OBSTETRICS

The preterm placental microbiome varies in association with excess maternal gestational weight gain

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OBJECTIVE: Although a higher maternal body mass index is associated with preterm birth, it is unclear whether excess gestational weight gain (GWG) or obesity drives increased risk. We and others have shown that the placenta harbors microbiota, which is significantly different among preterm births. Our aim in this study was to investigate whether the preterm placental microbiome varies by virtue of obesity or alternately by excess GWG.

STUDY DESIGN: Placentas (n = 320) were collected from term and preterm pregnancies. Genomic DNA was extracted and subjected to metagenomic sequencing. Data were analyzed by clinical covariates that included the 2009 Institute of Medicine's GWG guideline and obesity.

RESULTS: Analysis of 16S recombinant RNA—based metagenomics revealed no clustering of the microbiome by virtue of obesity ($P = .161$). Among women who spontaneously delivered preterm, there was again no clustering by obesity ($P = .480$), but there was significant clustering by excess GWG ($P = .022$). Moreover, among preterm births, detailed analysis identified microbial genera (family and genus level) and bacterial metabolic gene pathways that varied among pregnancies with excess GWG. Notably, excess GWG was associated with decreased microbial folate biosynthesis pathways and decreased butanoate metabolism (linear discriminate analysis, >3.0-fold).

CONCLUSION: Although there were no significant alterations in the microbiome by virtue of obesity per se, excess GWG was associated with an altered microbiome and its metabolic profile among those women who experienced a preterm birth.

Key words: excess gestational weight gain, maternal obesity, metagenomics, microbiome, preterm birth

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The human microbiome encompasses the totality of the microbes that live on and within our bodies and is the core of the emerging fields of metagenomic science and medicine (Table 1).[1,2] In 2012, the *healthy microbiome* in nonpregnant[3-5] and pregnant[6,7] women was defined across body sites and was recently expanded to now include in-depth characterization of the placental microbiome[8] and longitudinal profiling of the vaginal (posterior fornix) microbiome throughout pregnancy.[9-11]

Contrary to the commonly held paradigm that the upper reproductive tract and placenta are "sterile," we and others have shown that microbiota is present, even in the absence of any clinical evidence of intraamniotic infection.[8,12-25]

Using metagenomic approaches, we have observed that the placental microbiome profile significantly varied in association with spontaneous preterm birth[8] and that the taxonomic profiles that are associated with term or preterm pregnancies were accompanied by variations in bacterial-encoded metabolic pathways. Collectively, these findings are consistent with multiple other published reports over the years that have demonstrated the presence of microbes in previously held "sterile" sites, including the endometrium,[19-21] placenta,[8,13-18,22] and chorion/amnion and amniotic fluid.[23-25]

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TABLE 1
Terms and definitions

Term	Definition
Microbiota	Microbial population present in different ecosystems (niches) of the body
Microbiome	The totality of the microbes (and their genomes) who share our body space[1]: can also refer to the totality of microbes (and their genomes) in a given body site or niche
Metagenome	Entire community genomic repertoire and metabolic profile.
Community	The entirety of microbes in a given body site or niche
Dysbiosis	Microbial imbalance; altered community structure
16S recombinant RNA sequencing	Highly conserved gene in different species of bacteria that enables its use as a universal primer
Whole genome shotgun sequencing	*WGS*: used for metagenomic analysis
Operational taxonomic units	*OUT*: a distinct sequence
Diversity	Difference in the bacteria, either within a group of samples (alpha-diversity) or between a group of samples (beta-diversity)
Richness	Number of OTUs (or species) in a sample: richness reflects the abundance of microbes across multiple taxa
Alpha-diversity	Biodiversity within a group of samples[6]
Beta-diversity	Biodiversity between groups of samples[6]
Principle Coordinate Analysis	*PCoA*: used to examine beta-diversity between 2 groups of samples
UniFrac	Used to calculate a distance measure between organismal communities with the use of phylogenetic information; with the use of UniFrac, all taxa that are found are placed on a phylogenetic tree. A branch leading to taxa from both samples is marked as "shared"; branches that lead to taxa that appear in only 1 sample are "unshared."
Phylogenetic distance	Uses UniFrac: *distance* is defined as the sum of unshared branch lengths divided by the sum of all shared and unshared branches
Permutational multivariate analysis of variance	*PERMANOVA*: does not assume a normal distribution.

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Although the cause of preterm birth is multifactorial, its association with infection and inflammation has long been suspected.[22,26-44] The aforementioned variations in commensal microbiota–driven metabolism may result in the placental microbiome emerging as a key mediator in preterm birth. For example, although bacterial species that are present in preterm pregnancies may not be pathogenic necessarily, a relatively altered microbial community structure (dysbiosis) may convey an environment of localized inflammation that results in preterm birth.[29,30]

Previously, obesity has been shown to be associated with variation in placental[45,46] and systemic[47] inflammation. Additionally, obesity is associated with preterm birth.[48-51] Altogether, these observations have been assumed to suggest that obesity may contribute to preterm birth through increased inflammation. However, excess gestational weight gain (GWG) has also been associated with increased risk of preterm birth[50-53] and alterations in metabolic markers.[54] Thus this begs the question, is it obesity or excess GWG that modulates the triad of inflammation, an altered placental microbiome, and spontaneous preterm birth?

We hypothesized that excess GWG (but not obesity per se) would associate with distinct alterations in the placental microbiome and that these alterations would be pronounced significantly among women who spontaneously deliver preterm. The aims of this study were (1) to discriminate whether maternal obesity or GWG was associated with significant variation in the placental microbiome profile, (2) to determine whether this was observed in both the term and preterm interval, and (3) to determine which placental microbial metabolic pathways were affected by excess maternal GWG.

Materials and Methods

Subjects and sample materials

This study was approved by the Institutional Review Board at Baylor College of Medicine (H-26589) and Harris Health System and was performed with the use of specimens from our universal perinatal database and biospecimen repository (PeriBank database, Institutional Review Board H-26364; Baylor College of Medicine). All pregnant women who come to labor and delivery for delivery are recruited for enrollment into PeriBank, which is Baylor College of Medicine's universal perinatal database and biospecimen repository. A detailed description of PeriBank and the selection criteria for this study, including the clinical metadata that are extracted, is available in our previous publication on the placental microbiome.[8] Subjects for this study were recruited between August 2011 and November 2012 by trained PeriBank study personnel. As part of the consent process, we discussed with participants the potential risks of participation, including the physical risks that are associated with specimen collection and the possibility that protected health information or deidentified project data