54–0.92), hypertensive disorder complicating pregnancy (HDCP, RR = 4.7, 95%CI = 1.18–18.72), and maternal depression in the second trimester (RR = 3.71, 95%CI = 1.31–12.16). Pregnant women with depression had higher placental methylation of CRH and DIO3 genes, and there was a correlation between placental methylation of CRH and DIO3 genes.

Conclusion

Our study suggested that the changes in the promoter region of the placental DIO3 and CRH genes were involved in maternal depression in the second trimester induced preterm birth and small for gestational age birth.

Introduction

Both preterm birth and small for gestational age (SGA) birth are major adverse birth outcomes of pregnancy and are a significant public health problem because of their potential short-term and long-term outcomes (1, 2). Depression and anxiety are the most common emotional problems during pregnancy, influencing 10–40% of pregnant woman (3). Recent studies suggested that the incidence of preterm and SGA births are correlated with maternal depression (4, 5), but the mechanism is unclear. Maternal

depression during pregnancy can influence the hypothalamic-pituitary-adrenal (HPA) axis of both mother and fetus (6). Moreover, thyroid hormone, secreted by the hypothalamus-pituitary-thyroid (HPT) axis, is closely related to fetal growth and prepartum maturation (7) and can regulate HPA axis hormones by mediating corticotropin-releasing hormone (CRH) (8). Placental CRH can modulate the synthesis of estrogen and progesterone, which is involved in the timing of birth (9). Three key deiodinase D3 is highly expressed in the placenta for most of gestation, and placental D3 activity decreases in the 2 weeks before birth (7). The deiodinase 3 (DIO3) gene encodes D3, which is largely expressed in the placenta and is linked to prenatal growth in humans (10). According to the above, we hypothesized that changes in placental CRH and D3 may determine the time of birth and fetal growth, but we were unable to detect placental CRH and D3 during pregnancy. Placental epigenetic changes, which are susceptible to the maternal environment, including physical and psychological disorders, can functionally regulate gene expression and phenotype (11). Therefore, the changes in the promoter region of placental DIO3 and CRH gene may be alternative index to explore the mechanism that involved in the relationship between maternal depression and preterm birth and SGA birth.

In this study, we use a prospective cohort study of pregnant women to analyze the relationship between preterm birth and SGA birth and maternal depression during pregnancy, and then investigate the mechanism by detecting methylation levels of the promoter regions of the placental DIO3 and CRH genes.

Materials And Methods

Ethics approval and consent to participate

This is a prospective cohort study conducted on pregnant women. The study protocol was approved by the research institute's committee of human research in the Second Affiliated Hospital of Shantou University Medical College (NO.2016027), and abided by the standards of the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from pregnancy women.

Participants

We collected data of pregnant women in the obstetrics clinic from January 2018 to January 2020 for prospective analysis. The inclusion criteria of pregnant women were as follows: (1) singleton pregnancy, (2) intended to have regular antenatal care and give birth in our hospital, and (3) be able to understand the relevant scale options in this subject. The pregnant women were excluded from this study if they and the baby's father met the following criteria: (1) had thyroid, liver, kidney, lung or heart disease before and after pregnancy, (2) had depression, anxiety disorder, somnipathy, schizophrenia, mania, dissociative personality disorder, and other mental disorders prior to pregnancy, and (3) pregnancy was by assisted reproduction.

Questionnaire survey

Participants were required to complete a questionnaire, at the first visit for antenatal care, which was used to collect variables including the age of the pregnant women, pre-pregnancy body mass index (BMI), education level, economic level, history of adverse pregnancy, pregnancy complications, and life style (smoking, drink, diet, sleep).

Data collection

Data collection was performed after delivery, including age of mother, BMI, exposure to passive smoking, adverse pregnancy history, education level, economic level, vegetarianism, pregnancy anemia, hypertensive disorder complicating pregnancy (HDCP), pregnancy diabetes, pregnancy hypothyroidism, placenta previa, uterine infection, method of delivery, preterm birth, gestational age, birth weight, and gender. Adverse pregnancy history included spontaneous abortion, induced abortion, stillbirth, and preterm birth (12). Education level was divided into with and without a college education. Economic level was divided into two levels according to the minimum taxable income, which is 5000 yuan in China. Diagnosis of pregnancy anemia (13), hypertensive disorder complicating pregnancy (HDCP) (14), pregnancy diabetes (15), uterine infection (16), and placenta previa (17) were according to the diagnosis guidance.

Assessment of maternal depression in the second trimester

Pregnancy women were asked to fill out a self-rating depression scale (SDS) during the second trimester and third trimester, respectively. The SDS consisted of 20 items, including two items of psycho-emotional symptoms, eight items of physical disorders, two items of psychomotor disorders, and eight items of depressive psychological disorders. The score for each item ranged from 1 to 4, and the total score was multiplied by 1.25 to obtain a standard SDS scale. A standard score $\geq$ 50 indicates depression (18). The total reliability coefficient of the SDS was 0.784 (Cronbach's alpha) in Chinese women of rural areas, and it has been proven to be a valid and efficient tool for screening depression in the Chinese population (18).

Diagnosis of preterm birth and SGA birth

Preterm birth is a delivery before 37 weeks gestational age. The diagnosis of SGA is a birth weight of infants less than the 10th percentile for gestational age, using the Chinese neonatal birth weight curve (19).

Placenta sample collection

Approximately 1 g tissue from the maternal side of the placenta in each participant was obtained immediately after delivery. Samples of placental parenchyma were carefully dissected by trained research assistants to assure the maternal decidua was separated from the sample, from which the DNA extracted was of fetal origin. The samples were snap-frozen in liquid nitrogen, and after at least 1 hour later, stored in sample tubes at 80℃ for further examination.

Selection of placental samples for methylation detection

Methylation levels of the promoter region of the CRH and DIO3 genes were assessed by next generation sequencing-based bisulfite sequencing PCR (NGS-BSP). Because NGS-BSP is expensive, we used a nested case-control study to select specimens for examination. There were 97 pregnant women with depression in the second trimester in this study. We selected placental samples from these pregnant women and were matched 1:1 with samples from pregnant women without depression but the same maternal age, BMI, educational level, economic level, adverse pregnancy history, HDCP, pregnancy diabetes, uterine infection, and method of delivery.

Assessment of methylation levels of the promoter regions of placental CRH and DIO3 genes by NGS-BSP

Methylation was assessed by NGS-BSP. BSP primers were designed using the online MethPrimer website. DNA samples were extracted using the QI Amp DNA Mini Kit (Qiagen, Inc.). Purified DNA was quantified using an ND-1000 spectrophotometer (Nanodrop), and DNA samples (1μg) were bisulfite-modified using an EZ DNA Methylation Kit (Zymo Research). For each sample, BSP products of target genes were generated, pooled equally, and subjected to adaptor ligation. Barcoded libraries from all samples were sequenced on the Illumina HiSeq platform using a paired-end 150 bp strategy.

Statistical analysis

For continuous variables, the Shapiro-Wilk test was used to determine the normal distribution of the continuous variables, and the Wilcoxon-Mann-Whitney U-test was conducted for skewed distributions (presented as the median and the interquartile range). Descriptive statistics for categorical variables were reported as frequency (percentage) and compared using the Pearson chi-square test or Fisher's exact test, as appropriate. We used the Spearman coefficient to assess the correlation among the level of methylation of CpG sites, and to analyze the relationship among the SDS score. Logistic regression was used to examine the risk factors of preterm birth and SGA birth, the correlation between any two independent variables in the logistic regression equation should be less than 0.8. Skewed distribution data were log-transformed to obtain a normal distribution. Odds ratios with 95% CIs were calculated. All statistical analyses were performed with SPSS version 24.0, and a p-value < 0.05 was considered statistically significant.

Results

Occurrence and characteristics of preterm birth and SGA birth in this study population

A total of 345 pregnant women were enrolled in this study. There were 97 (28.1%) and 95 (27.5%) pregnant women who had depression in the second trimester and third trimester, respectively. Twenty-five pregnant women had a preterm birth, and thirty-five pregnant women had an SGA birth. The incidence of preterm births and SGA births in this prospective cohort was 7.2% and 10.1%, respectively. In order to analyze the effect of clinical variables on preterm and SGA births, we examined the association between

characteristics and clinical variables in the pregnant women with and without preterm births (Table 1) and with and without SGA births (Table 2).

Table 1
Characteristics of pregnant women with and without preterm birth in this study.

	All	With preterm	Without preterm	*P*
	345	25 (7.2%)	320 (92.8%)	
Maternal age	29 (27–33)	32.5 ± 5.3	29 (27–32)	0.02
BMI	20.1 (18.5–22.1)	22.5 ± 3.1	20 (18.4–21.9)	0.007
Passive smoking				0.10
Yes	218	13 (6%)	205 (94%)	
No	127	12 (9.4%)	115 (90.6%)	
Adverse pregnancy history				0.09
Yes	24	4 (16.7%)	20 (83.3%)	
No	321	21 (6.5%)	300 (93.5%)	
Education level				0.89
Non-high education	225	16 (7.1%)	209 (92.9%)	
High education	120	9 (7.5%)	111 (92.5%)	
Economic level				0.52
Low income	131	8 (6.1%)	123 (93.9%)	
Non-low income	214	17 (7.9%)	197 (91.1%)	
Vegetarianism				0.99
Yes	41	3 (7.3%)	38 (92.7%)	
No	304	22 (7.2%)	282 (92.8%)	
Pregnancy anemia				0.81
Yes	89	6 (6.7%)	83 (92.3%)	
No	256	19 (7.4%)	237 (92.6%)	
Pregnancy hypothyroidism				0.99
Yes	4	0 (0%)	4 (100%)	
No	341	25 (7.3%)	316 (92.7%)	
Pregnancy diabetes				0.008
Yes	66	10 (15.2%)	56 (84.8%)	

	All	With preterm	Without preterm	*P*
No	279	15 (5.4%)	264 (94.6%)	
HDCP				0.63
Yes	19	1 (5.3%)	18 (94.7%)	
No	326	24 (7.4%)	302 (92.6%)	
Second trimester Maternal depression				0.01
Yes	97	13 (13.4%)	84 (86.6%)	
No	248	12 (4.8%)	236 (95.2%)	
Delivery method				0.34
Vaginal	148	13 (8.8%)	135 (91.2%)	
Cesarean	197	12 (6.1%)	185 (93.9%)	
Uterine infection				0.005
Yes	12	4 (33.3%)	8 (66.7%)	
No	333	21 (6.3%)	312 (93.7%)	
Placenta previa				0.05
Yes	12	3 (25.0%)	9 (75.0%)	
No	333	22 (6.6%)	311 (93.4%)	
Gestational age	39.14	36.00	39.29	< 0.001
	(38.43–39.86)	(32.86–36.71)	(38.71-40.00)	
Birth weight	3.15 (2.95–3.40)	2.38 ± 0.56	3.20 (3.00-3.40)	< 0.001
Gender of infants				0.19
Male	177	16 (9.1%)	161 (90.9%)	
Female	168	9 (5.4%)	159 (94.6%)	

Table 2
Characteristics of pregnant women with and without SGA birth.

	All	With SGA	Without SGA	P
	345	35 (10.1%)	310 (89.9%)	
Maternal age	29 (27–33)	29 (27–33)	28 (26–32)	0.22
BMI	20.1 (18.5–22.1)	19.2 (17.3–21.0)	20.3 (18.6–22.1)	0.07
Passive smoking				0.43
Yes	218	20 (9.2%)	198 (90.8)	
No	127	15 (11.8%)	112 (88.2%)	
Adverse pregnancy history				0.99
Yes	24	2 (8.3%)	22 (91.7%)	
No	321	33 (10.3%)	288 (89.7%)	
Education level				0.15
Non-high education	225	19 (8.4%)	206 (91.6%)	
High education	120	16 (13.3%)	104 (86.7%)	
Economic level				0.01
Low income	131	20 (15.3%)	111 (84.7%)	
Non-low income	214	15 (7.0%)	199 (93.0%)	
Vegetarianism				0.99
Yes	41	4 (9.8%)	37 (90.2%)	
No	304	31 (10.2%)	273 (89.8%)	
Pregnancy anemia				0.25
Yes	89	6 (6.7%)	83 (93.3%)	
No	256	29 (11.3%)	227 (88.7%)	
Pregnancy hypothyroidism				0.08
Yes	4	1 (25%)	3 (75%)	
No	341	34 (10%)	307 (90%)	
Pregnancy diabetes				0.75
Yes	66	6 (9.1%)	60 (90.9%)	

	All	With SGA	Without SGA	P
No	279	29 (10.4%)	250 (89.6%)	
HDCP				0.02
Yes	19	5 (26.3%)	14 (73.7%)	
No	326	30 (9.2%)	296 (90.8%)	
Second trimester Maternal depression				0.04
Yes	97	17 (17.5%)	80 (82.5%)	
No	248	18 (7.3%)	126 (92.4%)	
Third trimester Maternal depression				0.39
Yes	95	12 (12.6%)	83 (87.4%)	
No	250	23 (9.2%)	227 (90.8%)	
Delivery method				0.72
Vaginal	197	21 (10.7%)	176 (89.3%)	
Cesarean	148	14 (9.5%)	134 (90.5%)	
Preterm delivery				0.09
Yes	25	5 (20.0%)	20 (80.0%)	
No	320	30 (9.4%)	290 (90.6%)	
Uterine infection				0.78
Yes	12	2 (16.7%)	10 (83.3%)	
No	333	33 (9.9%)	300 (90.1%)	
Placenta previa				0.78
Yes	12	2 (16.7%)	10 (83.3%)	
No	333	33 (9.9%)	300 (90.1%)	
Gestational age	39.14	38.86	39.29	< 0.001
	(38.43–39.86)	(37.43–39.29)	(38.57-40.00)	
Birth weight	3.15 (2.95–3.40)	2.65 (2.40–2.75)	3.20 (3.00-3.45)	0.006
Gender of infants				0.47

	All	With SGA	Without SGA	P
Male	177	20 (11.3%)	157 (88.7%)	
Female	168	15 (8.9%)	153 (91.1%)	

Risk factors for preterm and SGA births

Because preterm delivery often occurs in the third trimester, we did not use the result of SDS of third trimester for analysis of preterm birth. According to logistic regression analysis of the pregnant women in this study (Table 3), the risk factors for preterm birth were older maternal age (RR = 1.43, 95%CI = 1.01–2.03), uterine infection (RR = 129.31, 95%CI = 2.16-7725.55), and second trimester maternal depression (RR = 79.97, 95%CI = 3.57-1792.56). The mean age of pregnant women with preterm birth in this study was 32.5 years, but it was 29 years for those without preterm birth. The incidence of preterm birth was 6.3% in the pregnant women who did not have uterine infection, which was increased to 33.3% in those with uterine infection. For pregnant women who did not have depresssion in the second trimester, there were 4.8% preterm births, but the incidence of preterm births rose to 13.4% in those with depression (Table 1).

Table 3
Logistic regression of the risk factors for preterm birth.

Variables	RR	95%CI	*P*-value
Age of mother	1.43	1.01–2.03	0.045
BMI	1.36	0.87–2.13	0.172
Adverse pregnancy history	0.81	0.04–19.06	0.897
Placenta previa	0.67	0.01–45.19	0.853
Pregnancy diabetes	5.20	0.37–73.11	0.222
Uterine infection	129.31	2.16-7725.55	0.020
Maternal depression	79.97	3.57-1792.56	0.006
BMI: body-mass index, RR: relative risk			

According to logistic regression analysis, the risk factors for SGA birth were low maternal BMI (RR = 0.71, 95%CI = 0.54–0.92), HDCP (RR = 4.7, 95%CI = 1.18–18.72), and second trimester maternal depression (RR = 3.71, 95%CI = 1.31–12.16) (Table 4). The incidence of SGA births was 9.2% in pregnant women who did not have HDCP, but it was increased to 26.3% in those who had HDCP. Pregnant women who did not have depression had 7.3% SGA births, but the incidence of SGA births rose to 17.5% in pregnant women who had depression in the second trimester (Table 2).

Table 4
Logistic regression of the risk factors for SGA birth.

Variables	RR	95%CI	*P*-value
BMI	0.71	0.54–0.92	0.010
Economic level	0.54	0.19–1.51	0.239
Pregnancy hypothyroidism	2.15	0.48–8.36	0.642
HDCP	4.70	1.18–18.72	0.028
Preterm delivery	5.74	0.74–44.68	0.095
Maternal depression	3.71	1.13–12.16	0.031
BMI: body-mass index, HDCP: hypertensive disorder complicating pregnancy, RR: relative risk,			

Methylation levels of the promoter regions of the placental CRH and DIO3 genes in mothers with and without depression in the second trimester

We found that methylation levels of the promoter region of placental CRH and DIO3 gene were higher in maternal depression group than non-depressed group (Fig. 1), and the methylation levels of the placental DIO3 CpG sites were correlated with those of CRH (Fig. 2).

Discussion

In our study, the occurrence of depression during pregnancy is in line with other findings that reported the prevalence of prenatal depression to range from 10–40% (3), and the incidence of preterm and SGA births is consistent with the preterm birth occurrence in prior Chinese studies (20, 21). Moreover, we found uterine infection is the most important risk factor of preterm births, and advance maternal age could also cause a higher incidence of preterm birth, as previously shown (22, 23). Consistent with results from other studies, low maternal BMI and HDCP are the major risk factors for SGA birth (24, 25). The above mention results indicate that our study population is representative.

Many studies suggest maternal depression during pregnancy can increase the cortisol level both in the pregnant women and fetus (26). Cortisol can stimulate production of CRH in the placenta (27). Epigenetic mechanisms have been suggested to mediate the lasting molecular embedding of exposures to abnormal cortisol and CRH levels (28). In this study, increased methylation of the placental CRH gene may be one of the indications that maternal depression during pregnancy causes changes in placental hormones. CRH is involved at the time of birth to regulate estrogen and progesterone levels as they control the contractile properties of the myometrium (29). Therefore, changes in the promoter region of placental CRH gene may be involved in maternal depression at second trimester inducing preterm birth.

Preterm birth and SGA are clinically relevant complications. The increase of cortisol levels due to depression during pregnancy could inhibit the HPT axis in pregnant women (8), resulting in decreased

circulating concentrations of thyroid hormones (7). Maternal thyroid hormones have an important role in fetal development, especially in the first and second trimester (7). The above mention results suggest that maternal depression could result in SGA births by CRH-mediated inhibition of the HPT axis of both mother and fetus. We did not find prenatal hypothyroidism be a risk factor for SGA births in this study. There are two possible reasons for this discrepancy. On the one hand, the sample size might not be enough in this study. On the other hand, a decreased level of thyroid hormones in the mother, caused by depression, could not result in hypothyroidism. However, the changes in the promoter region of the placental DIO3 gene can still reflect the changes of placental thyroid hormone.

Maternal depression has been shown to reduce placental expression of enzymes that alter specific epigenetic modifications, resulting in long-term consequences for endocrine responses (29). A decrease in the level of maternal thyroid hormones can decrease its transfer through the placenta, and then inhibit the activity of D3, whereby the reduced thyroid hormones are converted to an inactive form (7). We postulate that increases in the promoter region of the placental DIO3 gene may result from the increase of cortisol levels due to maternal depression in the second trimester. The ratio of D3 to D1 activity is high during gestation. Developmental changes occur in which D3 activity decreases before delivery (30). Therefore, the increase in placental DIO3 methylation shown here can reduce D3 activity, which may result in early delivery.

Conclusion

In summary, the results in this study suggest that maternal depression in the second trimester could disrupt the HPA axis, but also influence thyroid hormone transfer to the fetus from the placenta to result in a high incidence of preterm birth and SGA birth. The mechanism may be related to changes in methylation in the promoter region of the placental DIO3 and CRH genes. A better understanding of placental epigenetic changes in the CRH and DIO3 genes, resulting from maternal depression, may eventually result in more optimal management of maternal thyroid dysfunction, and this may also include aspects of emotional management at the second trimester. Moreover, because epigenetic alterations can result in long-term consequences for offspring behavior and endocrine response, screening and intervention of those offspring whose mothers had depression during pregnancy is also a consideration.

Abbreviations

BMI
body mass index; CRH:corticotropin-releasing hormone; DIO3:Deiodinase 3; HDCP:hypertensive disorder complicating pregnancy; HPA:hypothalamic-pituitary-adrenal; HPT:hypothalamus-pituitary-thyroid; NGS-BSP:generation sequencing based on bisulfite sequencing PCR; SDS:self-rating depression scale; SGA:small for gestational age birth.

Declarations

Ethics approval and consent of participate

This is a prospective cohort study conducted on pregnant women. The study protocol was approved by the research institute's committee of human research in the Second Affiliated Hospital of Shantou University Medical College (NO.2016027), and abided by the standards of the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from pregnancy women.

Consent for publication

All authors provided approval for publication of the content.

Competing interests

The authors declare that they have no competing interests in our study.

Availability of data and material

The data in this study are available from the corresponding author on reasonable request.

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Author Contributions Statement

JHY performed next generation sequencing-based bisulfite sequencing PCR, statistical analysis, and helped to write the manuscripts. YMZ wrote the manuscript and collected the data of preterm infants. JHD helped to collect the data of preterm infants. XML, LLX, XCD, and HLL collected the data of mother. PSC and YJH contributed in experiment design and reviewing the final manuscripts. The author reviewed and approved the final manuscripts.

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Figures

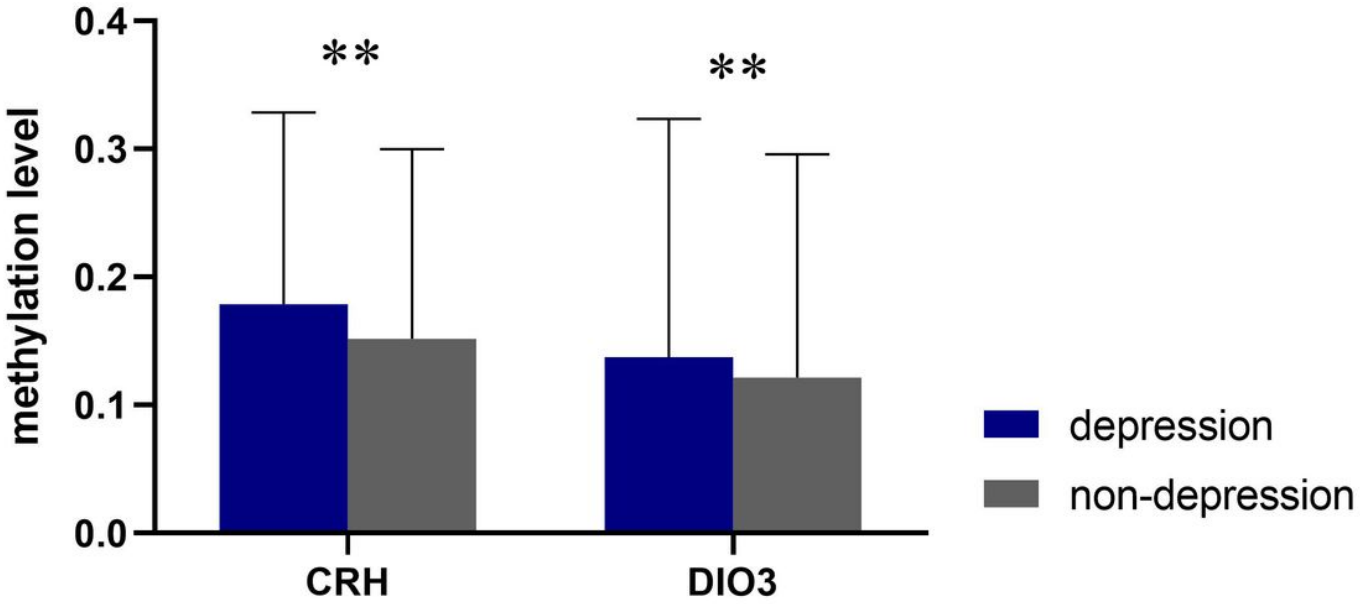

Figure 1

Comparison of DNA methylation levels of the placental DIO3 and CRH genes between pregnant women with and without depression in the second trimester. Placenta samples were divided into two groups according to maternal depression in the second trimester. DNA methylation levels in the promoter region of the placental DIO3 and CRH genes between pregnant women with and without depression were compared. The paired t-test was used for statistical analysis (*P<0.05; **P<0.01).

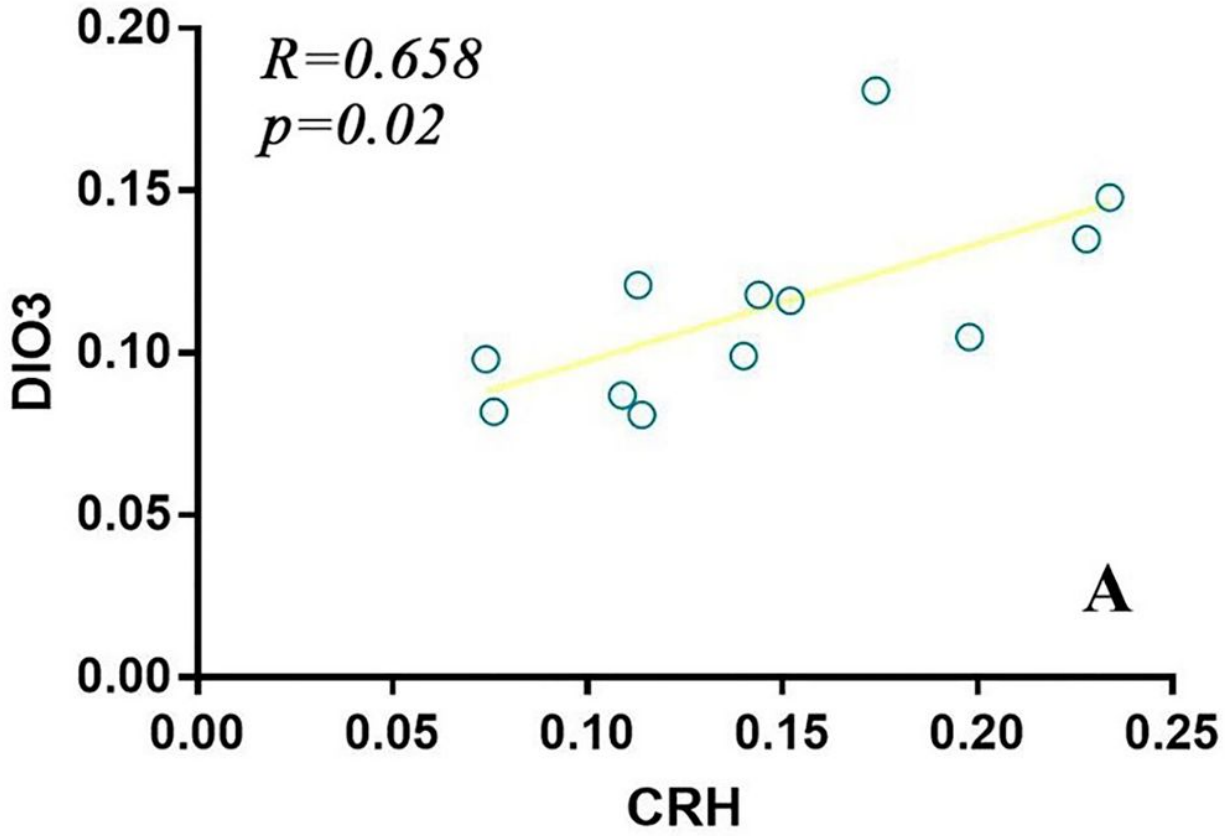

Figure 2

Correlation of DNA methylation levels of the placental DIO3 and CRH genes. Scatter plot showing the correlation of DNA methylation levels between the DIO3 and CRH genes in placenta. Pearson correlation statistics are shown in the top left corner.