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Profiles of gene expression in maternal blood predict offspring birth weight in normal pregnancy

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Abstract

The association between lower birth weight and increased disease risk in adulthood has drawn attention to the physiological processes that shape the gestational environment. We implement genome-wide transcriptional profiling of maternal blood samples to identify subsets of genes and associated transcription control pathways that predict offspring birth weight. Female participants ($N = 178$, mean = 27.0 years) in a prospective observational birth cohort study were contacted between 2009 and 2014 to identify new pregnancies. An in-home interview was scheduled for early in the third trimester (mean = 30.3 weeks) to collect pregnancy-related information and a blood sample, and birth weight was measured shortly after delivery. Transcriptional activity in white blood cells was determined with a whole-genome gene expression direct hybridization assay. Fifty transcripts were differentially expressed in association with offspring birth weight, with 18 up-regulated in relation to lower birth weight, and 32 down-regulated. Examination of transcription control pathways identified increased activity of NF-κB, AP-1, EGR1, EGR4, and Gfi families, and reduced the activity of CEBP, in association with lower birth weight. Transcript origin analyses identified non-classical CD16+ monocytes, CD1c+ myeloid dendritic cells, and neutrophils as the primary cellular mediators of differential gene expression. These results point toward a systematic regulatory shift in maternal white blood cell activity in association with lower offspring birth weight, and they suggest that analyses of gene expression during gestation may provide insight into regulatory and cellular mechanisms that influence birth outcomes.

Introduction

The gestational environment is an important influence on fetal development, with enduring effects on a wide range of organs and systems.[1–4] Research on the developmental origins of health and disease, in particular, has consistently reported associations between lower weight at birth – a readily available, albeit imperfect, proxy measure of the gestational environment – and increased risk for metabolic, cardiovascular, and autoimmune diseases in adulthood.[5–7] Recent studies have documented negative associations between birth weight and cognitive development and adult educational attainment,[8,9] expanding the reach of the gestational environment beyond health to other forms of human capital.

The lasting impact of prenatal environments has drawn attention to the physiological processes that shape the gestational milieu and that may contribute to lower birth weight deliveries. The transfer of nutrients from mother to fetus is an important part of this process, and several studies have shown that fetal nutrient restriction reduces birth weight and increases future risk for diabetes, hypertension, and other cardiovascular diseases.[16–18] Shifts in immune function during pregnancy are also evident, with exaggerated or dysregulated immune activity impairing nutrient transfer and contributing to preterm delivery and fetal growth restriction.[10,11] The circumstances that lead to dysregulated immunity during gestation are difficult to discern but may include infection, loss of fetal tolerance, and activation of neuroendocrine pathways responsive to psychosocial stressors.[12–14]

In this paper, we evaluate patterns of gene expression in whole blood samples collected during the third trimester of pregnancy in a community-based cohort of women in the Philippines. Genome-wide transcriptional profiling, in conjunction with promoter-based bioinformatic

analyses, allows us to assess the activity of all ~20,000 genes in the human genome and to identify the subsets of genes and transcription control pathways that predict offspring birth weight. In particular, examining profiles of gene expression in white blood cells may provide a more comprehensive analysis of the axes of maternal immune activity that are up- and down-regulated, and the cells primarily involved, than is afforded by the measurement of a circumscribed set of biomarkers in maternal circulation. Further, this approach complements prior studies linking birth weight with profiles of gene expression in placental tissue harvested at delivery[15,16] but has the advantage of using white blood cells that are accessible through minimally invasive means during gestation, prior to delivery.

The primary objective of this paper is to determine whether molecular signatures of lower birth weight can be detected in transcription profiles in maternal blood samples, collected early in the third trimester. A secondary objective is to identify the biological themes that characterize the sets of genes that are relatively over- or under-expressed in relation to birth weight. Previously, we have reported that the concentration of C-reactive protein (CRP) – a key biomarker of inflammation – during pregnancy is negatively associated with offspring birth weight in this cohort, consistent with prior research in the USA and elsewhere.[17] We therefore hypothesized that bioinformatic analyses will identify activation of transcription control pathways related to inflammation and immune activation as important predictors of lower birth weight.

Method

Participants and data collection

The Cebu Longitudinal Health and Nutrition Survey (CLHNS) is the largest and longest running birth cohort study in the Philippines. The cohort consists of 3,080 singletons born between May 1, 1983, and April 30, 1984, in urban and rural communities within Metro Cebu. The participants continue to be followed up in multiple surveys, and as of the latest full survey round in 2009, approximately 55% remained in the study.[18,19] Between 2009 and 2014, pregnancies were tracked among the remaining 823 female cohort participants (then 24–30 years old).[20]

During this period, women were contacted at 6-month intervals to identify new pregnancies and an in-home interview was scheduled for the 3rd trimester. Early 3rd trimester was targeted for consistency with prior research linking maternal physiology with offspring birth outcomes and to avoid losing observations associated with preterm delivery. Over the period of tracking, $n = 383$ women became pregnant one or more times ($n = 94$ were pregnant twice and $n = 15$ were pregnant three times), for a total of 507 tracked pregnancies. All data were collected under conditions of informed consent with institutional review board approval from Northwestern University (Evanston, IL) and the Office of Population Studies Foundation (Cebu, Philippines).

Of the $n = 383$ women, $n = 197$ were randomly selected for gene expression analyses (budgetary limitations prevented analysis of all available samples). In the case of women with more than one pregnancy during the tracking period, analyses focused on the first identified pregnancy. Compared with women remaining in the cohort as of 2005 when participants were ~21 years old, women included in the gene expression analysis did not differ in years of formal education (11.1 vs. 11.5 years, $p = 0.14$ for two-sample t test), weekly household income (584 vs. 651 pesos, $p = 0.6$), household material assets (5.2 vs. 5.3, $p = 0.5$), or the urbanicity of their neighborhood (41.1 vs. 41.2, $p = 0.9$; based on 7-component scale). Similarly, they did not differ in height (151.4 vs. 151.2 cm, $p = 0.7$) or weight (46.6 vs. 46.7 kg, $p = 0.9$).

During the in-home interview, women provided information on household demographics, pregnancy-related behaviors and complications, and timing of last menstrual period (LMP). Anthropometric measures were collected using standardized procedures.[21] The mean gestational timing of data collection was 30.3 weeks from LMP (SD = 4.1). Presence of pregnancy complications was based on maternal reports of diagnosed problems, including gestational diabetes, high blood pressure, urinary tract infection, and placenta previa.

A finger-stick dried blood spot (DBS) sample was collected as part of the in-home interview. Each participant's finger was cleaned with alcohol, and a sterile, disposable microlancet was used to deliver a controlled, uniform puncture. Whole blood was placed directly on standardized filter paper commonly used for neonatal screening (Whatman #903, GE Healthcare, Pascataway, NJ).[22] After collection, DBS cards were allowed to dry at room temperature for approximately 4 hours and were stored in gas-impermeable bags at −20°C prior to shipment. Samples were express shipped to the USA where they were stored at −30°C prior to analysis.

A follow-up in-home interview ($n = 187$) was implemented as soon as possible after delivery to collect information on birth outcomes and anthropometric measures, using standardized procedures 20. The median and mean timing of newborn weight measurement was 3 and 4.3 days post-delivery, respectively, with a range of 1–27 days. To minimize the impact of postnatal growth on infant weight, we excluded 7 infants who were measured more than 14 days after delivery, and we adjusted for day of measurement in all models. In addition, we further limited analyses to newborns born at gestational ages between 32 and 44 weeks, which excluded two additional observations for a final sample of 178-term singleton births.

Measurement of gene expression

DBS samples were shipped on dry ice to the UCLA Social Genomics Core Laboratory for RNA extraction, quality assurance assays, and transcriptome profiling. Details on the protocol for measuring gene expression in DBS samples have been published previously.[23] Briefly, total RNA was extracted by placing excised DBS in 370 uL RLT in RNAse-free sterile 1.5-ml microcentrifuge tubes, transferring eluate into a QIAshredder column, and processing through the QIAcube nucleic acid extraction system using RNeasy Micro Kit reagents, the manufacturer's standard operating protocol (including DNAse treatment), and a 20 µL elution volume. Extracted RNA was submitted to cDNA amplification using the NuGEN Ovation PicoSL WTA System, and 100 ng of the resulting cDNA was fragmented and fluorescently labeled using the NuGEN Encore BiotinIL Module for Illumina Whole Genome Expression BeadChips. The resulting fluorescent target sample was assayed using Illumina Human HT-12 v4.0 BeadChips, following the manufacturer's standard protocol for cDNA hybridization, with scanning on an Illumina iScan instrument in the UCLA Neuroscience Genomics Core following the manufacturer's standard protocol.

Statistical analyses

Raw gene expression data were quantile normalized and $\log_2$ transformed for analysis. Differential gene expression was assessed

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using standard linear statistical models with continuously measured birth weight as the dependent variable. Additional analyses included the following covariates as potential confounders/modifiers of the association between 3rd trimester gene expression and offspring birth weight: maternal age (years), sex of the baby (male = 1), maternal body mass index (BMI) prior to pregnancy (kg/m^2), primiparity (0, 1), cigarette smoking during pregnancy (0, 1), access to prenatal care (0, 1), pregnancy complications (0, 1), education level (years of schooling), and household material assets (0–16). Initial models did not include gestational age. Subsequent models adjusted for gestational age to help clarify the extent to which associations with birth weight were due to fetal growth rate or gestational duration. We similarly considered models before and after adjusting for the distribution of leukocyte subsets. Leukocyte subset prevalence was identified by the relative abundance of mRNAs encoding canonical markers for the following cell types: monocytes, CD4+ and CD8+ T lymphocytes, B lymphocytes, and natural killer cells (*CD14*, *CD3D*, *CD3E*, *CD4*, *CD8A*, *CD19*, *FCGR3A/CD16*, *NCAM1/CD56*).

Primary analyses identified genes that were differentially expressed in association with lower birth weight. All genes showing an association point estimate > 1.20-fold difference in average expression level over a 4-SD range in birth weight (i.e., from 2 SD below average to 2 SD above, spanning the sample's range of variation) were subsequently advanced to secondary bioinformatics analyses examining the transcription control pathways underlying the empirical profile of differential gene expression. These analyses tested the hypothesis that lower birth weight would be associated with greater activity of transcription control pathways involved in inflammation and immune activation (NF-κB and AP-1) and myeloid cell activation and differentiation (EGR1, EGR4/NGFIC, and Gfi1). Retained genes served as inputs for TELiS bioinformatics analyses assessing the prevalence of transcription factor-binding motifs (TFBMs) in the promoters of genes up- versus down-regulated in association with low birth weight.[24] NF-κB, AP-1, EGR1, EGR4, and Gfi family TFBMs were defined by TRANSFAC position-specific weight matrices V$NFKB_Q6, V$AP1_C, V$EGR1_01, V$NGFIC_01, and V$GFI1_01, respectively.[25] Log_2 TFBM prevalence ratios (up-/down-regulated) were averaged over nine parametric combinations of core promoter length (−300, −600, and −1000 to +200 bp relative to the RefSeq gene transcription start site) and TFBM detection stringency (TRANSFAC mat_sim values of .80, .90, and .95), with sampling variability in the mean log ratio quantified by 200 cycles of bootstrap resampling of linear model residual vectors (accounting for the correlation among genes).[26] Given this study's sample size and observed magnitude of differential gene expression, statistical power to detect a representative twofold (1 log_2 unit) TFBM prevalence ratio at $p < 0.05$ is 0.90.

With this analytic approach we tested each substantive, biologically informed hypothesis (e.g., differential NF-κB pathway activity) at an aggregated error rate of $p < 0.05$, using a single integrated statistical test (*p*-value). Because each biological hypothesis was distinct, pre-specified (not exploratory/discovery analyses of individual genes), and tested using a single integrated test statistic/p-value, no correction for multiple testing is needed (or possible, given that only a single *p*-value is computed). Our use of a single integrated test of each substantive hypothesis is consistent with recommended statistical practice, which only indicates correction for multiple testing within a family of analyses that involves multiple outcomes being tested in parallel to evaluate a single substantive hypothesis.[27]

Table 1. Descriptive statistics for study participants ($n = 178$)

Age (years)	27.0 ± 1.5
Primiparity (%)	29.8
Used prenatal care (%)	98.9
Smoked during pregnancy (%)	5.1
Presence of pregnancy complications (%)	18.5
BMI, pre-pregnancy (kg/m^2)	21.2 ± 3.3
Education (years)	11.0 ± 3.3
Household assets (# items)	4.8 ± 3.6
Infant birth weight (g)	2995 ± 337
Gestational age (wks)	39.3 ± 1.9
Male infants (%)	51.1

Values are mean ± SD for continuous variables, percentage for categorical variables.

Additional analyses used transcript origin analysis (TOA)[28] of the same input gene set to test whether lower birth weight was associated with altered activation of the myeloid lineage immune cells involved in tissue maintenance and homeostasis, particularly in pregnancy (monocytes and dendritic cells).[29–31] These analyses examined differentially expressed genes for preferential expression in cell-type-specific reference profiles from CD4+ T cells, CD8+ T cells, B cells, NK cells, neutrophils, classical (CD16-) monocytes, non-classical (CD16+) monocytes, and three types of dendritic cells: CD1c/BDCA1+ "DC1" cells, CD303/BDCA2+ plasmacytoid "DC2" cells, and CD141/BDCA3+ "DC3" cells.[32] To determine whether any observed differences in cell-specific transcript abundance stemmed from differences in the prevalence of specific immune cell subpopulations (as opposed to differential transcriptional activity of a particular cell population), we conducted transcriptome representation analysis (TRA) using the same reference profiles.[33] In both cases, statistical testing was again based on standard errors derived from 200 cycles of bootstrap resampling of residual vectors.

Results

Women were 24–30 years old when pregnant, with a mean age of 27.0 (Table 1). Mean birth weight was 2,995 g and ranged from 2,172 to 3,725 g. Genome-wide transcriptional profiling of whole blood samples identified 50 gene transcripts associated with birth weight (>1.2-fold difference in average expression) after adjustment for infant sex, pregnancy complications, maternal age, education level, pre-pregnancy BMI, material assets, smoking, parity, and access to prenatal care (18 of the identified transcripts were up-regulated in association with lower birth weight and 32 were down-regulated; all 50 are listed in the Supporting Information Table 1).

Subsequent analyses for biological themes examined the transcription control pathways underlying this pattern of differential gene expression. TELiS promoter-based bioinformatics analyses implicated increased activity of NF-κB (log_2 TFBM ratio: mean 1.27 ± 0.57 SE, $p = 0.025$) and AP-1 (1.05 ± 0.53, $p = 0.049$) transcription factors in driving the differential gene expression associated with lower birth weight (Fig. 1a).

To identify cellular mechanisms of these transcriptional alterations, parallel TOA analyses were conducted and identified non-classical CD16+ monocytes and CD1c+ myeloid dendritic cells as primary cellular mediators of genes up-regulated in association

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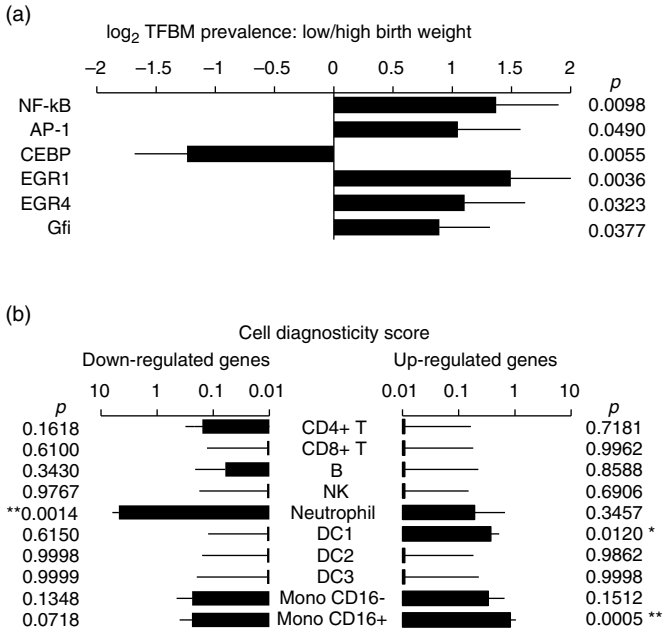

Fig. 1. Offspring birth weight in association with up-/down-regulated transcription factor-binding motifs (a) and cellular mediators of differential gene expression (b).
* p< 0.05
** p< 0.01

with lower birth weight (both $p < 0.05$; Fig. 1b). Neutrophils were identified as cellular mediators of gene down-regulation in association with lower birth weight ($p = 0.001$, Fig. 1b).

Consistent with the involvement of these myeloid lineage cell types, follow-up TELiS analyses also implicated key myeloid-regulating transcription factors, EGR1 (1.49 ± 0.50, $p = 0.0036$), EGR4/NGFIC (1.10 ± 0.51, $p = 0.032$), and Gfi family factors (0.89 ± 0.42, $p = 0.037$) in structuring the transcriptional correlates of lower birth weight. Analyses also indicated down-regulation of the C/EBP family of transcription factors involved in neutrophil differentiation ($-1.24 \pm 0.44, p = 0.006$). These findings are also consistent with the functional characteristics of the genes associated with birth weight, which include genes involved in neutrophil function (*MPO, DEFA1, DEFA1B, DEFA3, JUN, S100P*), oxygen transport and hemostasis (*HBG1, HBG2, HBQ1, HEMGN, VWCE*), and Type I interferon responses mediated by dendritic cells and non-classical monocytes (*APOBEC3A, IFI44L, IFI2, IFITM3, IFIT1, HERC5, HERC6A*). Collectively, these results suggest a regulatory shift in myeloid lineage cell differentiation patterns, with lower birth weight predicted by reduced activation and/or prevalence of neutrophils and increased activation and/or prevalence of non-classical monocytes and CD1c+ dendritic cells.

To determine whether the observed shift in myeloid lineage gene transcription reflected changes in cell subset prevalence or merely changes in the activation state of a fixed myeloid lineage population, we conducted two additional analyses. In the first, TRA was used to directly quantify cell subset prevalence and failed to identify any significant difference in the prevalence of any analyzed cell type in association with birth weight (all $p > 0.11$). In the second, we re-examined the transcription factor correlates of lower birth weight after controlling for the abundance of mRNAs encoding canonical markers of each leukocyte subset. With these additional controls, the results associating lower birth weight with greater activity of NF-κB (1.56 ± 0.52, $p = 0.003$) and AP-1 (1.14 ± 0.44, $p = 0.010$) were modestly strengthened. Similar results also emerged when analyses were adjusted for gestational age at delivery (NF-κB: 1.72 ± 0.51, $p = 0.0009$; AP-1: 1.42 ± 0.46, $p = 0.002$).

In order to estimate the magnitude of association between gene expression and birth weight, we created a summary variable based on the relative value for each of the 50 differentially expressed genes. Transcription values were standardized and summed, with reverse coding for the 18 genes for which increased expression predicted lower birth weight. Cronbach's alpha for the summary variable was 0.89, indicating a high level of consistency within individuals in the relative level of transcription across this subset of genes. In a fully adjusted model, gene expression was a significant predictor of birth weight, as expected (Table 2). The magnitude of association was substantial, with each standard deviation increase in transcription predicting an additional 109 g of birth weight (Fig. 2). Furthermore, a fully adjusted model including the summary gene expression variable explained 24.3% of the variance in birth weight, in comparison with 14.5% for the same model, but omitting gene expression.

Discussion

Lower birth weight is a robust predictor of increased risk for morbidity and mortality in adulthood, but the physiological processes that shape the gestational environment and reduce weight among healthy, full-term neonates remain poorly understood. Using genome-wide transcriptional profiling, we identify a subset of genes that are differentially expressed in maternal white blood cells in association with offspring birth weight. We also identify biological themes that may provide promising directions for future research into the mechanisms through which developmental process and environmental exposures influence the gestational milieu, with implications for trajectories of health in the next generation.

Our results suggest that offspring birth weight is associated with a systematic shift in gene expression profiles in maternal white blood cells during the third trimester of gestation. Specifically, lower birth weight is predicted by increased activity of two key

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Table 2. Coefficients from least squares regression models predicting offspring birth weight (g)

	Model 1			Model 2			Model 3		
	B	95% CI		B	95% CI		B	95% CI	
Transcription level				300.6***	179.9	421.2	279.8***	163.7	395.9
Age (years)	−4.5	−35.6	26.7				−6.9	−36.2	22.4
Primiparity (0, 1)	−96.5	−207.3	14.2				−87.9	−192.2	16.3
Prenatal care (0, 1)	628.1**	164.6	1091.6				541.4*	103.8	979.0
Smoked (0, 1)	127.0	−87.3	341.3				94.7	−107.4	296.8
Pregnancy complications (0, 1)	−12.4	−140.4	115.6				−49.1	−170.5	72.3
BMI, pre-pregnancy (kg/m^2)	97.5***	43.3	151.7				102.2***	51.2	153.2
Education (years)	9.4	−40.2	59.0				6.3	−40.4	53.0
Household assets (#)	8.0	−9.0	24.9				5.4	−10.6	21.4
Gestational age (wks)	46.1***	21.4	70.9				35.9***	12.2	59.6
Male infant (0,1)	57.9	−36.0	151.8				46.4	−42.0	134.9
Constant	595.9	−800.6	1992.4	2994.5***			1148.0	−185.8	2481.8
Adjusted R^2	0.145			0.116			0.243		

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

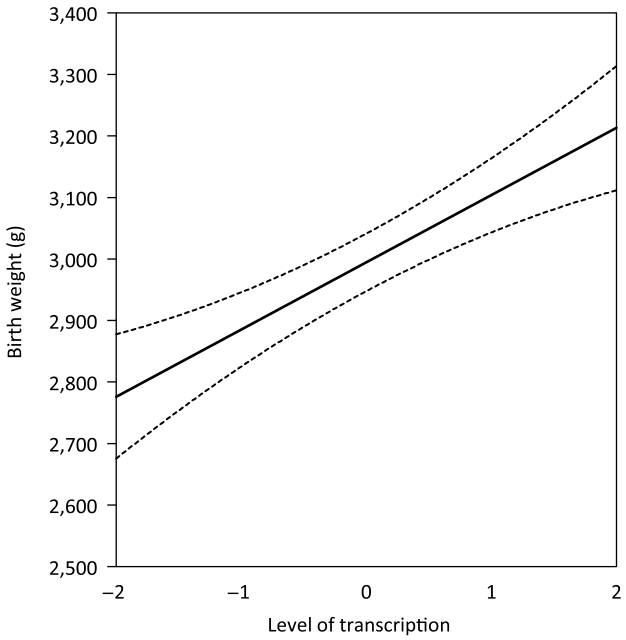

Fig. 2. Predicted birth weight in relation to a summary measure of expression of 50 transcripts, based on coefficients from Table 2, model 3. The 95% confidence interval is represented by dashed lines.

transcription control pathways involved in both inflammation and immune activation: NF-κB and AP-1. Furthermore, the pattern of empirical differences in gene expression suggests systematic skew in myeloid lineage immune cell activation involving up-regulation of CD1c+ dendritic cells and non-classical monocytes, and down-regulation of neutrophils. These associations are independent of maternal demographic characteristics (e.g., age, education, material assets) and other pregnancy-related variables (infant sex, gestational age at birth, pregnancy complications, and access to prenatal care). They are also independent of the prevalence of major leukocyte subsets within the assayed RNA pool. As such, the observations here point toward a shift in the transcriptional patterns of non-classical monocytes and dendritic cells relative to neutrophils, rather than a change in cell population abundance.

The association between lower birth weight and expression of genes involved in inflammation and immune activation is consistent with a wide body of prior research. Several studies – including two with this cohort – have documented negative associations between inflammatory biomarkers (e.g., CRP, pro-/anti-inflammatory cytokines) in maternal circulation during gestation and offspring birth weight.[17,34] Further, analyses linking inflammatory lesions in the placenta with fetal growth restriction and preterm delivery underscore the role of inflammatory/immune processes in shaping the gestational milieu.[35,36] Our study is novel in that it is one of the few studies to evaluate the transcriptome in relation to birth weight, and we go beyond prior work on the placenta by focusing on gene expression in maternal blood cells collected several weeks in advance of delivery.

The processes that trigger a systematic shift in gene expression in association with lower birth weight remain to be illuminated in future research and may reflect – on a proximate level – the effects of endogenous physiological processes (e.g., hormonal influences) and/or acute exposures to infectious agents.[12–14] Indeed, the expression profile that predicts lower birth weight – down-regulated neutrophil activity and up-regulated non-classical monocyte and dendritic cell activity – reflects a shift away from anti-bacterial responses and toward responses stimulated by viral infections,[37,38] suggesting a potential microbial alteration in immune homeostasis that subsequently impacts the immunoregulatory status of the maternal–fetal tissue interface.

More distally, it is possible that these shifts represent responses to environmental circumstances over the course of development. Microbial exposures in infancy and early childhood, for example, have been shown to have lasting effects on the regulation of inflammation and other immune processes.[39–44] Similarly, patterns of infant feeding, stunted growth, and exposure to psychosocial adversity in childhood influence patterns of immune regulation and function in adulthood.[45–47] The implications for gestational

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biology and birth outcomes are not clear, but a recent study found that women who experience economic hardship in childhood are at higher risk for adverse birth outcomes, including low birth weight, and that this association is partially mediated by maternal inflammation during gestation.[48]

A limitation of this study is the observational design, which poses a challenge to causal inference in that we cannot rule out unmeasured exposures that may account for the association between gene expression and offspring birth weight. In addition, although the community-based sample is locally representative, recruiting participants from a circumscribed geographic and ecological setting may limit the generalizability of these findings to other populations. On the other hand, comparisons with future studies, in different ecological settings, may provide opportunities for highlighting the exposures (e.g., microbial, nutritional) that influence patterns of gene expression during pregnancy. Caution is also warranted in extrapolating the magnitude of association between maternal gene expression and offspring birth weight in that we used the same data set to identify both the specific transcriptional correlates of birth weight and to estimate their predictive power. Future research will be required to assess the performance of these predictors in other settings. Birth weight also has limitations as a measure of fetal growth restriction, even when adjusted for gestational age, and future analyses should consider additional measures of newborn anthropometry and growth relative to duration of gestation. Lastly, this study was not powered for de novo discovery of statistically reliable associations of low birth weight with individual gene transcripts: such an analysis would require >5-fold larger sample size than available here. Additional transcriptional correlates of lower birth weight likely exist and remain to be identified in future research using larger samples.

The clinical and biological significance of our findings is not clear and remains to be elaborated in future research. Results are promising in that we were able to detect a strong transcriptional signal of lower birth weight in maternal blood samples collected 2–3 months prior to delivery. Analyses also point to pathways of known importance to the gestational milieu but may help clarify the specific regulatory and cellular mechanisms that contribute to lower birth weight. As such, the study may provide a foundation for future research that identifies new targets for salutary interventions, as well as molecular biomarkers that may be used to gauge risk or proxy outcomes in future studies.

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Conflicts of Interest. None

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the Belmont Report and with the Helsinki Declaration of 1975, as revised in 2008, and have been approved by the institutional committees at Northwestern University (Evanston, IL) and the Office of Population Studies Foundation (Cebu, Philippines).

Supplementary Material. To view supplementary material for this article, please visit https://doi.org/10.1017/S2040174419000175.

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