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Expression of Placental Regulatory Genes Is Associated with Fetal Growth

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Expression of placental regulatory genes is associated with fetal growth

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Abstract: The placenta is the principal organ regulating respiratory, nutritional, endocrine and metabolic functions on behalf of the developing fetus. Changes in gene expression patterns of placenta-specific genes may influence fetal growth. We profiled the expression of 17 genes related to placenta functioning in term placentas (n = 677) to identify genes differentially expressed across birth weight categories [small (SGA), appropriate (AGA) and large (LGA) for gestational age]. *ABCG2, CEBPB, CRH, GCM1, GPC3, INSL4, PGF and PLAC1* were inversely associated with LGA status, with odds ratios (ORs) and 95% confidence intervals (CI) ranging from *GCM1* (OR = 0.44, 95% CI: 0.29, 0.70) to *CRH* (OR = 0.73, 95% CI: 0.61, 0.88). *NR3C1* was positively associated with LGA status (OR = 2.33, 95% CI: 1.43, 3.78). *PLAC1* (OR = 0.66, 95% CI: 0.47, 0.92) and *ABCG2* (OR = 0.63, 95% CI: 0.44, 0.91) were additionally inversely associated with SGA status, and *PGF* was positively associated with SGA status (OR = 1.59, 95% CI = 1.08, 2.35). General trends were confirmed in an independent cohort (n = 306). Given that aberrant fetal growth may have long-lasting effects, our results suggest the potential utility of placental gene expression profiles as potential early markers of disease onset later in life.

Keywords: Birth weight; gene expression, placenta.

Introduction

Abnormal fetal growth, both undergrowth and overgrowth, has been linked to adverse health outcomes later in life, including metabolic as well as cognitive and neurobehavioral disorders [1–3]. The placenta is a transient organ situated at the maternal-fetal interface that regulates fetal growth and development by facilitating nutrient intake and waste removal, gas exchange, immune protection and neuroendocrine functions on behalf of the developing fetus [4]. Given its important role in overseeing fetal development, placental gene expression patterns likely influence fetal growth.

While several studies have reported associations between genes relevant to placental function and birthweight, a proxy of fetal growth, many of these studies, including our own [5], focus on the relevance of imprinted genes as placental regulators of fetal growth [5, 6]. Recent studies have also implicated the importance of a broad range of non-imprinted genes, including genes involved in processes critical to the appropriate development of the placenta, such as differentiation, invasion, migration and syncytialization, as well as genes that play a role in regulating maternal and fetal physiology, such as neuroendocrine hormones and receptors [7]. However, studies to date linking regulators of placental function to fetal growth have focused on a small subset of genes and were limited by small sample sizes. Additional studies have linked genes with known relevance to placental function to severe pregnancy complications, including preeclampsia [8]. Given the important role of these genes in regulating placental function, variability of these genes in uncomplicated pregnancies may also bear relevance on fetal growth. Hence, a more comprehensive assessment to explore genes relevant to a broader range of placental functions in relation to fetal growth is warranted.

We assembled a panel of 17 genes that were previously shown to play a critical role in placenta function. The

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Table 1: Panel of selected genes.

Gene symbol	Function	Birth outcome
ABCG2	Placental drug efflux transporter [9]	Placental choriocarcinoma [10]
CEBPB	Transcription factor that induces decidualization of stromal cells [11]	
CRH	Neuroendocrine hormone that likely plays an important role in gestational length and parturition [12]	Predictor of pre-term birth; fetal growth restriction [13]
CSH1	Vasodilator hormone [14]	Altered expression in SGA and LGA infants [7, 13]
CSHL1	Placental growth hormone; regulation of maternal/fetal metabolism [15]	Altered expression in SGA and LGA infants [7]
EPAS1	Placental angiogenic factor [16]	Preeclampsia [17]
GCM1	Trophoblast differentiation [18]	Preeclampsia [19]
GH2	Growth hormone; regulation of maternal/fetal metabolism [20]	Altered expression and levels in SGA infants [7]
GPC3	Trophoblast differentiation [21]	Simpson-Golabi-Behmel fetal overgrowth syndrome [22]
HIF1A	Angiogenesis [23]	Preeclampsia [24]
INSL4	Trophoblast invasion and migration [25]	Placenta accrete [26]
NR3C1	Glucocorticoid receptor [27]	Infant neurobehavior [28] Birth weight [29]
NR3C2	Mineralocorticoid receptor; decidualization of stromal cells [30]	
PGF	Placental angiogenic factor [31]	Preeclampsia [32]
PLAC1	Trophoblast invasion and migration into maternal decidua [33]	Preeclampsia [34]
PLAC4	Promotes syncytialization [35]	Preeclampsia [35]
PLAC8	Embryo development and implantation [36]	

selection was based on the following criteria: (1) highly/uniquely expressed in placental tissue; (2) demonstrated functional role in fetal development (*ABCG2*, *CEBPB*, *CRH*, *CSHL1*, *GH2*, *INSL4*, *NR3C1*, and *PLAC8*); or (3) demonstrated association with pregnancy complications (*CSH1*, *EPAS1*, *GCM1*, *GPC3*, *HIF1A*, *NR3C2*, *PGF*, *PLAC1*, and *PLAC4*). The selected genes and their relevance to placental function and birth outcomes are shown in Table 1. The objective of this study is to determine whether the expression levels of these "placenta regulatory genes" are associated with fetal growth.

Materials and methods

Study population

This study involves two independent birth cohorts. The first consisted of mother-infant pairs enrolled in the Rhode Island Child Health Study (RICHS) with available placental RNA (n = 684), as previously described [37]. Briefly, the study selectively recruited small for gestational age (SGA, lowest 10th percentile), large for gestational age (LGA, highest 10th percentile) and matched appropriate for gestation age (AGA) infants based on the 2013 Fenton growth chart from Women and Infants Hospital of Rhode Island. Lifestyle/demographic characteristics and exposure histories were obtained from structured chart reviews of medical records and interviewer-based questionnaires. All subjects provided written informed consent approved by the Institutional Review Boards at Women and Infants Hospital and Emory University.

As the RICHS population is predominantly Caucasian (78%) and oversampled for SGA and LGA infants, we conducted a replication study in the Stress in Pregnancy (SIP) study [38], an on-going urban birth cohort that is more ethnically diverse and has a birth weight distribution comparable to general population. The current study includes mother-infant pairs recruited from prenatal clinics at the Icahn School of Medicine at Mount Sinai, New York Hospital of Queens and Queens College with available placental RNA. We restricted our analysis to singleton pregnancies with gestational age ≥37 and maternal age ≥18 to match exclusion criteria implemented in the RICHS cohort (n = 307).

Placenta collection and RNA extraction

Placental biopsies free of maternal decidua were excised from each of four quadrants midway between the cord insertion site and the placental rim within 2 h of delivery to optimize RNA integrity. The tissues were subsequently snap-frozen in liquid nitrogen, homogenized with mortar and pestle, and stored at −80°C. RNA was extracted from RICHS placenta samples using the RNeasy mini kit (Qiagen, #74106) and from SIP samples using the Maxwell simplyRNA Tissue Kit (Promega, #AS1280), following the manufacturer's protocol. Extracted RNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific Inc., #ND-2000) and stored at −80°C until use.

Gene expression profiling

Placental RNA was profiled using a custom-designed code-set (nanoString Technologies, Seattle, WA, USA) as previously described [5]. Briefly, RNA was hybridized to reporter and capture probes, and purified target-dual probe complexes were aligned and immobilized

on imaging cartridges using an nCounter Prep Station II. Cartridges were scanned in an nCounter Digital Analyzer for code count detection. The nanoString Norm R package was used to normalize nCounter data. The raw code count data was normalized against the geometric mean of spike-in positive control probes and against the geometric mean of the housekeeping genes, *GAPDH*, *RPL19*, and *RPLP0*. The limit of detection (LOD) for each sample was set at 2 standard deviations above the mean of the included negative control probes. For each sample, expression values that fell below the LOD were set to LOD/sqrt2. Seven samples in the RICHS data-set and one sample in the SIP dataset were removed due to poor quality control metrics, leaving a sample size of 677 and 306, respectively.

Statistical analysis

Genes differentially expressed across birth weight categories (SGA, AGA, LGA) were identified using the limma R package (FDR < 0.05). The identified differentially expressed genes were further evaluated using multinomial logistic regression models (nnet R package), with the outcome modeled as SGA and LGA infants referenced against AGA infants in both the RICHS and SIP data-sets. In the SIP data-set, we additionally conducted generalized linear models (GLM) to assess the association between gene expression levels and Fenton growth percentile, modeled as a continuous variable. All regression models were adjusted for *a priori* determined covariates, including batch, infant ethnicity and maternal age. Gender and gestational age were accounted for in the Fenton growth chart defined outcome variable. Additional covariates, including maternal pre-pregnancy body mass index (BMI), maternal insurance, and maternal education were not included in the final models as their effect size estimates were less than 10%. All data was analyzed using R version 3.2.3 [39].

Results

The demographic and clinical characteristics of the RICHS [5] and SIP study [38] populations have been previously described and are summarized in Table 2. Briefly, the RICHS study population is predominately Caucasian (78.2%), with SGA, AGA and LGA infants constituting approximately 20%, 56% and 24%, respectively. Male and female infants were equivalently represented. The gestational age spanned the term range of 37–41 weeks.

Compared to the RICHS cohort, the birth weight distribution of the SIP study more closely reflects the general population, with SGA and LGA infants constituting approximately 10% each of the study population [40]. Mothers enrolled in SIP tended to be younger (maternal age <21 = 17.3%) than RICHS participants (maternal age <21 = 6.0%) A greater proportion of late term pregnancies were observed among the SIP participants (gestational age >40 weeks = 24.5%) compared to the RICHS participants (gestational age >40 weeks = 7.4%). As the SIP study represents an urban, ethnically diverse cohort, a significantly greater proportion of SIP population are non-white (78.4%) compared to RICHS (21.4%). No differences in the gender distribution were observed between the two populations.

As shown in Figure 1, nine genes were differentially expressed between LGA and AGA infants; eight genes (*ABCG2*, *CEBPB*, *CRH*, *GCM1*, *GPC3*, *INSL4*, *PLAC1*, *PGF*) were downregulated in LGA infants, and *NR3C1* was upregulated in LGA infants. Meanwhile, *PGF* was the only gene differentially expressed between SGA and AGA infants, with an upregulation observed among SGA infants in addition to the downregulation observed among LGA infants compared to AGA infants.

We further analyzed the association between the identified differentially expressed genes and fetal growth using multinomial logistic regression analysis, adjusting the models for plate batch, infant ethnicity, and maternal age. As seen in Figure 2, compared to AGA status, a log2 unit increase in expression of eight genes (*ABCG2*, *CEBPB*, *CRH*, *GCM1*, *GPC3*, *INSL4*, *PGF* and *PLAC1*) was associated with a significant decrease in odds of LGA status, ranging from *GCM1* (OR = 0.44, 95% CI: 0.29, 0.68) to *CRH* (OR = 0.73, 95% CI: 0.61, 0.88). A concomitant significant increase in the odds of SGA status was observed with a log2 unit increase in *PGF* expression (OR = 1.59, 95% CI: 1.08, 2.35). In contrast, a log2 unit increase in *NR3C1*

Table 2: Comparison of the study populations.

Characteristics	RICHS (n = 677)		SIP (n = 306)		P-value
	n	%	n	%	
Birth weight category					<0.01
SGA	130	19.2	38	12.4	
AGA	387	57.2	245	80.1	
LGA	160	23.6	23	7.5	
Infant gender					0.44
Male	332	49.0	159	52.0	
Female	345	51.0	147	48.0	
Maternal age (year)					<0.01
18–21	40	6.0	53	17.3	
22–30	295	39.7	140	45.8	
≥31	342	54.3	69	22.5	
Missing	0	0	44	14.4	
Infant race					<0.01
White	515	76.1	26	8.5	
Black	54	8.0	64	20.9	
Hispanic/other	78	11.5	156	51.0	
Asian	13	1.9	20	6.5	
Missing	17	2.5	40	13.1	
Gestational age (week)					<0.01
≤38	132	19.5	68	22.2	
39–40	494	73.1	163	53.3	
>40	51	7.4	75	24.5	

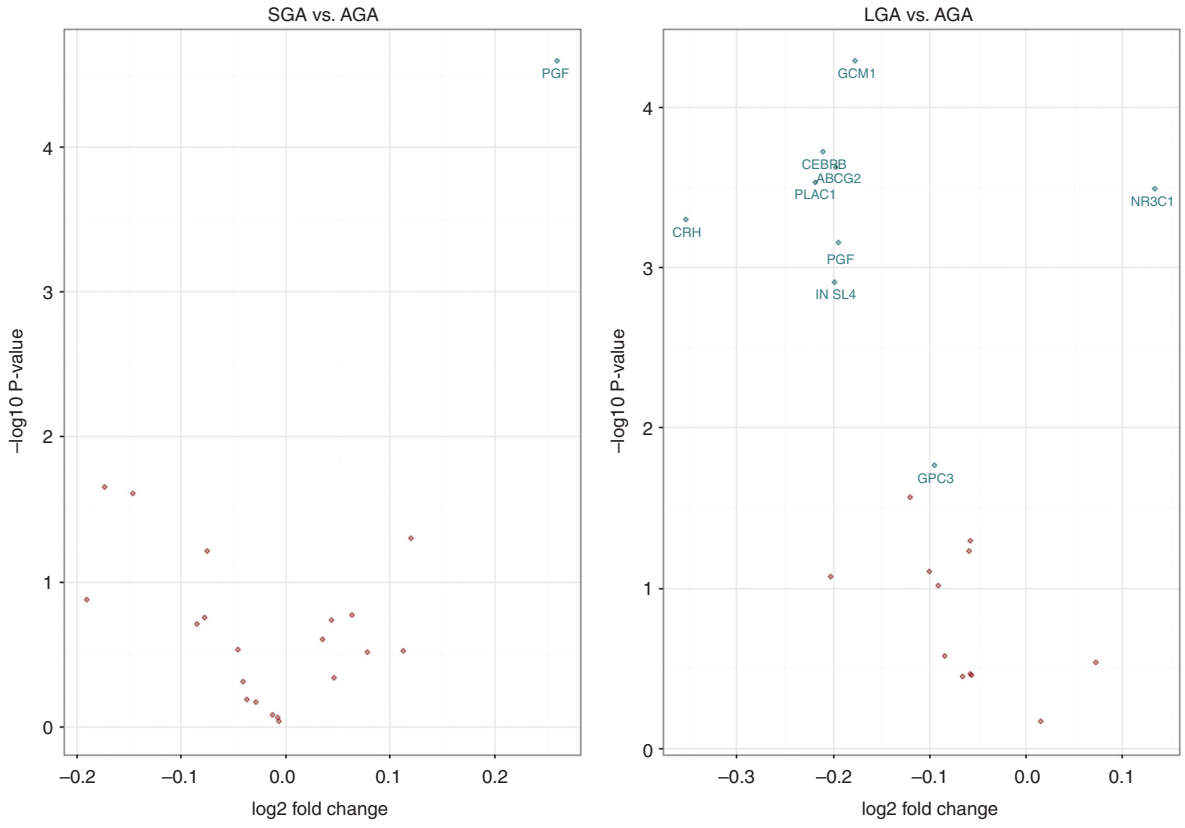

Figure 1: Genes differentially expressed between aberrant and normal birth weight categories.
Genes differentially expressed between SGA/AGA and LGA/AGA birth weight categories (FDR < 0.05) are labeled in blue.

expression was associated with increased odds of LGA status (OR = 2.33, 95% CI: 1.43, 3.78). For two genes, *ABCG2* and *PLAC1*, a log2 unit increase in expression was associated with lower odds of both LGA and SGA status.

Because the RICHS cohort was designed to study aberrant fetal growth, the study population was oversampled at both ends of fetal growth (SGA and LGA), resulting in a birthweight distribution that differs from the general population. To assess the robustness of our findings, we conducted a replication study in the SIP cohort to determine whether associations observed in the RICHS also present in a study population with a more normal birth weight distribution and a more diverse ethnic composition.

In the SIP study, gene expression data was available for 8 (*ABCG2*, *CEBPB*, *EPAS*, *GCM1*, *NR3C1*, *GCM1*, *PGF*, *PLAC1*, *PLAC4*) of the 17 assessed placenta regulatory genes in the RICHS study. As shown on Table 3, increased expression levels of *CEBPG* and *GCM1* were significantly associated with decreased odds of LGA status, consistent with findings in the RICHS study. Consistent trends were also observed between *NR3C1* expression and fetal growth in both cohorts, with a significant positive association between *NR3C1* expression and LGA status in the RICHS cohort and a significant inverse association between *NR3C1* expression and SGA status in the SIP cohort. In the SIP cohort, *GCM1* and *EPAS* were additionally associated with increased odds of SGA status. Interestingly, borderline significant positive associations were observed between *ABCG2* and *PLAC1* expression levels and SGA status, contrary to findings in the RICHS study, where these two genes were inversely associated with SGA status.

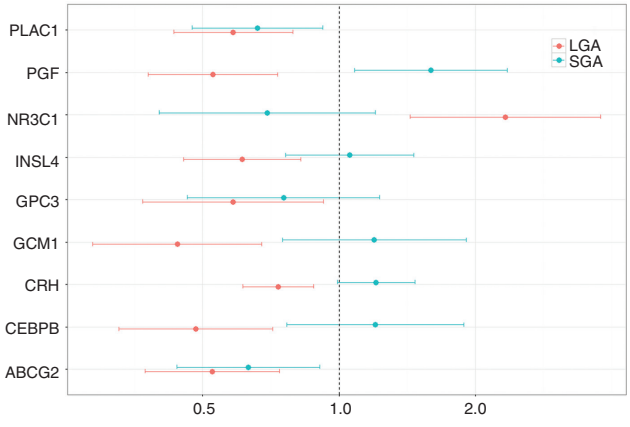

Figure 2: Association between placental gene expression and birth weight categories in the RICHS cohort.
Odds ratios (OR) and 95% CI for SGA or LGA status referenced against AGA status for a log2 unit increase in each gene.

Table 3: Associations between placental regulatory genes and fetal growth in RICHS and SIP[a].

	RICHS [OR (95% CI)]		SIP [OR (95% CI)]	
	SGA	LGA	SGA	LGA
ABCG2	**0.63 (0.44, 0.91)**	**0.52 (0.37, 0.74)**	1.43 (0.97, 2.12)	0.68 (0.40, 1.15)
CEBPB	1.20 (0.77, 1.88)	**0.48 (0.33, 0.71)**	1.36 (0.77, 2.40)	**0.48 (0.23, 0.97)**
EPAS	1.28 (0.66, 2.48)	0.56 (0.32, 1.00)	**2.32 (1.01, 5.33)**	0.59 (0.24, 1.46)
GCM1	1.20 (0.75, 1.91)	**0.44 (0.29, 0.68)**	**1.78 (1.11, 2.87)**	**0.56 (0.34, 0.91)**
NR3C1	0.69 (0.40, 1.20)	**2.33 (1.43, 3.78)**	**0.29 (0.12, 0.73)**	1.15 (0.34, 3.91)
PGF	**1.59 (1.08, 2.35)**	**0.53 (0.38, 0.73)**	1.19 (0.85, 1.66)	0.83 (0.56, 1.22)
PLAC1	**0.66 (0.47, 0.92)**	**0.58 (0.43, 0.79)**	1.26 (0.97, 1.64)	0.76 (0.56, 1.04)
PLAC4	0.82 (0.60, 1.13)	0.87 (0.64, 1.17)	1.12 (0.91, 1.38)	0.85 (0.69, 1.06)

[a]Models adjusted for infant race and maternal age. Significant associations with the outcome are indicated in bold.

Given the normal distribution in Fenton growth percentiles in the SIP cohort, we further explored the relationship between placental gene expression levels and fetal growth modeled as a continuous outcome using generalized linear models. Significant associations with fetal growth were observed for six of the eight assessed genes. Consistent with the findings of the multinomial models, *NR3C1* was the only gene positively associated with fetal growth while among the genes inversely associated with fetal growth, *GCM1* was among the genes with the largest negative effect size (Table 4).

Table 4: Generalized linear models assessing the association between placental regulatory gene expression and Fenton growth percentiles in the SIP cohort[a].

Gene	β	95% CI
ABCG2	−0.04	[−0.07, −0.01]
CEBPB	−0.06	[−0.11, −0.02]
GCM1	−0.07	[−0.10, −0.03]
EPAS1	−0.07	[−0.13, −0.01]
NR3C1	0.12	[0.04, 0.20]
PGF	−0.03	[−0.06, −0.01]
PLAC1	−0.03	[−0.05, 0.00]
PLAC4	−0.02	[−0.04, 0.00]

[a]Models adjusted for infant race and maternal age.

Discussion

Findings from our study demonstrate that changes in the expression of several placenta-specific genes are associated with fetal growth. An inverse association between fetal growth and the expression levels of *CEBPB*, *GCM1* and *PLAC1* and a positive association between fetal growth and *NR3C1* expression was consistently observed across two independent study populations with different sociodemographic characteristics; these robust results implicate the relevance of these genes to fetal growth.

The three genes inversely associated with LGA status predominately function in processes related to placental differentiation processes (*CEBPB*, *GCM1*, and *PLAC1*). For example, *CEBPB* is a transcription factor that is involved in the induction of stromal decidualization [11] and both *GCM1* [41, 42] and *PLAC1* [33, 43] promote trophoblast syncytialization and invasion. The observed inverse association between the expression of these genes and fetal growth may reflect the increased activity of these genes in hastening placental maturation among growth-restricted infants.

The glucocorticoid receptor *NR3C1* is a critical component of the placental regulatory pathway that controls fetal exposure to glucocorticoids, including cortisol. Elevated glucocorticoid levels at term have been previously associated with fetal growth retardation [44]. More recently, increased cord blood *NR3C1* methylation levels have been associated with both stress during pregnancy and decreased offspring birth weight, suggesting that this gene may act as a potential mediator linking maternal stress during pregnancy with fetal growth [29]. Given an inverse relationship between methylation and gene expression, our findings of a positive association between *NR3C1* expression and birth weight are consistent with the previous report of an inverse association between *NR3C1* methylation and birth weight.

While findings between the two cohorts were largely consistent with one another, contrary findings were observed for two genes, *ABCG2* and *PLAC1*. For both genes, U-shaped associations were observed in the RICHS study, with significant inverse associations with both SGA and LGA status. In the SIP study, however, borderline positive associations with SGA status were observed for both genes. These differences may point to the sensitivity of our findings related to these genes to underlying differences

in these populations. Additional studies are warranted to further evaluate contributing drivers in the association between these genes and aberrant fetal growth. The observed discrepancy in our findings highlights the importance of replicating studies across multiple cohorts of varying characteristics to identify robust associations.

While several studies have suggested an association between fetal development and the expression of genes implicated to play placental-specific roles in development, this study represents the most comprehensive profiling of the expression of placental regulatory genes in healthy, term pregnancies. The large sample size and sampling scheme based on fetal growth categories ensured sufficient power to detect meaningful risk factors for birth weight. Expression was profiled in the placenta, the principal regulator of fetal development and the organ in which the selected genes are almost uniquely expressed. Additionally, the inclusion of a replication study provides a means to assess the robustness of the presented findings.

However, certain limitations were also inherent to our study design. For example, the over-representation of infants at the extremes of the birth weight spectrum could limit the generalizability of the findings to the general population. We attempted to address this issue by including a replication study with a more normal birth weight distribution. Also, we are unable to capture the dynamics in the expression of these genes throughout the *in utero* period and, therefore, are unable to identify potential critical windows of susceptibility. Finally, while we observe associations between placental markers and birth weight, we cannot infer the directionality of the associations due to the cross-sectional nature of the study. Hence, it is unclear whether the changes in expression reflect an adaptive or pathologic response to the processes underlying abnormal fetal growth. Additional longitudinal studies are warranted to evaluate whether the observed changes in placental gene expression have implications on health outcomes later in life.

In summary, we identified associations between fetal growth and the expression pattern of a panel of genes involved in placental regulatory processes. Given the implications of aberrant fetal growth on health effects throughout the lifespan, our findings suggest that placental regulatory gene expression profiles have the potential to be leveraged as early markers of disease onset later in life.

Author's statement

Conflict of interest: Authors state no conflict of interest.

Material and methods: Informed consent: Informed consent has been obtained from all individuals included in this study.

Ethical approval: The research related to human subject use has complied with all the relevant national regulations, and institutional policies, and is in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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References

[1] Godfrey K, Inskip H, Hanson M. The Long-Term Effects of Prenatal Development on Growth and Metabolism. Semin Reprod Med. 2011;29:257–65.

[2] Moore GS, Kneitel AW, Walker CK, Gilbert WM, Xing G. Autism risk in small- and large-for-gestational-age infants. Am J Obstet Gynecol. 2012;206:314.e1–9.

[3] Colman I, Ataullahjan A, Naicker K, Van Lieshout RJ. Birth weight, stress, and symptoms of depression in adolescence: evidence of fetal programming in a national Canadian cohort. Can J Psychiatry. 2012;57:422–428.

[4] Burton GJ, Jauniaux E. What is the placenta? Am J Obstet Gynecol. 2015;213:S6.e1–4.

[5] Kappil MA, Green BB, Armstrong DA, Sharp AJ, Lambertini L, Marsit CJ, et al. Placental expression profile of imprinted genes impacts birth weight. Epigenetics 2015;10:842–9.

[6] Moore GE, Ishida M, Demetriou C, Al-Olabi L, Leon LJ, Thomas AC, et al. The role and interaction of imprinted genes in human fetal growth. Philos Trans R Soc Lond B Biol Sci. 2015;370:20140074.

[7] Männik J, Vaas P, Rull K, Teesalu P, Rebane T, Laan M. Differential expression profile of growth hormone/chorionic somatomammotropin genes in placenta of small- and large-for-gestational-age newborns. J Clin Endocrinol Metab. 2010;95:2433–42.

[8] Kurtoglu E, Avci B, Kokcu A, Celik H, Cengiz Dura M, Malatyalioglu E, et al. Serum VEGF and PGF may be significant markers in prediction of severity of preeclampsia. J Matern Fetal Neonatal Med. 2016;29:1987–92.

[9] Mao Q. BCRP/ABCG2 in the placenta: Expression, function and regulation. Pharm Res. 2008;25:1244–55.

[10] Bircsak KM, Gupta V, Yuen PYS, Gorczyca L, Weinberger BI, Vetrano AM, et al. Genetic and dietary regulation of glyburide efflux by the human placental breast cancer resistance protein transporter. J Pharmacol Exp Ther. 2016;357:103–13.

[11] Li DD, Yang ZQ, Guo CH, Yue L, Duan CC, Cao H, et al. Hmgn1 acts downstream of C/EBPβ to regulate the decidualization of uterine stromal cells in mice. Cell Cycle. 2015;14:3461–74.

[12] King BR, Smith R, Nicholson RC. The regulation of human corticotrophin-releasing hormone gene expression in the placenta. Peptides 2001;22:795–801.

[13] Whitehead C, Walker S, Tuohey L, McNamara H, Alexiadis M, Hannan N, et al. Placental specific mRNA in the maternal blood identifies pregnancies at risk of both preterm and term fetal growth restriction. Reprod Sci. 2014;21:360A.

[14] Wei J, Lau SY, Blenkiron C, Chen Q, James JL, Kleffmann T, et al. Trophoblastic debris modifies endothelial cell transcriptome in vitro: a mechanism by which fetal cells might control maternal responses to pregnancy. Sci Rep. 2016;6:30632.
[15] Männik J, Vaas P, Rull K, Teesalu P, Laan M. Differential placental expression profile of human growth hormone/chorionic somatomammotropin genes in pregnancies with pre-eclampsia and gestational diabetes mellitus. Mol Cell Endocrinol. 2012;355:180–7.
[16] Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev. 1997;11:72–82.
[17] Jarvenpaa J, Vuoristo JT, Savolainen E-R, Ukkola O, Vaskivuo T, Ryynanen M. Altered expression of angiogenesis-related placental genes in pre-eclampsia associated with intrauterine growth restriction. Gynecol Endocrinol. 2007;23:351–5.
[18] Anson-Cartwright L, Dawson K, Holmyard D, Fisher SJ, Lazzarini RA, Cross JC. The glial cells missing-1 protein is essential for branching morphogenesis in the chorioallantoic placenta. Nat Genet. 2000;25:311–4.
[19] Chen CP, Chen CY, Yang YC, Su TH, Chen H. Decreased placental GCM1 (glial cells missing) gene expression in pre-eclampsia. Placenta 2004;25:413–21.
[20] Handwerger S, Freemark M. The roles of placental growth hormone and placental lactogen in the regulation of human fetal growth and development. J Pediatr Endocrinol Metab. 2000;13:343–56.
[21] Khan S, Blackburn M, Mao DL, Huber R, Schlessinger D, Fant M. Glypican-3 (GPC3) expression in human placenta: localization to the differentiated syncytiotrophoblast. Histol Histopathol. 2001;16:71–8.
[22] Golabi M, Leung A, Lopez C. Simpson-Golabi-Behmel syndrome type 1. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. GeneReviews [Internet]. Seattle: University of Washington, Seattle; 1993-2017 [cited 2017 Jun 19]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1219/#top.
[23] Yamakawa M, Liu LX, Date T, Belanger AJ, Vincent KA, Akita GY, et al. Hypoxia-inducible factor-1 mediates activation of cultured vascular endothelial cells by inducing multiple angiogenic factors. Circ Res. 2003;93:664–73.
[24] Zhang D, Liu H, Zeng J, Miao X, Huang W, Chen H, et al. Glucocorticoid exposure in early placentation induces preeclampsia in rats via interfering trophoblast development. Gen Comp Endocrinol. 2016;225:61–70.
[25] Janneau J-L, Maldonado-Estrada J, Tachdjian G, Miran I, Motté N, Saulnier P, et al. Transcriptional expression of genes involved in cell invasion and migration by normal and tumoral trophoblast cells. J Clin Endocrinol Metab. 2002;87:5336–9.
[26] Goh W, Yamamoto SY, Thompson KS, Bryant-Greenwood GD. Relaxin, its receptor (RXFP1), and insulin-like peptide 4 expression through gestation and in placenta accreta. Reprod Sci. 2013;20:968–80.
[27] Palma-Gudiel H, Córdova-Palomera A, Leza JC, Fañanás L. Glucocorticoid receptor gene (NR3C1) methylation processes as mediators of early adversity in stress-related disorders causality: a critical review. Neurosci Biobehav Rev. 2015;55:520–35.
[28] Paquette AG, Lester BM, Lesseur C, Armstrong DA, Guerin DJ, Appleton AA, et al. Placental epigenetic patterning of glucocorticoid response genes is associated with infant neurodevelopment. Epigenomics 2015;7:767–79.
[29] Mulligan CJ, D'Errico NC, Stees J, Hughes DA. Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. *Epigenetics* 2012;7:853–857.
[30] Kuroda K, Venkatakrishnan R, Salker MS, Lucas ES, Shaheen F, Shaheen F, et al. Induction of 11β-HSD 1 and activation of distinct mineralocorticoid receptor- and glucocorticoid receptor-dependent gene networks in decidualizing human endometrial stromal cells. Mol Endocrinol. 2013;27:192–202.
[31] Aasa KL, Zavan B, Luna RL, Wong PG, Ventura NM, Tse MY, et al. Placental growth factor influences maternal cardiovascular adaptation to pregnancy in mice. Biol Reprod. 2015;92:44.
[32] Rätsep MT, Hickman AF, Croy BA. The Elsevier trophoblast research award lecture: impacts of placental growth factor and preeclampsia on brain development, behaviour, and cognition. Placenta 2016;48:S40–6.
[33] Chang W-L, Yang Q, Zhang H, Lin H-Y, Zhou Z, Lu X, et al. Role of placenta-specific protein 1 in trophoblast invasion and migration. Reproduction 2014;148:343–52.
[34] Kodama M, Miyoshi H, Fujito N, Samura O, Kudo Y. Plasma mRNA concentrations of placenta-specific 1 (PLAC1) and pregnancy associated plasma protein A (PAPP-A) are higher in early-onset than late-onset pre-eclampsia. J Obstet Gynaecol Res. 2011;37:313–8.
[35] Tuohey L, Macintire K, Ye L, Palmer K, Skubisz M, Tong S, et al. PLAC4 is upregulated in severe early onset preeclampsia and upregulated with syncytialisation but not hypoxia. Placenta 2013;34:256–60.
[36] Li M, Liu D, Wang L, Wang W, Wang A, Yao Y. Expression of placenta-specific 8 in human oocytes, embryos, and models of in vitro implantation. Fertil Steril. 2016;106:781–9.e2.
[37] Paquette AG, Lester BM, Koestler DC, Lesseur C, Armstrong DA, Marsit CJ. Placental FKBP5 genetic and epigenetic variation is associated with infant neurobehavioral outcomes in the RICHS cohort. Potash JB, Ed. PLoS One 2014;9:e104913.
[38] Finik J, Nomura Y. Cohort profile: Stress in Pregnancy (SIP) Study. Int J Epidemiol. 2017:dyw264. [Epub ahead of print].
[39] R: A language and environment for statistical computing. R Foundation for Statistical Computing. Version 3.2.3. Vienna, Austria: R Core Team; 2015.
[40] Dietz PM, Rizzo JH, England LJ, Callaghan WM, Vesco KK, Bruce FC, et al. Health care utilization in the first year of life among small- and large- for-gestational age term infants. Matern Child Health J. 2013;17:1016–24.
[41] Yu C, Shen K, Lin M, Chen P, Lin C, Chang GD, et al. GCMa regulates the syncytin-mediated trophoblastic fusion. J Biol Chem. 2002;277:50062–8.
[42] Chiu YH, Chen H. GATA3 inhibits GCM1 activity and trophoblast cell invasion. Sci Rep. 2016;6:21630.
[43] Chang W-L, Wang H, Cui L, Peng N-N, Fan X, Xue LQ, et al. PLAC1 is involved in human trophoblast syncytialization. Reprod Biol. 2016;16:218–24.
[44] Goland RS, Jozak S, Warren WB, Conwell IM, Stark RI, Tropper PJ. Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. J Clin Endocrinol Metab. 1993;77:1174–9.