Fetal growth restriction and methylation of growth-related genes in the placenta

Aim: To examine the associations between fetal growth restriction (FGR) and DNA methylation of six growth-related genes in human placenta. **Materials & methods:** A total of 181 mother-newborn pairs (80 FGR cases and 101 controls) were enrolled in this case-control study. Placental DNA methylation was measured by bisulfite pyrosequencing. **Results:** DNA methylation levels of *IGF2* and *AHRR* were positively associated with newborn birth weight and Quetelet's index, while DNA methylation levels of *HSD11B2* and *WNT2* were negatively associated with those fetal growth indicators. In addition, significantly elevated odds of FGR birth were associated with increasing DNA methylation of *HSD11B2* and *WNT2*, and decreasing DNA methylation of *IGF2*. **Conclusion:** Our findings demonstrated that placental DNA methylation levels of *IGF2*, *AHRR*, *HSD11B2* and *WNT2* were associated with measures of fetal growth.

First draft submitted: 17 June 2015; **Accepted for publication:** 2 October 2015; **Published online:** 18 December 2015

Keywords: birth weight • case-control study • DNA methylation • fetal growth restriction • placenta

Background

Infants who fail to achieve their own growth potential are considered to have fetal growth restriction (FGR) [1]. Such infants have an increased risk for perinatal morbidity and mortality, as well as impaired postnatal growth, neurodevelopment and cognitive abilities [2]. FGR infants also have increased risk for certain diseases later in life, particularly those comprised in the metabolic syndrome [3–5]. Because of these immediate and long-term outcomes associated with FGR, understanding the molecular mechanisms involved in the loss of fetal growth potential is critically important.

Throughout *in utero* development, the placenta, through the production of various enzymes and hormones, plays an important role in controlling fetal growth and development [6]. Recent work has made it increasingly clear that proper epigenetic regulation of both imprinted and nonimprinted genes

in placenta is important in placental development and fetal growth [7]. Investigating epigenetic alterations in placenta may add to our understanding of the molecular mechanisms behind reduced fetal growth *in utero* and increased disease susceptibility in later life.

Imprinted genes are highly expressed in placenta and have been shown to be indispensable for proper placental morphology and function [8,9]. *IGF2*, which is imprinted in tandem with *H19*, is the most intensively studied imprinted gene. Expression of *IGF2/H19* is regulated by differential and coordinated methylation of two imprinting control regions (ICRs; ICR1 and ICR2) in chromosome 11 imprinting cluster [10]. Recently, investigators have reported an association between placental DNA methylation of these ICRs and fetal growth indices (e.g., birth weight, head circumference and thorax circumferences) [11–13].

In addition to imprinted genes, some non-imprinted genes in placenta are also involved

Xirong Xiao^{†,1}, Yan Zhao^{†,2}, Rong Jin³, Jiao Chen², Xiu Wang², Andrea Baccarelli⁴ & Yunhui Zhang^{*,2}

¹Obstetrics & Gynecology Hospital, Fudan University, Shanghai 200032, China

²Department of Environmental Health, School of Public Health, Fudan University, Shanghai 200032, China

³The International Peace Maternity & Child Health Hospital of China Welfare Institute, Shanghai 200032, China

⁴Departments of Environmental Health, Harvard School of Public Health, 677 Huntington Ave, Boston, MA 02115, USA

*Author for correspondence:

Tel.: +86 215 423 7085

Fax: +86 216 404 6351

yhzhang@shmu.edu.cn

[†]Authors contributed equally

in fetal growth, and DNA methylation of their promoters and exons has been shown to be associated with fetal growth indices. One gene to such effect is *HSD11B2*. *HSD11B2* is a key gene involved in glucocorticoid metabolism [14]. High methylation of this gene in human placenta has been associated with reduced fetal growth [15,16].

Although a few studies have begun to examine the associations of placental DNA methylation with fetal growth, most of these studies lacked adjustment for potentially important confounders and thus few of them provided consistent results [11–13,17–18]. In addition, no study to date has examined the role of placental DNA methylation in the development of FGR. Thus, we conducted this case–control study to investigate how placental DNA methylation of six growth-related genes associates with measures of fetal growth, and further evaluate the risk of FGR birth in relation to placental DNA methylation.

Material & methods

Study population

This study is an extension of our previous case–control study, which was designed to investigate the association between prenatal environmental endocrine disruptors exposure and FGR [19]. Low birth weight (LBW) was defined as fetal birth weight <2500 g and gestational age (GA) ≥37 weeks [20]. Intrauterine growth restriction (IUGR) was defined as estimated fetal weight below the 10th centile for GA, based on ultrasound measurements of fetal biparietal diameter, head circumference, abdominal circumference and femur length [21–23]. Since in our study there is no significant difference in birth weight, birth length and Quetelet’s index between IUGR infants and LBW infants (see [Supplementary Table 1](#)), infants with either IUGR or LBW were diagnosed as FGR cases, and equal numbers of healthy newborns were included as controls. During December 2011 and November 2013, 220 mother–newborn pairs, includ-

Table 1. General characteristics of subjects by birth outcome status.				
Characteristics	Total (n = 181)	FGR (n = 80)	Control (n = 101)	p-value [†]
Birth weight (kg) [‡]	2.85 ± 0.61	2.29 ± 0.37	3.29 ± 0.34	<0.001**
Birth length (cm) [‡]	48.80 ± 2.31	46.97 ± 2.35	50.23 ± 0.74	<0.001**
Quetelet index (kg/m ²) ^{‡,§}	11.84 ± 1.86	10.34 ± 1.33	13.02 ± 1.26	<0.001**
Gestational age (year) [‡]	38.07 ± 2.51	36.60 ± 2.93	39.25 ± 1.18	<0.001**
Maternal age (year) [‡]	26.73 ± 4.32	26.26 ± 4.82	27.11 ± 3.86	0.196
Prepregnant BMI (kg/m ²) [‡]	19.66 ± 2.18	19.65 ± 2.50	19.67 ± 1.92	0.965
Gender:				0.063
– Male	93	38	55	
– Female	88	42	46	
Prenatal vitamin use:				0.302
– Yes	161	69	92	
– No	20	11	9	
Birth type:				0.051
– Vaginal delivery	135	54	81	
– Cesarean section	46	26	20	
Maternal education:				0.261
– Middle school	49	26	23	
– High school	49	18	31	
– College	83	36	47	
Monthly income (RMB):				0.062
– <5000	81	42	39	
– ≥5000	100	38	62	
[†] p-value calculated using the χ^2 -test and t-test. [‡] Values are shown as mean ± standard deviation. [§] Quetelet index was calculated as weight (in kg) divided by the square of height (in m). **p < 0.01. FGR: Fetal growth restriction; RMB: Ren Min Bi.				

Gene name	Median (25th–75th percentile)			p-value [†]
	Total (n = 181)	Case (n = 80)	Control (n = 101)	
<i>HSD11B2</i>	13.58 (11.77–14.77)	13.92 (12.25–15.25)	13.25 (11.30–14.50)	0.003**
<i>NR3C1</i>	63.96 (59.79–68.63)	64.11 (58.90–68.34)	63.79 (60.34–68.71)	0.620
<i>WNT2</i>	12.98 (8.49–43.01)	12.53 (8.63–43.45)	13.46 (8.42–43.17)	0.678
<i>IGF2</i> :				
– Pos.1	58.55 (55.72–61.49)	57.92 (54.65–61.27)	58.66 (56.74–61.87)	0.128
– Pos.2	45.77 (40.18–48.60)	44.03 (35.96–47.69)	46.35 (42.98–49.25)	0.005**
<i>IL6</i> :				
– Pos.1	6.13 (4.71–8.27)	6.23 (4.82–8.28)	6.10 (4.54–8.28)	0.796
– Pos.2	14.92 (11.34–18.51)	14.84 (12.04–18.22)	15.00 (10.89–18.53)	0.948
<i>AHRR</i> :				
– Pos.1	57.20 (52.49–61.99)	55.84 (51.23–60.07)	57.95 (53.95–63.48)	0.021*
– Pos.2	73.78 (67.97–80.39)	72.06 (66.60–77.93)	75.02 (69.88–80.91)	0.075
– Pos.3	41.23 (36.81–46.39)	41.01 (36.71–46.00)	41.44 (37.04–46.72)	0.673

[†]p-value calculated using the Mann–Whitney U test.
 *p < 0.05; **p < 0.01.
 Pos.1: The first studied CpG site; Pos.2: The second studied CpG site; Pos.3: The third studied CpG site.

ing 110 FGR cases and 110 healthy controls and their mothers, were enrolled in our case–control study. The mothers answered a detailed questionnaire concerning maternal weight, and height before pregnancy, maternal age, maternal smoking, drinking, dietary habits, etc., after delivery within 2 days. All subjects signed written informed consents approved by Fudan University's Institutional Review Board.

Placental samples were collected immediately after delivery. For each subject, eight biopsies of apparently normal tissue were collected (two from each of the four quadrants). All samples were taken from the maternal side of the placenta (the genes we studied mainly express on this side), 2 cm from the umbilical cord insertion site, free of maternal decidua. After washing three times in sterile phosphate-buffered saline, samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

One hundred and eighty-one subjects donated placental samples, thus we enrolled those 181 subjects (including 80 FGR cases and 101 healthy controls) in this present study.

Quantification of placental DNA methylation

A pooled sample (120 mg) for each placenta that comprised four biopsies (two from the upper left-hand quadrant and two from the lower right-hand quadrant) was used for DNA extraction. Genomic DNA was extracted using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Purified DNA was quantified using a ND1000

spectrophotometer (Nanodrop, DE, USA) and about 1 µg DNA was bisulfite modified using the EZ Methylation Gold-Kit (Zymo Research, CA, USA). Final elution was performed with 30 µl M-Elution Buffer.

Placental DNA methylation of six growth-related genes (*HSD11B2*, *NR3C1*, *WNT2*, *IGF2*, *IL6* and *AHRR*) was quantitated using bisulfite-PCR and pyrosequencing. *HSD11B2* and *NR3C1* are two genes involved in glucocorticoid metabolism. *WNT2*, *IGF2* and *IL6* are crucial for normal development of the placenta. *AHRR* serves to inhibit aryl hydrocarbon receptor transcription, which is involved in mediating xenobiotic metabolism. All those studied genes are involved in metabolic and endocrine function of the placenta and their expression levels have been associated with fetal growth restriction [24–26]. The PCR primers and position of each CpG site are shown in [Supplementary Table 2](#). Analysts were blind to all information concerning our subjects. To prevent batch effects from bisulfite treatments interfering with the analysis, samples were randomized across batches. Methylation level of each CpG dinucleotide was expressed as methylated cytosines over the sum of methylated and unmethylated cytosines. Each sample was tested in triplicate and the average was used for statistical analysis.

Some controls were included in every pyrosequencing run. To verify bisulfite conversion efficiency, a C outside a CG site was used in every assay as built-in control. After bisulfite treatment, the conversion of this C into T is expected to be 100%. We insert a C/T single-nucleotide polymorphism into the sequence to be analyzed that will result in a 100% T if conversion

Table 3. Crude and adjusted association between DNA methylation and birth weight.

Methylation	Birth weight			
	Crude model		Adjusted model [†]	
	β (95% CI)	p-value	β (95% CI)	p-value
<i>HSD11B2</i>	-0.048 (-0.083 to -0.012)	0.009**	-0.027 (-0.053 to -0.002)	0.033*
<i>NR3C1</i>	0.008 (-0.005–0.021)	0.229	-0.003 (-0.012–0.006)	0.557
<i>WNT2</i> :				
– High	-0.018 (-0.040–0.005)	0.121	-0.019 (-0.037 to -0.002)	0.034*
– Low	0.011 (-0.014–0.037)	0.387	0.010 (-0.005–0.025)	0.198
<i>IGF2</i> :				
– Pos.1	0.017 (0.001–0.034)	0.043*	0.009 (-0.002 to 0.020)	0.125
– Pos.2	0.010 (-0.001–0.020)	0.072	0.008 (0.001–0.015)	0.026*
<i>IL6</i> :				
– Pos.1	-0.003 (-0.032–0.026)	0.819	-0.008 (-0.027–0.011)	0.386
– Pos.2	0.006 (-0.010–0.021)	0.468	0.002 (-0.008–0.013)	0.683
<i>AHRR</i> :				
– Pos.1	0.020 (0.006–0.033)	0.005**	0.010 (0.001–0.019)	0.027*
– Pos.2	0.010 (-0.001–0.022)	0.076	0.006 (-0.001–0.013)	0.086
– Pos.3	0.001 (-0.012–0.012)	0.956	0.004 (-0.003–0.012)	0.250
Methylation level of each gene was modeled as the independent variable associated with birth weight as the dependent variable.				
[†] Adjusted for gestational age, infant gender, birth type, prenatal vitamin use and environmental tobacco smoke during pregnancy.				
*p < 0.05.				
**p < 0.01.				
High: High <i>WNT2</i> methylation group; Low: Low <i>WNT2</i> methylation group; Pos.1: The first studied CpG site; Pos.2: The second studied CpG site; Pos.3: The third studied CpG site.				

is efficient. To ensure that pyrosequencing sequenced the correct pattern, two wells were filled with oligonucleotide with a known sequence. Moreover, a human unmethylated (0%) standard and fully methylated (100%) standard were used as sample controls.

Statistical analysis

Statistical analyses were performed by the SPSS 16.0 statistical package (SPSS, Inc., IL, USA). Normally distributed continuous variables were expressed as mean $\pm$ standard deviation. Parametric t-test and χ^2 test were used to compare demographic characteristics in the FGR mother-newborn pairs and controls. Potential differences in placental DNA methylation levels between FGR cases and controls were evaluated through Mann–Whitney U test.

Multiple linear regression models were used to model the associations of placental DNA methylation with fetal growth indices (birth weight, birth length and Quetelet index). Variables considered in regression models included infant gender, GA, birth type, maternal prepregnancy BMI, prenatal vitamin use, maternal age, maternal environmental tobacco smoke during pregnancy, maternal education and monthly income. A backward stepwise method (critical level of $p < 0.20$

for variable removal) was used to determine which variables should be included in final models.

Multivariate logistic regression was used to estimate the odds of FGR birth in association with placental DNA methylation. Variables considered in logistic regression models included GA, infant gender, maternal age, maternal prepregnancy BMI, maternal physical activity, environmental tobacco smoke during pregnancy, maternal education and monthly income. All potential confounders were added to models in a forward stepwise procedure and were finally included if they altered the association between placental DNA methylation and FGR birth by >10%. $p < 0.05$ was considered significant, and all statistical tests were two-sided.

Results

Population characteristics

Of the 220 subjects enrolled in our case–control study, 181 subjects (80 FGR cases, 101 controls) donated placental samples. There was no difference in characteristics between subjects who provided samples compared with those who did not (data not shown). The maternal and pregnancy characteristics of FGR and control subjects are listed and compared as shown in Table 1. Cases and controls differed with regard to birth weight, birth

length, Quetelet index and GA. Cases and controls did not differ by maternal age, prepregnant BMI, infant gender, prenatal vitamin use, birth type, maternal education background and monthly income.

Placental DNA methylation levels

Four sites of *HSD11B2*, three sites of *WNT2* and *AHRR*, two sites of *IL6* and *IGF2*, and one site of *NR3C1* were measured. In each assay, correlation between DNA methylation at the individual CpG site was examined. All correlations were above 0.7 in the assay of *HSD11B2* and *WNT2*. Thus, the average methylation across four sites of the *HSD11B2* locus and three sites of the *WNT2* promoter was reported. In contrast, the one site evaluated in *NR3C1*, two sites in *IGF2*, two sites in *IL6* and three sites in *AHRR* were each reported separately.

Mann–Whitney U test was used to compare the difference in DNA methylation between FGR cases and controls. As shown in Table 2, mean methylation across four CpG sites of *HSD11B2* was significantly higher in FGR group than in control group (13.92 vs 13.25; $p = 0.003$). By contrast, DNA methylation at position 2 of *IGF2* and position 1 of *AHRR* was significantly lower in FGR group than in control group (44.03 vs 46.35; $p = 0.005$; 55.84 vs 57.95; $p = 0.021$).

Placental DNA methylation levels & fetal growth indicators

Multiple linear regression models were used to model the associations of placental DNA methylation with fetal growth indicators. For *WNT2*, histogram of methylation data showed a bimodal distribution (Supplementary Figure 1). Thus, when we did regression analysis of *WNT2*, all subjects were divided into high and low methylation group.

Table 3 shows the relationship between placental DNA methylation and newborn birth weight. In adjusted model, DNA methylation of *HSD11B2* and *WNT2* (high methylation group) had significant negative association with newborn birth weight. Every 1% unit increase in DNA methylation was associated with 27 and 19 g reduction in newborn birth weight, respectively ($\beta = -0.027$; $p = 0.033$; $\beta = -0.019$; $p = 0.034$). In contrast, DNA methylation at position 1 of *AHRR* and position 2 of *IGF2* had significant positive association with newborn birth weight. Every 1% unit increase in DNA methylation was associated with 8 and 10 g increase in newborn birth weight, respectively ($\beta = 0.008$; $p = 0.026$; $\beta = 0.010$; $p = 0.027$).

Table 4 shows the relationship between placental DNA methylation and newborn birth length. In both crude and adjusted model, DNA methylation of

Table 4. Crude and adjusted association between placental DNA methylation and birth length.

Methylation	Birth length			
	Crude model		Adjusted model†	
	β (95% CI)	p-value	β (95% CI)	p-value
<i>HSD11B2</i>	-0.162 (-0.302 to -0.022)	0.023*	-0.118 (-0.219 to -0.016)	0.023*
<i>NR3C1</i>	0.049 (-0.002–0.099)	0.059	0.008 (-0.029–0.045)	0.682
<i>WNT2</i> :				
– High	-0.015 (-0.087–0.057)	0.679	-0.014 (-0.082–0.053)	0.671
– Low	-0.002 (-0.104–0.101)	0.976	-0.005 (-0.070–0.061)	0.886
<i>IGF2</i> :				
– Pos.1	0.035 (-0.032–0.102)	0.301	0.008 (-0.039–0.056)	0.730
– Pos.2	0.028 (-0.013–0.068)	0.183	0.018 (-0.011–0.046)	0.226
<i>IL6</i> :				
– Pos.1	-0.013 (-0.128–0.102)	0.824	-0.033 (-0.133–0.047)	0.411
– Pos.2	0.032 (-0.030–0.095)	0.309	0.008 (-0.035–0.052)	0.702
<i>AHRR</i> :				
– Pos.1	0.039 (-0.017–0.095)	0.175	0.001 (-0.039–0.039)	0.933
– Pos.2	0.021 (-0.026–0.068)	0.380	0.005 (-0.027–0.037)	0.772
– Pos.3	-0.023 (-0.071–0.024)	0.332	-0.004 (-0.036–0.029)	0.827

Methylation level of each gene was modeled as the independent variable associated with birth length as the dependent variable.
†Adjusted for gestational age, birth type and environmental tobacco smoke during pregnancy
* $p < 0.05$.
High: High *WNT2* methylation group; Low: Low *WNT2* methylation group; Pos.1: The first studied CpG site; Pos.2: The second studied CpG site; Pos.3: The third studied CpG site.

Table 5. Crude and adjusted association between DNA methylation and Quetelet index.

Methylation	Quetelet index			
	Crude model		Adjusted model [†]	
	β (95% CI)	p-value	β (95% CI)	p-value
<i>HSD11B2</i>	-0.111 (-0.224–0.003)	0.056	-0.060 (-0.148 to -0.029)	0.184
<i>NR3C1</i>	0.011 (-0.029–0.052)	0.581	-0.018 (-0.050–0.014)	0.463
<i>WNT2</i> :				
– High	-0.070 (-0.144–0.003)	0.061	-0.093 (-0.150 to -0.036)	0.002**
– Low	0.048 (-0.028–0.124)	0.211	0.045 (-0.010–0.100)	0.107
<i>IGF2</i> :				
– Pos.1	0.057 (0.006–0.109)	0.030*	0.038 (-0.002–0.077)	0.064
– Pos.2	0.026 (-0.006–0.057)	0.113	0.018 (-0.007–0.042)	0.157
<i>IL6</i> :				
– Pos.1	0.007 (-0.083–0.097)	0.878	-0.008 (-0.076–0.061)	0.828
– Pos.2	0.024 (-0.025–0.072)	0.335	0.007 (-0.030–0.044)	0.720
<i>AHRR</i> :				
– Pos.1	0.067 (0.025–0.109)	0.002**	0.036 (0.005–0.067)	0.024*
– Pos.2	0.033 (-0.003–0.069)	0.070	0.019 (-0.007–0.045)	0.152
– Pos.3	0.006 (-0.031–0.042)	0.755	0.015 (-0.012–0.041)	0.267
Methylation level of each gene was modeled as the independent variable associated with Quetelet index as the dependent variable. [†] Adjusted for gestational age, infant gender, birth type, prenatal vitamin use and environmental tobacco smoke during pregnancy. * $p < 0.05$; ** $p < 0.01$. High: High <i>WNT2</i> methylation group; Low: Low <i>WNT2</i> methylation group; Pos.1: The first studied CpG site; Pos.2: The second studied CpG site; Pos.3: The third studied CpG site.				

HSD11B2 was inversely associated with newborn birth length. Every 1% unit increase in *HSD11B2* methylation was associated with a decrease of 0.118 cm in newborn birth length ($\beta = -0.118$; $p = 0.023$).

Table 5 presents the regression results for placental DNA methylation and Quetelet index (calculated as weight [in kg] divided by the square of height [in m]). In adjusted model, evaluated DNA methylation was associated with smaller Quetelet index within high *WNT2* methylation group ($\beta = -0.093$; $p = 0.002$), while DNA methylation at position 1 of *AHRR* was positively associated with the Quetelet index ($\beta = 0.036$; $p = 0.024$).

Although FGR newborns seemed to have shorter GA than control newborns, no significant association was found between placental DNA methylation and GA (data not shown).

Placental DNA methylation levels & relative odds of fetal growth restriction birth

Table 6 presents crude and adjusted odds ratios (OR) and 95% CI for the risk of FGR birth in relation to placental DNA methylation. In adjusted model, higher DNA methylation of *HSD11B2* and *WNT2* was significantly associated with higher relative odds of FGR birth. Every 1% unit increase in DNA methylation of

HSD11B2 and *WNT2* (high methylation group) was associated with 22.4 and 13.6% higher odds of FGR birth, respectively (OR: 1.224; $p = 0.011$; OR: 1.136; $p = 0.037$). In contrast, DNA higher methylation of *IGF2* was associated with significantly lower odds of FGR birth (position 1, OR: 0.932; $p = 0.041$; position 2; OR: 0.947; $p = 0.012$).

Discussion

Alterations to placental DNA methylation may result in altered expression of genes involved in metabolic/endocrine functions and may also affect the placenta's ability to transport water, gas, nutrients and waste products crucial for the proper growth and survival of the fetus [27]. In this analysis of an Asian population, we tested for the association of being born FGR with DNA methylation of *IGF2*, *WNT2*, *HSD11B2*, *NR3C1*, *AHRR* and *IL6*.

We observed significant differences in DNA methylation of *IGF2* between FGR and control placental samples. Elevated DNA methylation was associated with higher newborn birth weight. The CpG site associated with birth weight is located in differentially methylated regions of *IGF2*, which may play a role in regulating gene expression. This observation is in keeping with the study by St-Pierre *et al.* In their study, they found

DNA methylation at the same CpG site was positively associated with birth weight and thorax circumference without adjusting any confounders [11].

During development, the WNT pathway is involved in cell proliferation, fate, migration, polarity and cell death. Each of these mechanisms is crucial for normal development of the placenta to enable fetal growth [28]. Ferreira *et al.* demonstrated that high *WNT2* promoter methylation is significantly associated with reduced *WNT2* expression in placenta and low birth weight percentile in the neonate [17]. This is the only study to report an association of *WNT2* methylation with fetal growth indices. In our study, we found the distribution of *WNT2* methylation was bimodal, and *WNT2* methylation was inversely associated with birth weight and Que- telet index within high methylation group. The bimodal distribution of *WNT2* methylation might be driven by the SNPs within or near the promoter region [17].

Prenatal exposure to excess maternal glucocorticoids impaired fetal growth. *HSD11B2*, a key gene involved in glucocorticoid metabolism, interconverts hormonally active glucocorticoid to inactive cortisone [29,30]. In a cross-sectional study, Marsit *et al.* found that DNA methylation of *HSD11B2* was inversely associated with *HSD11B2* expression in placenta and with fetal growth indices in the neonate [15]. In line with this cross-

sectional study and our previous small case–control study [16] we found placental DNA methylation was inversely associated with newborn birth weight and birth length. In addition to *HSD11B2*, *NR3C1*, the glucocorticoid receptor gene, is also a known mediator of glucocorticoid signaling [31]. Filiberto *et al.* demonstrated that DNA methylation of *NR3C1* promoter region was inversely associated with large-for-gestational-age status in newborns [18], while in this present study, we did not find any association between *NR3C1* promoter methylation and measures of fetal growth. This might be due to the different assays, which focused on different CpG sites within *NR3C1* promoter.

Other genes highlighted in our study are *AHRR* and *IL6*. The relationship between DNA methylation of those two genes and fetal growth has not been addressed. *AHRR* is a known cancer susceptibility gene and a key regulator of the catabolism of xenobiotics [32,33]. Lower methylation of *AHRR* has been associated with higher levels of self-report or cotinine-based assessment of maternal smoking [34]. In our study, lower *AHRR* methylation was found to be associated with reduced fetal growth. Given maternal smoking is known to affect fetal growth, placental *AHRR* methylation might be one mechanism explaining the effect of maternal smoking

Table 6. Crude and adjusted odds ratios for placental DNA methylation and fetal growth restriction birth.

Methylation	Crude model		Adjusted model [†]	
	OR (95% CI)	p-value	OR (95% CI)	p-value
<i>HSD11B2</i>	1.208 (1.060–1.376)	0.005**	1.224 (1.047–1.430)	0.011*
<i>NR3C1</i>	0.989 (0.947–1.033)	0.748	1.022 (0.967–1.079)	0.444
<i>WNT2</i> :				
– High	1.060 (0.966–1.163)	0.216	1.136 (1.007–1.281)	0.037*
– Low	0.979 (0.906–1.058)	0.600	0.971 (0.872–1.081)	0.971
<i>IGF2</i> :				
– Pos.1	0.932 (0.879–0.988)	0.018*	0.932 (0.863–0.990)	0.041*
– Pos.2	0.951 (0.917–0.987)	0.008**	0.947 (0.908–0.988)	0.012*
<i>IL6</i> :				
– Pos.1	1.013 (0.922–1.113)	0.794	1.020 (0.906–1.148)	0.744
– Pos.2	0.998 (0.949–1.050)	0.947	0.995 (0.935–1.148)	0.878
<i>AHRR</i> :				
– Pos.1	0.947 (0.904–0.993)	0.023*	0.949 (0.895–1.006)	0.079
– Pos.2	0.967 (0.931–1.004)	0.077	0.955 (0.910–1.002)	0.060
– Pos.3	0.992 (0.955–1.030)	0.671	0.956 (0.909–1.006)	0.083

[†]Adjusted for gestational age, prenatal vitamin use and environmental tobacco smoke during pregnancy.

*p < 0.05; **p < 0.01.

High: High *WNT2* methylation group; Low: Low *WNT2* methylation group; OR: Odds ratio; Pos.1: The first studied CpG site; Pos.2: The second studied CpG site; Pos.3: The third studied CpG site.

on fetal growth. *IL6* is a kind of proinflammatory cytokine, which is also thought to play a role in placental development and fetal growth. One study reported that FGR subjects had significantly more *IL6* mRNA and protein in the placenta [26]. However, we did not find any difference in *IL6* DNA methylation.

Our study is one of the first to show an association between placental DNA methylation of six growth-related genes and risk of FGR birth. A significant strength of this study is that we were able to adjust for potential confounders. Moreover, we used the population-based ascertainment of placental sample, which is a target tissue directly responsible for fetal growth. The limitations of this study include the relatively small sample size, which may restrict our statistical power. Additionally, owing to the lack of mRNA collection, we could not test the association between placental DNA methylation and gene expression.

Conclusion

Our findings demonstrated that placental DNA methylation levels of *IGF2*, *AHRR*, *HSD11B2* and *WNT2* were associated with measures of fetal growth. Further investigation into the role of DNA methylation in the development of FGR is warranted.

Future perspective

Placental DNA methylation may serve as an early epigenetic marker of maternal exposure and may be involved in the pathway leading to adverse birth outcomes and

increased adult disease susceptibility. Elucidating the role of DNA methylation in the developmental origins of health and disease is the challenge in the next 5–10 years, with the goal of providing early interventions and prevention, and new treatment opportunities.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/epi.15.101

Financial & competing interests disclosure

The present work was in part supported by funding from the National Natural Science Foundation of China (grant 81072263 to Y Zhang) and from the NIH (R01ES020268, R01ES021357, R01NR013945, P30ES00002). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Difference in DNA methylation between fetal growth restriction cases & controls

- Mean methylation across four CpG sites of *HSD11B2* was significantly higher in fetal growth restriction (FGR) group than in control group.
- DNA methylation at position 2 of *IGF2* and position 1 of *AHRR* was significantly lower in FGR group than in control group.

Placental DNA methylation levels & fetal growth indices

- DNA methylation levels of *IGF2* and *AHRR* were positively associated with newborn birth weight and Quetelet's index.
- DNA methylation levels of *HSD11B2* and *WNT2* were negatively associated with newborn birth weight and Quetelet's index.

Placental DNA methylation levels & relative odds of fetal growth restriction

- Significantly elevated odds of FGR birth were associated with increasing DNA methylation of *HSD11B2* and *WNT2*, and decreasing DNA methylation of *IGF2*.

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