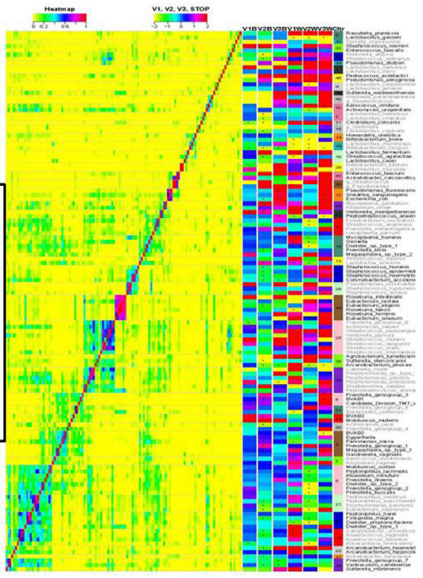

Figure 1: Heatmap of the correlation matrix of 127 phylotypes in the M&M 16S rRNA data, together with color bars for the effect of SPTB on the relative abundance of these phylotypes at V1, V2, V3 as well as in STOP. Significant associations are marked with *. First 3 columns are in AA women in M&M; columns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