OBSTETRICS

The placental microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis

Amanda L. Prince, PhD; Jun Ma, PhD; Paranthaman S. Kannan, PhD; Manuel Alvarez, MSc; Tate Gisslen, MD; R. Alan Harris, PhD; Emma L. Sweeney, PhD; Christine L. Knox, PhD; Donna S. Lambers, MD; Alan H. Jobe, MD, PhD; Claire A. Chougnet, PhD; Suhas G. Kallapur, MD*; Kjersti M. Aagaard, MD, PhD*

BACKGROUND: Preterm birth (PTB) is a leading cause of neonatal morbidity and mortality and is not uncommonly associated with chorioamnionitis. We recently have demonstrated that the placenta harbors a unique microbiome with similar flora to the oral community. We also have shown an association of these placental microbiota with PTB, history of antenatal infection, and excess maternal weight gain. On the basis of these previous observations, we hypothesized that the placental membranes would retain a microbiome community that would vary in association with preterm birth and chorioamnionitis.

OBJECTIVE: In the current study, we aimed to examine the differences in the placental membrane microbiome in association with PTB in both the presence and absence of chorioamnionitis and/or funisitis using state-of-the-science whole-genome shotgun metagenomics.

STUDY DESIGN: This was a cross-sectional analysis with 6 nested spontaneous birth cohorts (n = 9–15 subjects/cohort): Term gestations without chorioamnionitis, term with chorioamnionitis, preterm without chorioamnionitis, preterm with mild chorioamnionitis, preterm with severe chorioamnionitis, and preterm with chorioamnionitis and funisitis. Histologic analysis was performed with Redline's criteria, and inflammatory cytokines were analyzed in the cord blood. DNA from placental membranes was extracted from sterile swabs collected at delivery, and whole-genome shotgun sequencing was performed on the Illumina HiSeq platform. Filtered microbial DNA sequences were annotated and analyzed with MG-RAST (ie, Metagenomic Rapid Annotations using Subsystems Technology) and R.

RESULTS: Subjects were assigned to cohorts on the basis of gestational age at delivery and independent scoring of histologic chorioamnionitis. We found that preterm subjects with severe chorioamnionitis and funisitis had increases in cord blood inflammatory cytokines. Of interest, although the placental membrane microbiome was altered in association with severity of histologic chorioamnionitis (permutational multivariate analysis of variance $P = .005$), there was no observable impact with either betamethasone or antibiotic treatment. In preterm subjects with chorioamnionitis, we found a high abundance of both urogenital and oral commensal bacteria. These alterations in the microbiome were accompanied by significant variation ($P < .05$) in microbial metabolic pathways important in the glucose-fed pentose phosphate pathway (term subjects), or glycerophopholipid metabolism, and the biosynthesis of the siderophore group nonribosomal peptides (preterm subjects).

CONCLUSION: Consistent with ours and others previous findings, women who experienced spontaneous PTB harbor placental microbiota that further differed by severity of chorioamnionitis. Integrative metagenomic analysis revealed significant variation in distinct bacterial metabolic pathways, which we speculate may contribute to risk of preterm birth with and without severe chorioamnionitis.

Key words: chorioamnionitis, funisitis, microbiome, preterm birth, whole-genome shotgun metagenomics

Preterm birth (PTB; <37 weeks of gestation) is among the leading causes of global neonatal morbidity and mortality. On average, 9.6% of births in the United States are preterm, and an increased incidence of PTB in the United States occurred from 1981 to 2012, with a recent stabilization reported.[1,2] Although the etiology of PTB remains elusive, a near majority (40%–70%) of PTBs may be associated with chorioamnionitis that is clinically diagnosed by the occurrence of a sustained (>2 hours) maternal fever of greater than 100.4°C and at least 1 of the following symptoms: maternal tachycardia (>100 bpm), fetal tachycardia (>160 bpm), foul-smelling amniotic fluid, or uterine tenderness.[3] Of women diagnosed with clinical chorioamnionitis, however, less than 70% receive confirmation of histologic chorioamnionitis.[4,5] Conversely, a previous study demonstrated the presence of histologic chorioamnionitis without clinical symptoms, which suggests that placental inflammation may occur in the absence of an infectious agent.[4] In addition to maternal complications associated with chorioamnionitis, severe cases of chorioamnionitis are associated with neonatal sepsis, funisitis (inflammation of the umbilical cord), and fetal inflammatory response syndrome.[3,4]

Previously, it has been proposed that an ascending infection from the vagina to the placenta induces chorioamnionitis and PTB.[6] This hypothesis was proposed as a result of the association of bacteria of the urogenital tract, such as *Mycoplasma* species, *Ureaplasma* species, and group B *Streptococcus*, with chorioamnionitis and with colonization of placental/fetal membranes.[7-18] Although previous studies describing ascending infection have used primarily culture-based

Cite this article as: Prince AL, Ma J, Kannan PS, et al. The placental microbiome is altered among subjects with spontaneous preterm birth with and without chorioamnionitis. Am J Obstet Gynecol 2016;●●●:●●●●.

0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2016.01.193

FLA 5.4.0 DTD ■ YMOB10941_proof ■ 4 March 2016 ■ 1:51 pm ■ ce

techniques,[8,11-15,17-20] the advent of next-generation (NextGen) metagenomics sequencing has allowed for the culture-independent characterization of the vaginal microbiome.[21-26] Similar to previous studies that use culture-based techniques, the vaginal microbiome in the majority of women is dominated by *Lactobacillus* species in both nonpregnant and pregnant populations.[21-26] In addition to characterizing pregnancy-associated changes in the vaginal microbiome, recent studies that investigated the vaginal microbiome in association with PTB were performed. These studies from several distinct patient populations have similarly pyrosequenced the vaginal microbiome between term and preterm subjects longitudinally but with mixed and disparate findings.[25-27] Altogether, these studies emphasize the importance for further investigation into the role of other body niche microbiota in PTB.

Intriguingly, bacterial species not associated with the vagina have been implicated in chorioamnionitis and with placental/fetal colonization, such as the oral bacteria of *Streptococcus* species and *Fusobacterium* species.[7,9,28-31] Recently, bacteria has been found within the placenta of term and preterm subjects,[32-34] and we have previously provided an in-depth metagenomic characterization of the placental parenchyma microbiome.[35,36] We determined that the bacteria harbored in the placenta are associated most closely with the microflora of the oral cavity and vary in association with PTB and excess maternal gestational weight gain.[35,36] The association between the placental and oral microbiomes provides a potential explanation for the presence of commensal oral bacteria (such as *Streptococcus* and *Fusobacterium* spp) in chorioamnionitis, with a potential mechanism being hematogenous spread of bacteria during pregnancy.[31,37] When we further investigated the placental parenchyma microbiome, we found significant differences between term placentas and preterm placentas.[35,36] Given the known association between inflammation and PTB, we aimed to further examine the placental membrane microbiome in an independent cohort in the context of inflammation in the chorion and amnion by investigating term and preterm subjects with and without chorioamnionitis and funisitis undergoing spontaneous labor.

Materials and Methods

Subjects

The gravidae provided consent from 2009 to 2012 under a protocol approved by the Institutional Review Boards (IRBs) of Good Samaritan Hospital, Cincinnati, Ohio (09105-09-067) and Cincinnati Children's Hospital Medical Center (2009-0236); subsequent IRB approval for metagenomics studies was obtained from the Baylor College of Medicine IRB (H-27393). Informed consent was requested from mothers admitted for imminent preterm delivery. Inclusion criteria for the preterm cohort were mothers delivering between 32^0 and 36^6 weeks' gestation because of preterm premature rupture of membranes, spontaneous preterm labor, or clinically diagnosed chorioamnionitis. The inclusion criteria for the term infants were women presenting with spontaneous labor at $\geq$38 weeks of gestation. We excluded mothers delivering infants for medical indications, such as maternal hypertensive disorders including preeclampsia and HELLP syndrome, because these disorders would be expected to have a very low incidence of chorioamnionitis and therefore skew the comparisons. Only 3 subjects (Preterm 381, Term 225, and Term 239) had findings of incidental hypertension, which was not an indication for induction of labor or delivery. Also excluded were the following: gravidae with HIV; delivered infants with acute, life-threatening illness or requiring extensive resuscitation; or fetuses with congenital malformations or infections, such as syphilis or cytomegalovirus. Maternal and neonatal demographics were collected by interview and chart review.

Sample collection

All sample collection was performed with the use of standard operating procedures and followed a strict uniform protocol established before study initiation. Nurses specifically trained in study procedures performed sample collection. After delivery of the infant and before delivery of the placenta, cord blood was collected from the umbilical vein with a sterile ViaCord collection kit (ViaCord, Waltham, MA) containing citrate phosphate dextrose anticoagulant. Placenta was delivered by standard obstetrical practice and immediately collected in sterile bags. These were kept in a refrigerator until sampling. Placental sampling was performed in a dedicated room via strict sterile precautions to prevent exogenous contamination. Placenta was kept with the fetal surface facing the operator. The amnion surface was cleaned by swabbing with 70% ethanol and drying immediately. Then, the surface of the amnion was cut with sterile instruments. A second set of sterile instruments was used to perform a blunt dissection to create a pocket on the underside of the amnion without puncturing the amnion. Swabs for microbial collection were swirled to collect samples from fetal chorion and/or villous placental membranes adjacent to the fetal side while taking care to avoid contamination from the maternal side.

The following swabs were collected and subsequently cryofrozen until analyses: (1) Dry Dacron swab for DNA collection (part 220115; BD, Franklin Lakes, NJ), (2) UP transport medium (clear media, part 220221; BD), and (3) Port-A-Cul (for anaerobic and aerobic culture; pink media, part 221607; BD). This collection method differs from our previous study involving the placental tissue microbiome.[35] In our previous study, placental parenchyma tissue was collected 4 cm from the cord insertion site.[35] In our current study, we examined the placental membrane microbiome in association with chorioamnionitis. The niche site is ideal for examining associations between the placental microbiome and inflammation because of the presence of decidual leukocytes.

Histology

The placenta and umbilical cord were sampled from specific tissue locations via an aseptic-standardized protocol

FLA 5.4.0 DTD ■ YMOB10941_proof ■ 4 March 2016 ■ 1:51 pm ■ ce