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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). NR3CI 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
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.

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Table 1: Panel of selected genes.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Function</th>
<th>Birth outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRH</td>
<td>Neuroendocrine hormone that likely plays an important role in gestational length and parturition [12]</td>
<td>Altered expression in SGA and LGA infants [7, 13]</td>
</tr>
<tr>
<td>EPAS1</td>
<td>Placental angiogenic factor [16]</td>
<td>Preeclampsia [19]</td>
</tr>
<tr>
<td>GCM1</td>
<td>Trophoblast differentiation [18]</td>
<td>Altered expression and levels in SGA infants [7]</td>
</tr>
<tr>
<td>GPC3</td>
<td>Trophoblast differentiation [21]</td>
<td>Preeclampsia [24]</td>
</tr>
<tr>
<td>HIF1A</td>
<td>Angiogenesis [23]</td>
<td>Placenta accrete [26]</td>
</tr>
<tr>
<td>INSL4</td>
<td>Trophoblast invasion and migration [25]</td>
<td>Infant neurobehavior [28]</td>
</tr>
<tr>
<td>NR3C1</td>
<td>Glucocorticoid receptor [27]</td>
<td>Birth weight [29]</td>
</tr>
<tr>
<td>NR3C2</td>
<td>Mineralocorticoid receptor; decidualization of stromal cells [30]</td>
<td>Preeclampsia [32]</td>
</tr>
<tr>
<td>PGF</td>
<td>Placental angiogenic factor [31]</td>
<td>Preeclampsia [34]</td>
</tr>
<tr>
<td>PLAC1</td>
<td>Trophoblast invasion and migration into maternal decidua [33]</td>
<td>Preeclampsia [35]</td>
</tr>
<tr>
<td>PLAC4</td>
<td>Promotes syncytialization [35]</td>
<td></td>
</tr>
<tr>
<td>PLAC8</td>
<td>Embryo development and implantation [36]</td>
<td></td>
</tr>
</tbody>
</table>
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/√2. 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.

Comparison of the study populations. As shown in Figure 1, nine genes were differentially expressed between SGA and AGA infants; eight genes (ABCG2, CEBPB, CRH, GCM1, GPC3, INSL4, PLAC1, PGF) were downregulated in LGA infants, and NR3CI 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 NR3CI
expression was associated with increased odds of LGA status (OR = 2.33, 95% CI: 1.43, 3.78). For two genes, \textit{ABCG2} and \textit{PLAC1}, a log2 unit increase in expression was associated with lower odds of both LGA and SGA status.

Because the RICH 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 RICH cohort 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 (\textit{ABCG2}, \textit{CEBPB}, \textit{EPAS}, \textit{GCM1}, \textit{NR3C1}, \textit{GCM1}, \textit{PGF}, \textit{PLAC1}, \textit{PLAC4}) of the 17 assessed placenta regulatory genes in the RICH cohort. As shown on Table 3, increased expression levels of \textit{CEBPB} and \textit{GCM1} were significantly associated with decreased odds of LGA status, consistent with findings in the RICH cohort. Consistent trends were also observed between \textit{NR3C1} and fetal growth in both cohorts, with a significant positive association between \textit{NR3C1} expression and LGA status in the SIP cohort. In the SIP cohort, \textit{GCM1} and \textit{EPAS} were additionally associated with increased odds of SGA status. Interestingly, borderline significant positive associations were observed between \textit{ABCG2} and \textit{PLAC1} expression levels and SGA status, contrary to findings in the RICH cohort, where these two genes were inversely associated with SGA status.

**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.

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

**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 expression 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

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**Table 3:** Associations between placental regulatory genes and fetal growth in RICHS and SIP*.

<table>
<thead>
<tr>
<th>Gene</th>
<th>RICHS [OR (95% CI)]</th>
<th>SIP [OR (95% CI)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SGA</td>
<td>LGA</td>
</tr>
<tr>
<td>ABCG2</td>
<td>0.63 (0.44, 0.91)</td>
<td>0.52 (0.37, 0.74)</td>
</tr>
<tr>
<td>CEBPB</td>
<td>1.20 (0.77, 1.88)</td>
<td>0.48 (0.33, 0.71)</td>
</tr>
<tr>
<td>EPAS</td>
<td>1.28 (0.66, 2.48)</td>
<td>0.56 (0.32, 1.00)</td>
</tr>
<tr>
<td>GCM1</td>
<td>1.20 (0.75, 1.91)</td>
<td>0.44 (0.29, 0.68)</td>
</tr>
<tr>
<td>NR3C1</td>
<td>0.69 (0.40, 1.20)</td>
<td>2.33 (1.43, 3.78)</td>
</tr>
<tr>
<td>PGF</td>
<td>1.59 (1.08, 2.35)</td>
<td>0.53 (0.38, 0.73)</td>
</tr>
<tr>
<td>PLAC1</td>
<td>0.66 (0.47, 0.92)</td>
<td>0.58 (0.43, 0.79)</td>
</tr>
<tr>
<td>PLAC4</td>
<td>0.82 (0.60, 1.13)</td>
<td>0.87 (0.64, 1.17)</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>β</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCG2</td>
<td>-0.04</td>
<td>[-0.07, -0.01]</td>
</tr>
<tr>
<td>CEBPB</td>
<td>-0.06</td>
<td>[-0.11, -0.02]</td>
</tr>
<tr>
<td>GCM1</td>
<td>-0.07</td>
<td>[-0.10, -0.03]</td>
</tr>
<tr>
<td>EPAS1</td>
<td>-0.07</td>
<td>[-0.13, -0.01]</td>
</tr>
<tr>
<td>NR3C1</td>
<td>0.12</td>
<td>[0.04, 0.20]</td>
</tr>
<tr>
<td>PGF</td>
<td>-0.03</td>
<td>[-0.06, -0.01]</td>
</tr>
<tr>
<td>PLAC1</td>
<td>-0.03</td>
<td>[-0.05, 0.00]</td>
</tr>
<tr>
<td>PLAC4</td>
<td>-0.02</td>
<td>[-0.04, 0.00]</td>
</tr>
</tbody>
</table>

*Models adjusted for infant race and maternal age.
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**


