PREMATURITY

Abstracts 9-19

Moderators: Jay Iams, MD; Joe Simpson, MD, Senior Vice President for Research and Global Programs, March of Dimes

9 Progesterone promotes the expansion of proangiogenic immature myeloid cells and prevents their differentiation into inflammatory cells

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OBJECTIVE: Immature myeloid cells (IMCs) are bone marrow-derived cells that normally differentiate into granulocytes, macrophages, and dendritic cells (DCs) but expand in pathological conditions such as malignancy. DCs are antigen-presenting cells that regulate the immune response. We have shown that IMCs accumulate in the placenta and actively promote angiogenesis. IMCs peak in concentration during mid-pregnancy and their presence correlates with neonatal birthweight. Furthermore, labor and delivery are preceded by a decrease in IMCs and an increase in DCs populating the placenta. We hypothesized that progesterone may promote IMC population expansion while preventing their differentiation into inflammatory cells.

STUDY DESIGN: We derived bone marrow cells from C57Bl6 pregnant mice. We isolated the $CD11b^+$ myeloid subset by magnetic based immuno-separation, and cultured them in the presence of GMCSF and progesterone (10^{-7}, 10^{-8}, and 0 mM). After 5 days we analyzed cultures for the presence of $Ly6C^{int}Ly6G^{hi}$ granulocytic IMCs, $Ly6C^{hi}Ly6G^{int}$ monocytic IMCs and $CD11c^+MHCII^+$ DCs by flow cytometry. We also compared their presence in Lewis Lung Carcinoma tumors that were implanted subcutaneously in mice to that in placentas derived from pregnant mice.

RESULTS: Progesterone treatment caused a dose dependent increase in granulocytic IMCs ($n=3$, $p<0.05$), accompanied by a concomitant decline in the monocytic IMCs ($n=3$, $p<0.001$). Importantly, these changes were paralleled by a decrease in DCs ($n=3$, $p<0.05$). When analyzing $CD45^+$ hematopoietic cells in tumors and placentas, we detected a significant enrichment ($P<0.01$) of monocytic IMCs subpopulation in tumors compared to that in placentas. This was paralleled by a concomitant, more than 2-fold decrease ($P<0.01$) in granulocytic IMCs.

CONCLUSION: Progesterone enhances proliferation and/or survival of placenta specific- granulocytic IMCs but not that of tumor specific-monocytic IMCs, in a dose dependent manner. Importantly, the differentiation of IMCs into DCs was abrogated by progesterone. We thus speculate that progesterone may play role in the maintenance of proangiogenic IMCs in the placenta. Inhibition of their differentiation into inflammatory cells such as DCs and neutrophils might explain, at least in part, the protective effect of progesterone in preventing preterm labor.

10 Distinct microbiota in the cervicovaginal space are associated with spontaneous preterm birth: findings from a large cohort and validation study

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OBJECTIVE: Changes in microbial communities have been implicated in both health and disease. Investigations into the association between the cervicovaginal (CV) microbiota and spontaneous preterm birth (SPTB) have been limited in scope and number. This study sought to assess if longitudinal cohort of pregnant women and then to perform validation in a 2nd prospective cohort.

STUDY DESIGN: A prospective cohort of singleton pregnancies were enrolled ("M&M", n=1500). Biospecimens were collected at 3 time points in pregnancy (16-20 (V1), 20-24 (V2), 24-28 (V3) weeks). All cases of PTB were adjudicated by the PI. From the larger cohort, a nested case-control was performed with 80 SPTB cases and 320 term controls that were frequency matched by race to the cases. 16S rRNA gene analyses were performed to characterize the composition and structure of the CV microbiota. The effect of bacteria was quantified as the log ratio between the mean relative abundance at SPTB samples vs. TERM delivery samples. The log ratios were estimated using zero-inflated negative binomial models. A second cohort of woman ("STOP") with specimens collected between 22-32 weeks was used as validation (N=616).

RESULTS: When performing phylotype analyses, 127 phylotypes were detected in all samples from both cohorts. Significant associations were demonstrated between specific bacteria, in both a positive and negative manner, with SPTB. 37 bacteria were significantly associated with a decreased risk of SPTB while 13 were associated with an increased risk in the primary cohort. Racial differences in these associations were evident (figure 1). The validation cohort confirmed the highly significant associations between specific microbes and SPTB. Bifidobacterium species were noted to be significantly protective against SPTB at all gestational time points while BVAB2, BVAB3 and Mobiluncus were associated with a dramatic increase risk of SPTB (all q-values <0.0001).

CONCLUSION: CV microbiota are significantly associated with SPTB. Targeting the bacteria that are associated with an increased risk of SPTB and/or enhancing the presence of the protective bacteria may serve as new therapies to reduce the rate of PTB. With this new evidence, these types of studies should become a research priority. (R01NR014784).

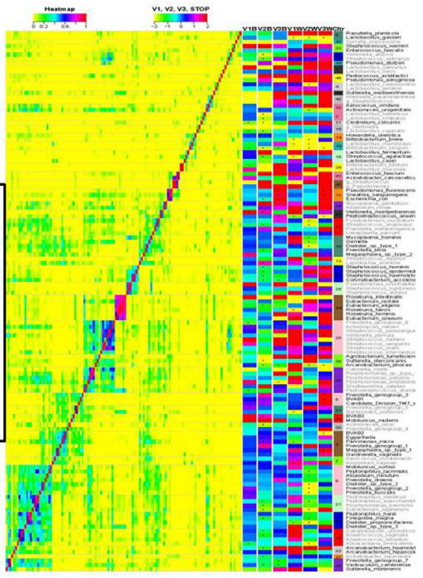

Figure 1: Heatmap of the correlation matrix of 127 phylotypes in the M&M 16S rRNA data, together with color bars for the effect of SPTB on the relative abundance of these phylotypes at V1, V2, V3 as well as in STOP. Significant associations are marked with *. First 3 columns are in AA women in M&M; columns 4-6 are non-AA, and the last column is STOP cohort

11 Use of evolutionary triangulation to refine genetic association studies of spontaneous preterm birth (SPTB)

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OBJECTIVE: Genetic association studies of SPTB have generally yielded inconsistent results. SPTB rates in the US are lowest in European-Americans compared to other groups including Hispanics, American Indians, and African-Americans. We hypothesized that genes identified by Evolutionary Triangulation (ET), a novel analytic technique exploiting evolutionary differentiation by comparing population structure among 3 populations with variable patterns of disease prevalence, could refine results from previous SPTB gene association studies.

STUDY DESIGN: We tested 2 SPTB gene lists: (1) Top maternal and fetal genes corresponding to top 20 maternal and fetal SNPs from GWAS of 1,025 SPTB cases < 34 wks and 1,015 term controls (Zhang, et al., 2015) (2) 640 genes on online dbPTB site. To generate the ET gene list, SNP allele frequency data were first obtained from CEU (Utah residents with Western and Northern European ancestry from the CEPH collection), GIH (Gujarati Indians in Houston, TX)/MEX (Mexican ancestry in Los Angeles, CA), and YRI (Yoruba in Ibadan, Nigeria)/ASW (African ancestry in Southwest USA) populations from HapMap. Next, we calculated Wright's F_{ST}, a metric assessing population genetic differences by pairwise allele comparisons. ET SNPs were selected according to the overlaps of high and low F_{ST} with CEU as the outlier population across several degrees of differentiation. Genes ±100 Kb of each ET SNP were considered ET genes and were compared to SPTB genes from List 1 and List 2.

RESULTS: ET identified 5/17 maternal and 8/16 fetal genes from Zhang (Table), several of which are expressed in the uterus (maternal) or placenta (fetal). Of 640 dbPTB genes, 79 were identified by CEU_GIH_YRI ET, and 57 were identified by the CEU_ASW_MEX ET gene list. In total, ET identified 123 unique genes of the 640 dbPTB genes (19.2%).

CONCLUSION: Applying ET analysis to SPTB provided independent support for multiple genes previously associated in GWAS and candidate gene studies, and presents an alternative filtering metric for genetic analyses based on evolutionary history. Genes identified in prior SPTB association studies confirmed by ET should be prioritized for further genetic prematurity research.

Table. Genes from List 1 identified by ET. *gene expressed in uterus #gene expressed in placenta

Gene	Chr.	Orig. Assn.	Orig. p-value	ET - CEU, GIH, YRI	ET - CEU, ASW, MEX
SHROOM3	4	Maternal	$5.6e^{-4}$	Yes	
LOC100128865	4	Maternal	$2.7e^{-5}$	Yes	Yes
MYPN*	10	Maternal	$3.3e^{-5}$	Yes	
ETNK1*	12	Maternal	$3.7e^{-5}$	Yes	
CNTN5*	11	Maternal	$4.1e^{-5}$	Yes	
LOC100128365	6	Fetal	$2.7e^{-12}$	Yes	
RNASET2#	6	Fetal	$1.4e^{-10}$		Yes
L3MBTL3	6	Fetal	$8.3e^{-7}$	Yes	
SMAD9#	13	Fetal	$1.1e^{-6}$		Yes
RREB1#	6	Fetal	$2.3e^{-6}$	Yes	
SORL1#	11	Fetal	$2.8e^{-6}$	Yes	
KCNH7#	2	Fetal	$6.2e^{-6}$		Yes
NOL10#	2	Fetal	$6.4e^{-6}$	Yes	

12 Alterations in the placental microbiome among spontaneous preterm births

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OBJECTIVE: We have previously demonstrated that the placenta harbors a low biomass microbiome which varies in association with preterm birth (PTB). However, with regard to examination of the placental microbiome, there are inherent limitations to longitudinal placenta collection in any given pregnancy, making it problematic to delineate causation from association. Here, we aimed to examine associations with the microbiome-encoded metabolic pathways and preterm birth using a large cohort with both longitudinal and cross-sectional sampling. We reasoned that robust causal inference analysis across multiple body sites in a prospective longitudinal cohort inclusive of both spontaneous and indicated PTB could potentially overcome such obstacles.

STUDY DESIGN: Subjects were enrolled (n=331) in the early third trimester or at delivery (196 term, 135 preterm). Extensive clinical metadata (such as comorbidities and indications for inductions) enabled covariate analytics and gestational age (GA) comparison. Oral, vaginal, stool, and placental swabs and tissue were uniformly collected from subjects and their infants. DNA was extracted and subjected to 16S and whole genome shotgun (WGS) metagenomics. Quality filtered sequences were analyzed (QIIME and MG-RAST) and causal inference approaches (hierarchical clustering by Manhattan distance and regression modeling).

RESULTS: Upon examination of term and preterm subjects, we saw minimal differences by virtue of GA and type of labor (spontaneous versus indicated) within the posterior fornix (p=0.053), the maternal oral cavity (p=0.534), and stool (p=0.585). However, with close examination of the preterm placental microbiome, we found differences in taxa abundance manifest as increases in *Ureaplasma* and Mycoplasmatales in subjects with sPTB. We found significant increases in Streptophyta in subjects with iPTB ($p<0.05$). Inferred