

COPD (11, 12). *RARB* is expressed in human lung tissue, bronchial epithelial cells, and airway smooth muscle cells (10), and knockout of this gene could lead to premature alveolar septation in mice (13), suggesting its fundamental function in lung development, normal physiology, and capacity. In this study, we observed the positive association between PAH exposure and hypermethylation in *RARB* promoter; a putative effect may exist on decreasing expression of *RARB* caused by PAHs. There was also a suggestive association of rs1529672-A allele with reduced *RARB* expression. Considering the important biological roles of *RARB* in lung development, we speculated that the decreased expression of *RARB* may act as the common target for PAH exposure and rs1529672, suggesting a possible mechanism underlying the statistical gene–environment interaction.

Strengths of this study were the prospective design and results validation by two independent cohorts of populations with a wide range of PAH exposure. The limitations of this study included not investigating the SNPs reported after March 2017, along with variants in genes involved in PAH metabolism and related biological pathways. Future longitudinal studies in other ethnic populations and the well-designed functional studies are warranted to validate these associations and elucidate putative mechanisms for the PAH–*RARB* interaction in lung function impairment.

In conclusion, the GWAS reported *RARB* rs1529672 may modify the effect of PAH exposure on annual FEV₁/FVC decline. The results emphasize the urgency of reducing environmental PAH levels and the importance of lung function monitoring in the routine physical examination, especially for high-risk populations. ■

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Human Fetal Lungs Harbor a Microbiome Signature

To the Editor:

The concept of the “sterile” fetus has been challenged recently (1–4). We previously reported the existence of an airway microbiome at birth in neonates (5) and speculated that this airway

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microbiome could have fetal origins. The exact source of the infant microbiome is unknown. Other investigators and we have shown that the neonatal microbiome after birth, especially that of the airway, is similar regardless of whether the neonate was delivered vaginally or by cesarean section (5), suggesting that the neonatal microbiome signature could possibly be transplacentally derived and acquired *in utero*. No studies to date have examined the presence of the fetal lung microbiome in humans from an *in utero* environment. Therefore, in this study we examined, for the first time, the presence of human fetal and placental microbiomes early in gestation, using state-of-the-art molecular approaches.

Methods

Ethics statement and tissue collection. After informed consent was obtained, deidentified human fetal samples were collected in the United States with institutional review board approval (USC-HS-13-0399 and CHLA-14-2211). The only information collected was regarding gestational age and genetic or structural abnormalities. All human tissues were collected after dilation and curettage or dilation and evacuation via well-established sterile surgical

procedures. Lungs and (when available) specimen-matched placentas were collected and snap-frozen for subsequent processing.

Sample collection and workflow. A total of 31 deidentified human fetal tissue samples (18 lungs, 3 intestines, and 10 placentas) from 11 weeks gestation (first trimester) to 20 weeks gestation (second trimester) were collected in the United States via sterile and standardized clinical procedures. The initial microbiome analysis was conducted using the whole genome sequencing (WGS) metagenomic shotgun method. Because these were low-biomass samples, bacteria were not detectable in the samples by WGS (undetectable at an average depth of 13 million reads per sample). Subsequently, we conducted a targeted 16S analysis of the same samples at two independent labs (Lee Kong Chian School of Medicine, Singapore, and the University of Alabama at Birmingham [UAB]). The two labs used different DNA extraction kits and different microbiome analysis pipelines. All 31 samples were analyzed at the UAB, and 26 out of 31 samples (17 lungs, 2 intestines, and 7 placentas) were analyzed in Singapore, owing to a smaller quantity of samples.

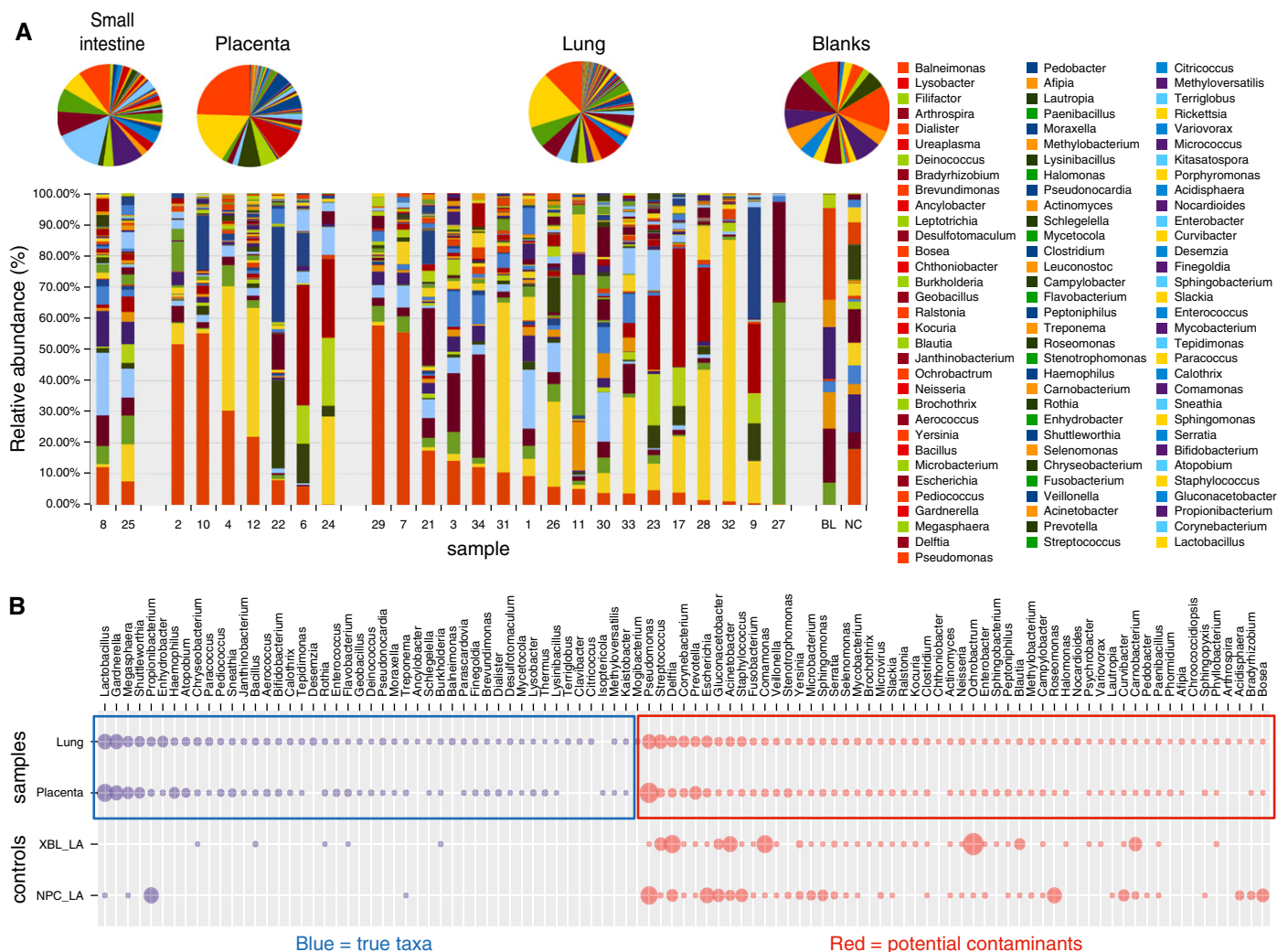


Figure 1. (A) Microbiome analysis of fetal lung, placenta, and small intestine conducted at Singapore. (B) True taxa versus potential contaminants.

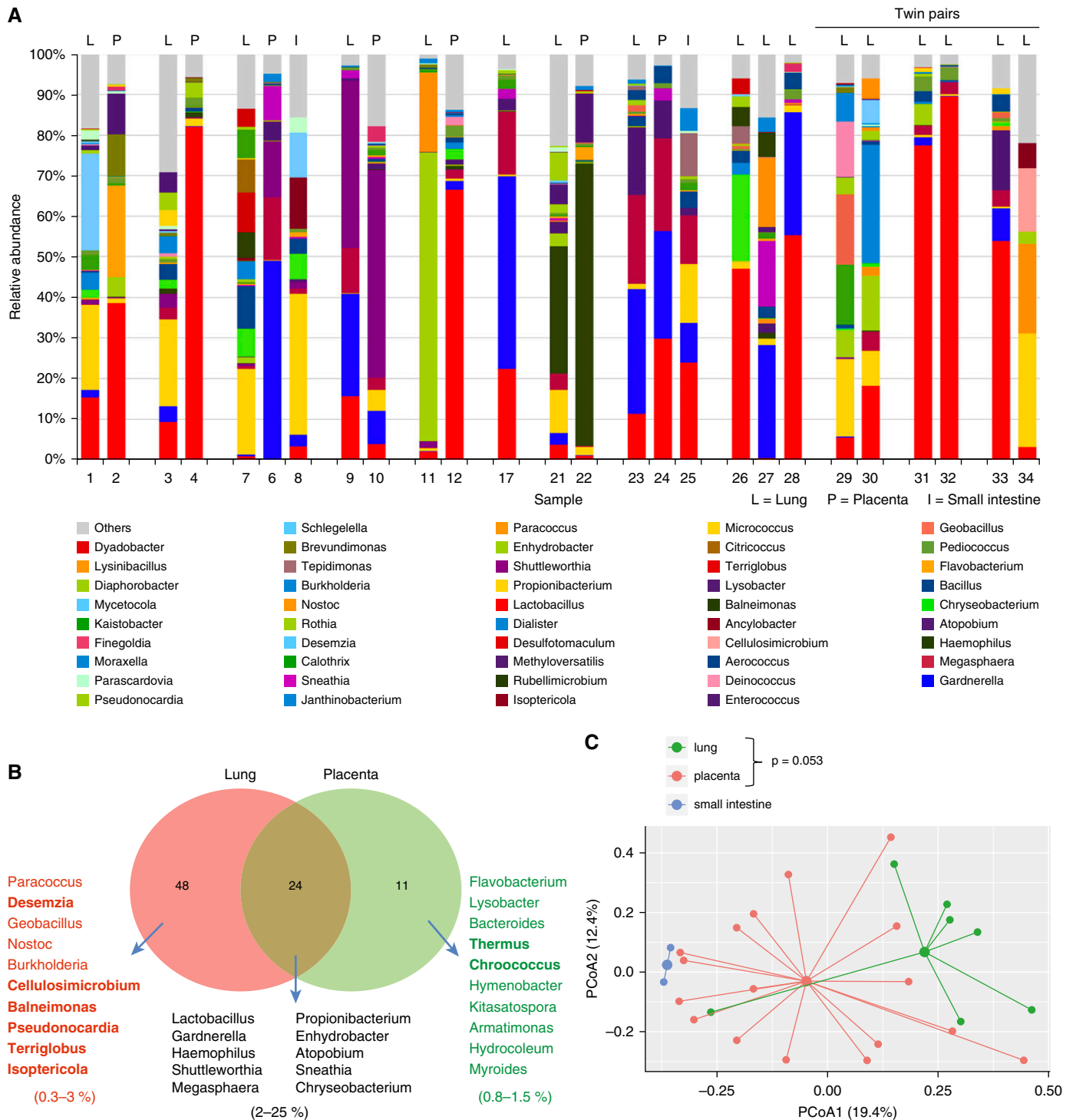


Figure 2. (A) Matched microbiome profiles of lung (L), placenta (P), and intestines (I) from the same patients. Lung microbiome profile of triplets (LLL) and twins (LL). (B) Shared microbiota in fetal lungs and placentas. (C) Principal coordinate plot showing the β diversity of the fetal and placental microbiomes. PCoA = principal coordinates analysis.

DNA extraction and sequencing. Blank DNA extractions from sterile phosphate-buffered saline were performed and served as negative extraction controls. Whole-genome metagenomic shotgun sequencing was performed on a HiSeq 2500 (Illumina) according

to previous workflows (6). In parallel, using the same DNA samples, libraries for targeted amplicon sequencing were prepared (7) and sequencing was performed on a MiSeq platform (Illumina).

Bioinformatic analysis. Metagenomic shotgun sequencing reads were aligned to the human reference genome (8). Nonhuman reads were classified using Kraken with default parameters matched (9). Controls from negative PCR and blank DNA extractions were sequenced and assessed using the decontam package to detect potential contaminants (10).

Details regarding the methods used for analyses conducted at the UAB are available in our previous publication (5). Raw sequence data have been deposited in the National Center for Biotechnology Information's Sequence Read Archive (BioProject Accession No. PRJNA550234).

Results

In this targeted analysis, we were able to detect bacterial DNA in all fetal samples at both labs. In the first 16S analysis conducted at Singapore, the lung samples were found to contain 48 unique taxa, whereas in the placenta samples only 11 unique taxa were identified, and 24 taxa were shared (Figure 1A). All data were adjusted for blank sequences, and decontam modules were used to rule out any possible contamination (Figure 1B). In a pairwise lung and placenta analysis, some overlap between lung and placenta microbiome profiles was seen and β -diversity plots suggested some distinct lung and placental microbiome profiles. Although some separation of the microbiome profiles was evident from a principal coordinates analysis (PCoA), the differences in β diversity between the placenta and lung samples did not reach statistical significance (permutational multivariate ANOVA [PERMANOVA], $P=0.053$; Figure 2).

The 16S analysis conducted at the UAB on the same samples (total $n=31$; 18 lungs, 3 intestines, and 10 placentas) also identified microbiome DNA signature in all samples with the PCoA plot analysis of the human fetal lung and placental microbiomes showing overlap, and no statistical differences ($P>0.1$, PERMANOVA). A PCoA plot analysis of the human fetal lung microbiomes split into two separate groups (11–15 wk gestation [$n=3$] and 16–20 wk gestation [$n=11$]) demonstrated increasing β diversity ($P<0.01$, PERMANOVA). Analysis of the distance and clustering (with closer clustering signifying a shared larger proportion of the phylogenetic tree) indicated a significant difference in microbiome diversity between the two gestational age groups. Overall, at both sites, analysis of the bacterial taxa distribution and diversity showed some overlap in the microbiome signatures of fetal lungs and matched placentas.

Discussion

Recent studies have confirmed the presence of a diverse microbiome in humans, including neonates, but the fetal presence of the lung microbiome remains questionable. Herein, we present the first microbiome study conducted on human fetal tissues, in which we demonstrate the presence of microbial DNA in human fetal lungs and placentas as early as 11 weeks gestation.

In summary, our major novel finding is the confirmation of the presence of a human fetal microbiome DNA signature, as early as the first trimester. Although it was not detected by WGS metagenomic analysis owing to low biomass, we were able to detect a microbiome DNA signature on a targeted 16S analysis in two independent analyses. In addition, we identified temporal changes in fetal lung microbiome diversity during development, suggesting maturational changes with advancing gestational age. Although the reason for these maturational changes is unknown, it is possible that

they could be related to maternal or intrauterine factors. Our analysis also confirms the existence of a placental microbiome that shows some overlap with the corresponding human fetal lung microbiome, based on the overall microbiome analysis, as well as α and β diversities. We speculate that materno–fetal transfer of microbial DNA (and perhaps of other microbial products and whole live or dead bacteria) is a realistic possibility and may serve to “prime” the developing innate immune system of the fetus and help to establish a normal host–commensal relationship. ■

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Hospital-Level Availability of Prone Positioning in Massachusetts ICUs

To the Editor:

Prone positioning (PP), a cost-effective therapy (1) with a mortality benefit in moderate-severe acute respiratory distress syndrome (ARDS) (2), is strongly recommended in guidelines for severe ARDS (3) but has been poorly adopted (4–6). Although clinician-level barriers to implementation of PP have been explored (4), institutional barriers may supersede physicians' beliefs regarding the effectiveness of PP. We sought to evaluate the institutional availability of PP.

Methods

We conducted a survey of all ICUs at acute-care hospitals in Massachusetts, April–July 2019. We e-mailed surveys to ICU nurse and physician leadership; if the surveys remained incomplete after four follow-ups, we completed the survey by phone. For hospitals that provided more than one response, one survey was randomly selected from hospitals with duplicate responses ($n = 6$) or the survey with the more complete responses ($n = 3$) was selected. The survey asked, “Does your ICU have the ability to prone patients?” (“yes,” “no,” or “case-by-case”). Hospitals that responded “yes” were termed “prone-ready.” Follow-up questions inquired about institutional protocols/guidelines describing indications/instructions for PP, nurse training in PP, and reasons for not using PP. Respondents were also presented with a list of adjunctive treatments for ARDS and asked to select treatments used at their institution. We collected descriptive data for each hospital (number of ICU and total hospital beds, profit status, teaching status, case mix index (7), and Centers for Medicare and Medicaid Services star rating [8]) using publicly available information (7–9).

Using the chi-square test and ANOVA, we compared hospital characteristics on the basis of the hospitals' ability to perform PP. Statistical testing was two-tailed, with $\alpha = 0.05$ using SAS 9.4 (SAS Institute). The Beth Israel Deaconess Medical Center Institutional Review Board deemed the study exempt from review.

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Results

Among 60 acute-care hospitals in Massachusetts with ICUs, 54 responded to the survey (90% response rate; six nonresponders were nonacademic hospitals, five of which had <250 total beds). Twenty-four respondents (44.4%) were “prone-ready”; 15 (27.8%) could provide PP on a case-by-case basis, and 15 (27.8%) could not provide PP. Prone-ready hospitals accounted for 358 ICU beds out of a total of 600 ICU beds in the state (59.7%); case-by-case hospitals and PP-unavailable hospitals accounted for 71 (11.8%) and 122 ICU beds (20.3%), respectively. Prone-ready hospitals were more likely to be larger teaching hospitals with a more severe case mix index (Table 1). Twenty-seven hospitals (37.0%) had a PP protocol/guideline [20 (83.3%) prone-ready, 6 (40.0%) case-by-case, and 1 (6.7%) PP-unavailable]. Thirty-four hospitals (63.0%) reported that some or all nurses had received training in PP [24 (100%) prone-ready, 9 (60.0%) case-by-case, and 1 (6.7%) PP-unavailable].

Twenty-three respondents (42.6%) indicated they did not use PP in the past year, accounting for 119 (19.8%) of ICU beds in the state. Common reasons for not using PP were physician or nurse discomfort ($n = 14$), lack of nurse training ($n = 13$), and/or lack of proper equipment ($n = 9$; in seven free-text comments, lack of “a rotating bed” was noted).

Forty-two respondents (77.8%) indicated they had transferred patients with ARDS to another facility in the past year; however, hospital transfer was not associated with PP availability (43% of transferring hospitals were prone-ready; $P = 0.49$). Use of adjunctive treatments for ARDS before transfer was common, regardless of PP availability (Figure 1).

Discussion

Although guidelines recommend PP for patients with severe ARDS, prior studies have shown underuse of PP. We found that most hospitals in Massachusetts were either unable, or not completely able, to routinely offer PP. Reasons identified by ICU leadership for hospital-level lack of PP availability included multiple modifiable factors, such as lack of training and misconceptions about equipment requirements. Our results suggest that institutional-level barriers to implementation of PP are a promising initial target to improve implementation.

Our findings also provide context to prior studies of PP adoption that focused on rates in ICUs participating in trial networks (3, 5) and/or within teaching centers (6). For example, Duan and colleagues evaluated only ICUs that offered PP, and reported that 10% of the appropriate patients received PP (6). Our finding of low real-world availability of PP suggests that the prevalence of PP use among eligible patients is likely even lower than previous estimates.

We found that other adjunctive treatments for ARDS were often used at centers where PP was unavailable. Notably, some adjunctive interventions are based on weaker evidence, are more expensive, or require levels of monitoring similar to those required for PP, suggesting that resource limitations or staffing constraints alone do not explain the lack of PP adoption at the institutional level. Some respondents reported a common misconception that “lack of equipment” was a barrier to instituting PP. However, PP does not require specialized equipment beyond basic cushioning to support the face, chest, and pelvis (10, 11).

Our study has limitations. First, we surveyed hospitals in one state, and Massachusetts is notable for having many geographically