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Association between Intrauterine Microbiome and Risk of Intrauterine Growth Restriction: A Case-Control Study Based on Guangxi Zhuang Birth Cohort in China

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Substantial evidence show that intrauterine growth restriction (IUGR) is linked to both short-term and long-term health consequences. Recent studies have shown that the intrauterine environment harbors a diverse community of microbes. However, the relationship between intrauterine microbiome and IUGR has been rarely studied. In our investigation of 35 neonates with IUGR and 187 neonates without IUGR, we found that the intrauterine microbiome was largely composed of nonpathogenic commensal microbiota from the *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* phyla. Carriage of genera *Afipia* [odds ratio (OR) 0.24; 95% confidence interval (CI) 0.10-0.60], *Hydrogenophaga* (OR 0.10; 95% CI 0.01-0.76), and *Perlucidibaca* (OR 0.25; 95% CI 0.10-0.61) were significantly associated with decreased risk of IUGR, while one log10-unit increasing of relative abundance the genera *Catenibacterium* (OR 2.56; 95% CI 1.09-6.01) and *Senegalimassilia* (OR 1.78; 95% CI 1.00-3.16), and carriage of *Holdemanella* (OR 4.07; 95% CI 1.54-10.76), *Parvimonas* (OR 3.33; 95% CI 1.16-9.57), *Sandaracinus* (OR 3.27; 95% CI 1.21-8.84), and *Streptococcus* (OR 3.52; 95% CI 1.13-10.95) were associated with increased risk of IUGR. The present study firstly demonstrated that carriage of *Afipia*, *Hydrogenophaga*, and *Perlucidibaca* in the intrauterine environment is associated with a decreased risk of IUGR, while carriage of *Holdemanella*, *Parvimonas*, *Sandaracinus*, and *Streptococcus*, and increased relative abundance of *Catenibacterium* and *Senegalimassilia* are associated with an increased risk of IUGR. The study provides evidence that the intrauterine microbiome may play a role in the etiology of IUGR.

Keywords: 16S; intrauterine growth restriction; microbiome; neonate; pregnacy
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Introduction

Intrauterine growth restriction (IUGR) is broadly defined as an estimated fetal weight that is below 10th percentile for gestational age as determined by an ultrasound. It is often used interchangeably with the term small for gestational age (SGA), which is diagnosed as a baby who has a birthweight smaller than the population norms on the growth charts (always below the 10th percentile for babies of the same gestational age) (Sharma et al. 2016). It is esti-

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mated that the incidence of IUGR is approximately 5% to 15% (Brodsky and Christou 2004). A growing number of evidences have shown that IUGR is linked to both short-term and long-term health consequences of the fetuses (ACOG, The American College of Obstericians and Gynecologists 2021), including neonatal morbidity and mortality, neurodevelopmental in childhood (Miller et al. 2016) and risk of chronic diseases in adulthood, such as hypertension, cardiovascular disease, diabetes mellitus, metabolic syndrome, and dyslipidemia (Barker 1995, 1998; Varvarigou 2010). These issues point out the importance of attempting to find out the underlying cause, which will help the development of those specific children.

IUGR has been attributed to multiple factors, among which the maternal factor is one of the most concerned. Prior studies have shown that a substantial amount of maternal factors are associated with IUGR, such as maternal disorders, maternal nutrition, infectious diseases, and substance use and abuse (ACOG 2021). A large number of studies have shown that risk factors mainly affect the development of the fetus by regulating the intrauterine environment, which is composed of the uterus, placenta, fetal membranes, and umbilical cord, and provides a key interface between the mother and the developing fetus during pregnancy (Chen and Gur 2019). Evidence shows that the underlying mechanisms may involve in the regulation of a milieu of hormones, metabolites, cytokines, and nutrients in intrauterine environment (Chen and Gur 2019). For example, ambient air pollutants may trigger systemic, pulmonary and placental inflammation, oxidative stress, endothelial and cardiovascular changes, decreased trans-placental nutrient and gas exchange and thereby restrict the intrauterine growth of the placenta and the fetus (Westergaard et al. 2017). For these reasons, it is believed that intrauterine environment has been one of the most important factors of the fetus development, and changes in intrauterine environment will bring huge risk to the fetus.

Based on 16S-based metagenomic and whole-genome shotgun sequencing, a large number of microbial DNA have been identified in placenta (Stout et al. 2013), amniotic fluid (DiGiulio 2012; Collado et al. 2016; Urushiyama et al. 2017), and even neonatal first-pass meconium (Jiménez et al. 2008) in recent studies, which indicates the existence of a microbiome in the intrauterine environment. Subsequent evidence shows that the intrauterine microbiome originated from the direct ascension of the vaginal canal microbiome (Zervomanolakis et al. 2007; Suff et al. 2018) or the blood transmission of the microbiome of distal sites such as the oral cavity and the gut (Jiménez et al. 2005; Fardini et al. 2010; Tan et al. 2013; Baker et al. 2018). Considering the important role of microbiome in those originated sites, the relationship between intrauterine microbiome diversity and birth outcomes has become a research hotspot. For example, Doyle and his colleagues showed that term and preterm labor were associated with distinct microbial community structures in placental membranes, which was independent of delivery modes (Doyle et al. 2014). A second group also suggested the placental microbiome was associated with low birth weight in full-term neonates (Antony et al. 2015). Only one pilot study with a small sample has focused on the relationship between IUGR and intrauterine microbiome diversity (Hu et al. 2021). It suggested that IUGR was associated with unique features of the placental microbiome. However, the results still need to be verified.

In the present study, we aimed to investigate whether the intrauterine microbiome was associated with IUGR by using a case-control study based on Guangxi Zhuang Birth Cohort (GZBC) in China. A previous study showed that the microbial communities in the amniotic fluid were very consistent with that in neonatal oropharyngeal aspirate samples (Wang et al. 2018). We used the oropharyngeal aspirates for intrauterine microbiota detection, as they may be rarely contaminated.

Materials and Methods

Study population

A case-control study was conducted based on a subset of the Guangxi Zhuang Birth Cohort (GZBC). GZBC was a prospective and Zhuang population-based birth cohort conducted in seven major counties of the Guangxi province in China, which has been introduced in our previous study in detail (Liang et al. 2020). To reduce confounding, cases and controls were extracted from just one hospital, Debao Maternal and Child Care Service Center in Debao county. Cases were those neonates with IUGR which was defined as a birth weight that was less than the 10th percentile for gestational age according to local standard (Crispi et al. 2010), while controls were those neonates without IUGR. Those neonates were excluded if their mothers were used to take any medicines disturbing intrauterine microbiome diversity during pregnancy (such as antibiotics and systemic steroids), suffered from a chronic disease (including gastritis, gastric ulcer, hepatitis, diabetes mellitus and hypertension), had labored a baby with a major birth defect, or were multiple gestations. At last, a total of 222 mother-infant pairs (35 IUGRs and 187 non-IUGRs) with completed baseline questionnaire and medical records were included for the final analyses. This study had been approved by the ethical committee of Guangxi Medical University (No.20140305-001). All participants had signed an informed consent form.

Baseline survey and other covariates data collection

The in-person interviews were performed at the hospitals by professionally trained interviewers through a standardized and structured questionnaire for the baseline survey. Information on maternal demographic characteristics (e.g., age, ethnicity, and self-reported pre-pregnancy weight) and lifestyles (e.g., drinking, smoking or passive smoking during pregnancy) was collected. Other maternal information (e.g., height, parity, and pregnancy complications) and birth characteristics (e.g., sex, gestational age,

and birth weight) were obtained from Maternal and Child Information System. Maternal pre-pregnancy body mass index (BMI) (kg/m^2) was defined using their self-reported pre-pregnancy weight and height measured at the first antenatal visit.

Sample collection

Standard methods were used to collect the oropharyngeal aspirates by trained professionals under strict aseptic conditions and a uniform protocol. To avoid bacteria contaminations, 2-5 mL of oropharyngeal aspirate was collected using sterile pipettes within 2 min of delivery. Then each sample was stored separately in 2-5 sterile frozen tubes and temporarily placed at −20°C. These samples were then transferred to a −80°C freezer for long-term storage.

DNA extraction and PCR amplification

Microbial community genomic DNA was extracted from oropharyngeal aspirate samples using the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to manufacturer's instructions. The hypervariable region V3-V4 of the bacterial 16S rRNA gene were amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, Waltham, MA, USA). The PCR amplification of 16S rRNA gene was performed as follows: initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 45 s, and single extension at 72°C for 10 min, and end at 4°C. The PCR mixtures contain 4 μL of 5 × *TransStart* FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.4 μL of *TransStart* FastPfu DNA Polymerase, template DNA 10 ng, and finally ddH_2O up to 20 μL. PCR reactions were performed in triplicate.

Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Majorbio, Shanghai, China).

Processing of sequencing data

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: (i) the 300 bp reads were truncated at any site receiving an average quality score of < 20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp and the reads containing ambiguous characters were discarded; (ii) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) errors in barcode matching and in primer matching were zero and 2 nucleotides, then samples were distinguished according to the barcode and primers.

Operational taxonomic units (OTUs) with a 97% similarity cutoff (Edgar 2013) were clustered using UPARSE (version 7.1, http://drive5.com/uparse/), and chimeric sequences were identified and removed. The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the 16S rRNA database (Silva SSU138) using confidence threshold of 0.7.

Statistical analysis

Comparisons of socio-demographic characteristics of participants for this study were based on *t*-test and chi-square, performed in IBM SPSS (Statistical Package for the Social Sciences, version 21) at a 5% significance level. Community richness and alpha-diversity were measured using Sobs, Chao and Shannon indexes. Difference in alpha-diversity between IUGRs and non-IUGRs was visualized using violin plots. Beta-diversity was evaluated using Bray-Curtis dissimilarity matrices (Bray and Curtis 1957) and visualized using principal coordinate analysis (PCoA) plots to illustrate differences of microbiome composition profiles at OTU level between IUGRs and non-IUGRs. For community state type (CST) analysis, a Jensen-Shannon distance matrix was calculated using the 'vegdist' function in the vegan package (Oksanen et al. 2020) with a custom distance function that calculates the square root of the Jensen-Shannon divergence (Endres and Schindelin 2003). This distance matrix was used for hierarchical clustering using the 'hclust' function in R, with Ward linkage.

We compared bacterial genus between cases and controls. We limited our analysis of bacterial genus to those with bacterial OTU greater than 11 samples (4.95%). We characterized individuals as carriers and non-carriers of the genus, with non-carriers having zero sequence reads for the specific genus. We also analyzed the relative abundance as continuous variables in the models. The relative abundance with non-carriers for the specific genus was replaced by half of the minus relative abundance in samples. Due to the relative abundance with skewed distribution, we made a log10 transform on all relative abundance. To explore the association of the relative abundance at genus level with IUGR risk, we conducted L1 penalized least absolute shrinkage and selection operator (LASSO) logistic regression (Tibshirani 1996) in R 'glmnet' package. Covariates (maternal age, ethnicity, pre-pregnancy BMI, drinking, smoking or passive smoking, parity, delivery mode, and neonate sex) were controlled in the LASSO genera selection process. To evaluate the risk associations for the selected genera, we fitted traditional logistic regression models using each selected genus (categorical and continuous) as a predictor of IUGR risk individually. Furthermore, we also conducted stratified analyses according to delivery mode. Analyses were carried out using SPSS 25 and R

V.4.0.3.

Results

Characteristics of mothers and infants between cases and controls

The characteristics of mothers and infants between cases and controls are summarized in Table 1. The cases and controls in the present study did not significantly differ in maternal age, ethnicity, pre-pregnancy BMI, parity, drinking status, smoking status, and delivery mode. However, there were statistically significant differences in maternal occupation, infants, sex, gestational age, birth length, and birth weight.

Characteristics of sequencing results

A total of 12,688,682 high-quality reads were produced from 222 samples in this study, with an average of 57,156 reads per sample (Table 2). The Good's coverage of each group was over 99%, which indicated a near complete sampling of the community. The operational taxonomic unit (OTU), observed richness (Sobs), estimators of community richness (Chao), and diversity (Shannon) are also shown in Table 2 and Fig. 1. Means of OTUs, Sobs, Chao indexes, and Shannon indexes in IUGRs were lower than non-IUGRs, demonstrating the lower richness and diversity in intrauterine microbiome of IUGRs, although there was no statistical significance.

Principal coordinates analysis (PCoA) between IUGR and non-IUGR groups

The overall difference in the intrauterine microbiome community structure between IUGRs and non-IUGRs was

Table 1. Characteristics of mothers and infants between intrauterine growth restriction (IUGR) cases and non-IUGR controls.

Characteristics	IUGR (n = 35)	Non-IUGR (n = 187)	P value
Mothers			
Age (Mean ± SD, year)	26.57 ± 5.51	26.56 ± 4.87	0.130
< 30	23 (65.71)	110 (58.82)	
≥ 30	12 (34.29)	77 (41.18)	
Ethnicity (n (%))			0.525
Zhuang	30 (85.71)	170 (90.91)	
others	5 (14.29)	17 (9.09)	
Pregnancy-related factors			
Pre-pregnancy BMI (n (%))			0.458
Underweight (< 18.5 kg/m²)	10 (28.57)	36 (19.25)	
Normal weight (18.5-24 kg/m²)	20 (57.14)	120 (64.17)	
Overweight (≥ 24 kg/m²)	5 (14.29)	31 (16.58)	
Parity (n (%))			0.197
Primigravida	21 (60)	90 (48.13)	
Multigravida	14 (40)	97 (51.87)	
Drinking (n (%))			0.585
No	32 (91.43)	163 (87.17)	
Yes	3 (8.57)	24 (12.83)	
Smoking or passive smoking (n (%))			0.656
No	13 (37.14)	77 (41.18)	
Yes	22 (62.86)	110 (58.82)	
Delivery mode (n (%))			0.271
Vaginal delivery	26 (74.29)	121 (64.71)	
C-section	9 (25.71)	66 (35.29)	
Infants			
Sex (n (%))			0.049
Male	23 (65.71)	89 (47.59)	
Female	12 (34.29)	98 (52.41)	
Gestational age (Mean ± SD, week)	38.91 ± 1.17	38.28 ± 1.45	0.044
Birth length (Mean ± SD, cm)	48.34 ± 1.08	49.99 ± 1.46	< 0.001
Birth weight (Mean ± SD, g)	2.63 ± 0.28	3.18 ± 0.45	< 0.001

Data are shown as mean ± SD or n (%). IUGR, Intrauterine growth restriction; Non-IUGR, Non-intrauterine growth restriction; BMI, body mass index.

Table 2. Sequencing data summary among IUGRs and non-IUGRs.

	IUGRs (n = 35)	Non-IUGRs (n = 187)	P value
Sequences	59,539.23 ± 8,099.88	56,710 ± 11,914.09	0.086
OTUs/Sobs	399.26 ± 252.04	471.65 ± 312.20	0.197
Chao	514.21 ± 259.46	582.18 ± 347.64	0.273
Shannon	2.28 ± 1.14	2.58 ± 1.25	0.178

Data are shown as mean ± SD. IUGR, Intrauterine growth restriction; non-IUGR, non-intrauterine growth restriction; OTUs, operational taxonomic units; Sobs, observed richness; Chao, estimators of community richness; Shannon, diversity.

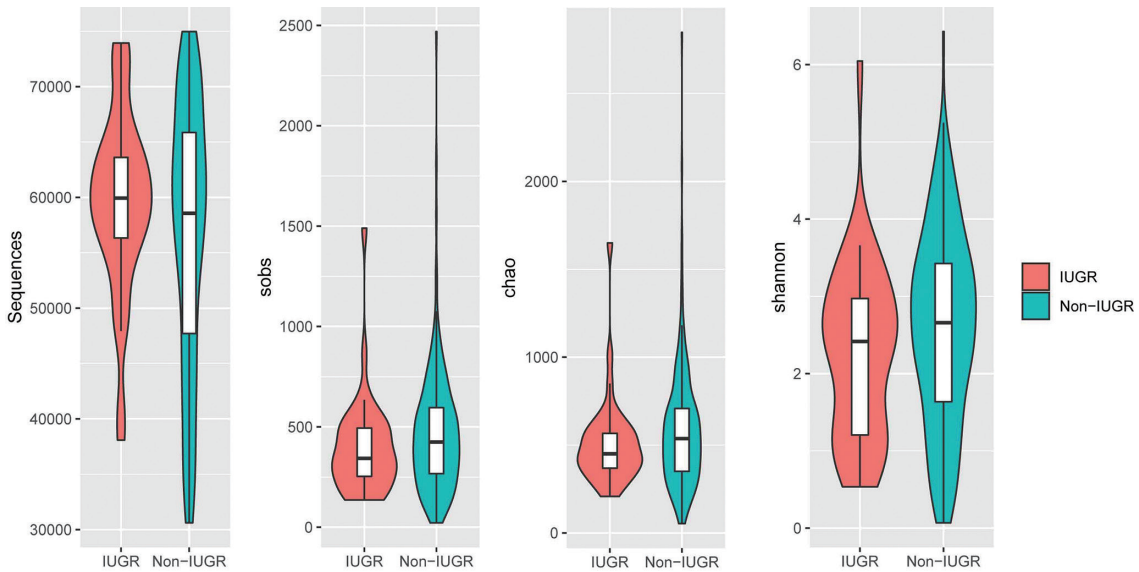

Fig. 1. Violin plots of sequences and alpha diversity estimators of the microbiome among intrauterine growth restriction (IUGRS) and non-IUGRS.
The violin with box plot shows the median and interquartile range (IQR) of the indices, and the width of the violin represents the density distribution of the indices. Medians of OTUs, Sobs, Chao indexes, and Shannon indexes in IUGRs were lower than non-IUGRs, although there was no statistical significance.

evaluated using Principal Coordinates Analysis (PCoA) of Bray-Curtis dissimilarity (Fig. 2). Principal coordinates 1 and 2 (PCo1 and PCo2) explained 16.07% and 11.07% of the variation in Bray-Curtis dissimilarity, respectively. There was a statistically significant clustering between IUGR group and non-IUGR group (P = 0.036). However, the clustering of the IUGR group was not separated completely from the non-IUGR group.

Intrauterine microbiome structures in non-IUGR and IUGR groups

Fig. 3A shows relative abundance of intrauterine microbiota in each sample at the genus levels. The overall microbiota structure for each group at the phylum and genus level are shown in Fig. 3B. At the phylum level, 58 bacterial phyla were identified in all samples. *Proteobacteria*, *Firmicutes*, and *Actinobacteria* were the top three dominant phyla among both IUGRs and non-IUGRs. The relative abundance of *Firmicutes*, *Fusobacteria* and *Tenericutes* among IUGRs was decreased, while *Proteobacteria* and *Actinobacteria* were increased, when compared with non-IUGRs.

At the genus level, a total of 1,609 bacterial genera were identified in all samples. The dominant genus was *Lactobacillus* (16.15% and 19.34%), followed by *Ralstonia* (16.64% and 17.31%), *Pseudomonas* (17.55% and 6.41%), *Gardnerella* (9.39% and 4.82%), and unclassified_k__norank (4.75% and 6.21%), in IUGR group and non-IUGR group. The relative abundance of *Lactobacillus*, *Ralstonia*, unclassified_k__norank, *Rhodococcus*, *Burkholderia-Paraburk*, *Acinetobacter*, *Corynebacterium_1*, *Sneathia*, *Ureaplasma*, *Afipia* and others in IUGR group was decreased, while *Pseudomonas*, *Gardnerella*, *Atopobium*, *Prevotella*, and *Stenotrophomonas* were increased, when compared with non-IUGR group.

Community state types analysis in non-IUGR and IUGR groups

A community state type is a cluster of community states that have similar composition and relative abundance

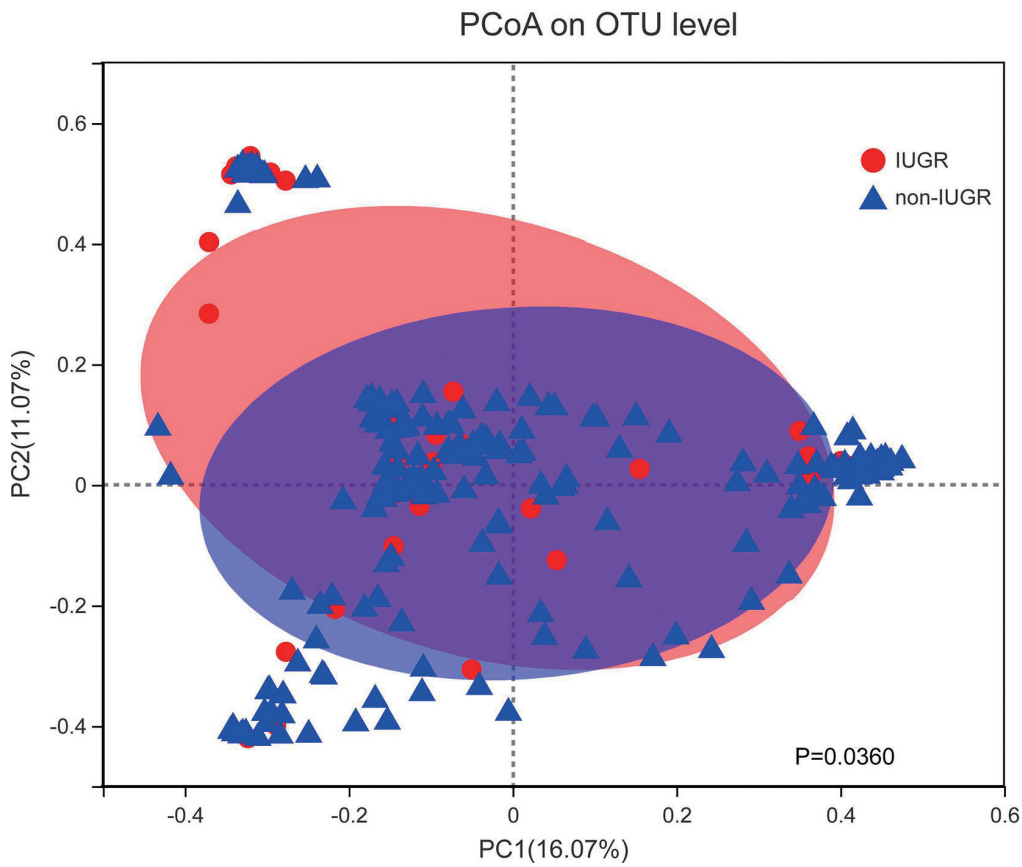

Fig. 2. Principal coordinates analysis (PCoA) between IUGR and non-IUGR groups.
Principal coordinates analysis (PCoA) was performed on distance matrices as indicated with significance of clustering determined by PERMANOVA or Adonis with 999 permutations. There was a statistically significant clustering between IUGR group and non-IUGR group (P = 0.036).

in terms of the phylotypes observed. In this study, hierarchical clustering of intrauterine microbiota profiles resulted in four community state types (CST): I (*Ralstonia* dominated), II (*Gardnerella* dominated), III (*Lactobacillus* dominated), and IV (*Pseudomonas* dominated) (Fig. 4). The dominant phylum was Ralstonia (36.43%) followed by unclassified phylum (8.71%) and Rhodococcus (5.78%) in CST I; Gardnerella (19.45%) followed by Sneathia (11.29%) and Acinetobacter (7.66%) in CST II; Lactobacillus (61.17%) followed by Ralstonia (7.42%) and Rhodococcus (3.12%) in CST III; and Pseudomonas (72%) followed by Stenotrophomonas (7.38%) and Lactobacillus (6.22%) in CST IV, respectively. Microbial profiles for IUGR group and non-IUGR group were assigned to intrauterine community state types (CST) I (31.43%, 43.85%), II (25.71%, 20.86%), III (20.00%, 27.27%), and IV (22.86%, 8.02%) (Table 3).

Associations between intrauterine microbial genus and risk of IUGR

LASSO regularized regression identified 15 intrauterine microbial genera associated with risk of IUGR, including *Afipia*, *Atopobium*, *Catenibacterium*, *Collinsella*, *Flavobacterium*, *Holdemanella*, *Hydrogenophaga*, *Megamonas*, *Orenia*, *Parvimonas*, *Perlucidibaca*, *Pseudomonas*, *Sandaracinus*, *Senegalimassilia*, and *Streptococcus* (Table 4). We next examined the associations of 15 selected intrauterine microbial genera with risk of IUGR by traditional logistic regression models. Without adjusting any factors (crude model), we found that all other selected microbial genera were significantly associated with risk of IUGR, except for *Collinsella*, *Megamonas*, *Orenia*, *Pseudomonas*, and *Streptococcus*.

After adjusting for maternal age, ethnicity, pre-pregnancy BMI, drinking, smoking or passive smoking, parity, delivery mode, and neonate sex, we found per log10-unit increase of relative abundance of *Catenibacterium* (OR 2.56; 95% CI 1.09-6.01), *Sandaracinus* (OR 5.86; 95% CI 1.26-27.22), and *Senegalimassilia* (OR 1.78; 95% CI 1.00-3.16) significantly increased risk of IUGR, while increased log10 relative abundance of *Perlucidibaca* (OR 0.20; 95% CI 0.06-0.65) significantly decreased risk of IUGR. We also found carriage of *Holdemanella* (OR 4.07; 95% CI 1.54-10.76), *Parvimonas* (OR 3.33; 95% CI 1.16-9.57), *Sandaracinus* (OR 3.27; 95% CI 1.21-8.84), and *Streptococcus* (OR 3.52; 95% CI 1.13-10.95) was significantly associated with an increased risk of IUGR, while carriage of *Afipia*, *Hydrogenophaga* (OR 0.10; 95% CI 0.01-0.76), *Perlucidibaca* (OR 0.25; 95% CI 0.10-0.61), was signifi-

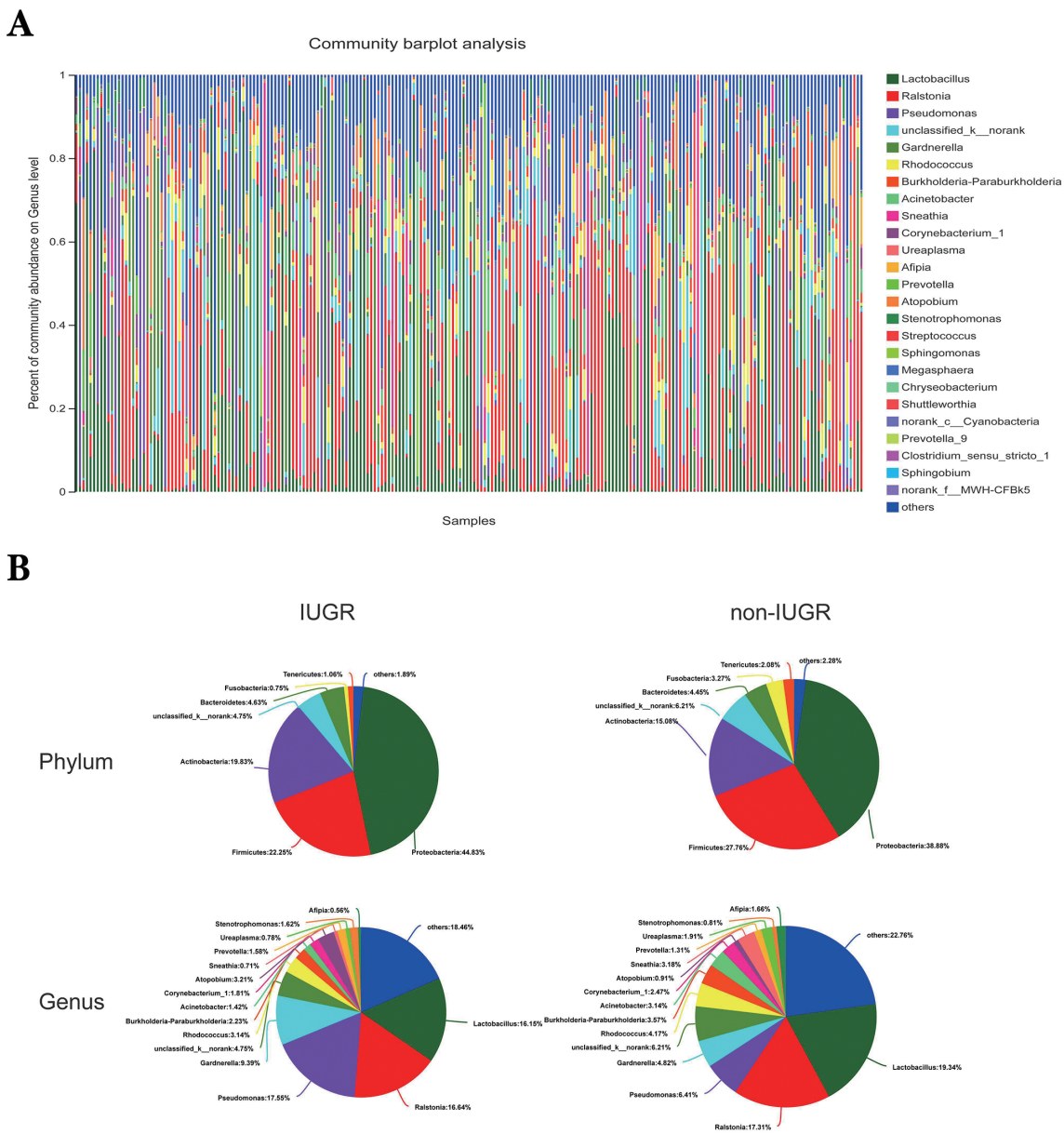

Fig. 3. Relative abundance (%) of intrauterine microbiota in each sample at the genus level (A), and Pie plots of intrauterine microbial abundance at the phylum and genus level in non-IUGR and IUGR groups (B).
A and B showed that the intrauterine microbiome was largely composed of nonpathogenic commensal microbiota from the *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* phyla.

cantly associated with a decreased risk of IUGR. The consistent results were observed for *Afipia*, *Hydrogenophaga*, *Perlucidibaca*, *Holdemanella*, *Parvimonas*, *Sandaracinus*, and *Streptococcus,* whether as a continuous variable or a categorical variable.

Discussion

There is increasing evidence that not only is the intrauterine environment as non-sterile as expected, but also the maternal-fetal transmission of the microbiota even occurs during pregnancy (Vandenplas et al. 2020). A fetus begins swallowing amniotic fluid at 10-14 weeks' gestation (Grassi et al. 2005; Li et al. 2020), which makes the microbiota interconnected from different sites in intrauterine fetal environment, such as amniotic fluid microbiota, oropharyngeal microbiota and intestinal microbiota. Wang et al. (2018) proposed that pharyngeal aspirates are located deeply in the neonatal body and can be collected immediately at birth, which makes pharyngeal aspirates be an ideal material as neonatal initial microbiota. Given the significance of oropharyngeal aspirate microbiota and its closeness of intrauterine fetal environment, we collected oropharyngeal aspirates of neonates immediately at birth to examine the association of the intrauterine microbiome with IUGR. Consistent with previous studies (Antony et al. 2015; Hu et al. 2021), we suggested that the intrauterine microbiome

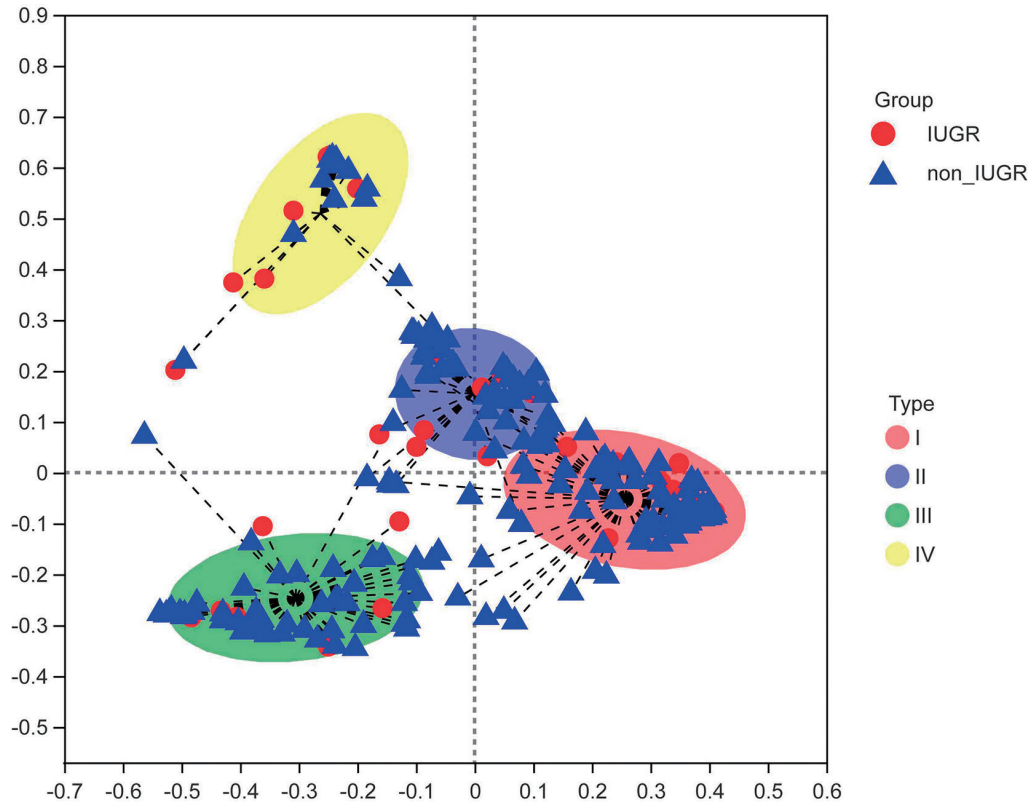

Fig. 4. Community state types of non-IUGR and IUGR groups.
Hierarchical clustering of intrauterine microbiota profiles resulted in four community state types (CST): I (Ralstonia dominated), II (Gardnerella dominated), III (Lactobacillus dominated), and IV (Pseudomonas dominated).

Table 3. Proportions of community state types in IUGR and non-IUGR groups.

Community state types	IUGR		Non-IUGR	
	n	%	n	%
I	11	31.43	82	43.85
II	9	25.71	39	20.86
III	7	20.00	51	27.27
IV	8	22.86	15	8.02

IUGR, Intrauterine growth restriction; non-IUGR, non-intrauterine growth restriction.

was largely composed of nonpathogenic commensal microbiota from the *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* phyla.

Our present study showed that the richness and diversity in intrauterine microbiome of IUGRs were lower than non-IUGRs. Similarly, Zheng et al. (2015) found that the placentas of low birth weight neonates had significantly lower bacterial richness and evenness than that of normal birth weight neonates. These findings indicated that a low bacterial richness of intrauterine environment may be associated with fetal development. A previous study (Le Chatelier et al. 2013) demonstrated that individuals with a low bacterial richness were characterized by more marked overall adiposity, insulin resistance, and dyslipidemia, which indicated that a low bacterial richness may be associated with abnormal glucose metabolism (Le Chatelier et al. 2013).

We found that the intrauterine microbial genera *Afipia*, *Hydrogenophaga*, and *Perlucidibaca* were associated with decreased risk of IUGR, while *Catenibacterium*, *Holdemanella*, *Parvimonas*, *Sandaracinus*, *Senegalimassilia*, and *Streptococcus* were associated with increased risk of IUGR. *Afipia*, *Hydrogenophaga*, and *Perlucidibaca* all belonged to the phylum *Proteobacteria* and Gram-negative (Thonnard et al. 1994; Song et al. 2008; Choi et al. 2020; Singh et al. 2020), while *Catenibacterium*, *Holdemanella*, *Parvimonas* and *Streptococcus* belonging to the phylum *Firmicutes*, and *Senegalimassilia* belonging to the phylum *Actinobacteria*

Table 4. Associations between selected intrauterine microbial genera† and risk of IUGR (n = 222).

Genus	Crude		Adjusted	
	OR (95% CI)	P value	OR‡ (95% CI)	P value
Afipia				
lg (abundance)§	0.62 (0.40-0.96)	0.034	0.64 (0.39-1.04)	0.074
carriers	0.25 (0.10-0.58)	0.001	0.24 (0.10-0.60)	0.002
Atopobium				
lg (abundance)	1.30 (0.96-1.76)	0.091	1.28 (0.92-1.78)	0.135
carriers	2.13 (1.02-4.45)	0.045	2.11 (0.94-4.70)	0.070
Catenibacterium				
lg(abundance)	2.43 (1.09-5.41)	0.030	2.56 (1.09-6.01)	0.031
carriers	2.34 (0.94-5.82)	0.067	2.55 (0.95-6.80)	0.062
Collinsella				
lg (abundance)	1.16 (0.73-1.86)	0.532	1.18 (0.73-1.93)	0.499
carriers	2.02 (0.87-4.69)	0.102	2.08 (0.87-4.98)	0.101
Flavobacterium				
lg (abundance)	1.34 (0.66-2.70)	0.420	1.38 (0.62-3.08)	0.435
carriers	2.18 (1.05-4.53)	0.037	2.13 (0.98-4.63)	0.057
Holdemanella				
lg (abundance)	1.44 (0.74-2.77)	0.282	1.77 (0.84-3.74)	0.133
carriers	3.00 (1.27-7.07)	0.012	4.07 (1.54-10.76)	0.005
Hydrogenophaga				
lg (abundance)	0.05 (0-0.93)	0.045	0.06 (0.00-1.13)	0.060
carriers	0.09 (0.01-0.66)	0.018	0.10 (0.01-0.76)	0.026
Parvimonas				
lg (abundance)	1.66 (0.85-3.24)	0.142	1.65 (0.81-3.35)	0.168
carriers	3.65 (1.32-10.05)	0.012	3.33 (1.16-9.57)	0.025
Perlucidibaca				
lg (abundance)	0.22 (0.07-0.69)	0.010	0.20 (0.06-0.65)	0.008
carriers	0.25 (0.11-0.61)	0.002	0.25 (0.10-0.61)	0.003
Pseudomonas				
lg (abundance)	1.27 (0.93-1.73)	0.132	1.28 (0.93-1.76)	0.134
carriers	-		-	
Sandaracinus				
lg (abundance)	6.01 (1.50-24.10)	0.011	5.86 (1.26-27.22)	0.024
carriers	3.06 (1.25-7.48)	0.014	3.27 (1.21-8.84)	0.020
Senegalimassilia				
lg (abundance)	1.85 (1.09-3.14)	0.023	1.78 (1.00-3.16)	0.049
carriers	2.87 (1.07-7.65)	0.036	2.56 (0.88-7.45)	0.084
Streptococcus				
lg (abundance)	1.02 (0.65-1.61)	0.921	1.15 (0.71-1.84)	0.573
carriers	2.53 (0.85-7.54)	0.096	3.52 (1.13-10.95)	0.029

†Genera were selected from LASSO logistic models with the optimal value of lambda from 100 repeated 10-fold cross-validation. Microbial OTU with the presence greater than 11 samples were eligible for the variable selection. In the table, ORs of genus *Megamonas* and genus *Orenia* were not showed, because IUGRs had no carrier of genus *Megamonas* and genus *Orenia*. Carriers of genus *Megamonas* and genus *Orenia* are 20.32% (38/187) and 18.18% (34/187) in non-IUGRs, respectively.

‡Adjusted for maternal age, ethnicity, pre-pregnancy BMI, drinking, smoking or passive smoking, parity, delivery mode, and neonate sex.

§lg (abundance) means that relative abundance of selected intrauterine microbial genera was log10-transformed [log10 (abundance)].

IUGR, Intrauterine growth restriction; non-IUGR, non-intrauterine growth restriction; OR, odds ratio; CI, confidence interval; BMI, body mass index; LASSO, least absolute shrinkage and selection operator.

were Gram-positive (Mohr et al. 2012; Lagier et al. 2013; Marchand-Austin et al. 2014; Berry et al. 2019; Kageyama and Benno 2000; Wylensek et al. 2020). We speculate that Gram-positive intrauterine bacterium may impede fetal growth compared to Gram-negative intrauterine bacterium. A prior study (Kandasamy et al. 2017) reported that the selected Gram-negative probiotic had higher beneficial effects in inducing protective immunity against pathogens as compared with the selected Gram-positive probiotics in humans and animal models. This may be the reason why the effects of gram-negative bacteria and gram-positive bacteria were inconsistent. Notably, decidual macrophages present unique phenotypes to play a key role in the establishment of the immunological aspects of maternal-fetal interaction (Sun et al. 2021). Dysfunction of decidual macrophages gives rise to pregnancy complications such as preeclampsia, recurrent spontaneous miscarriage, preterm labor and fetal growth restriction (Sun et al. 2021). A delicate immune balance is critical for the maintenance of a successful pregnancy, while disruption of this balance can induce complications such as implantation failure, miscarriage, preterm birth/labor, preeclampsia and fetal growth restriction (Negishi et al. 2018).

To our knowledge, this is the first study to demonstrate the relationship between intrauterine microbiome and risk of IUGR by using oropharyngeal aspirate samples collected immediately at delivery, which expands our understanding of intrauterine microbiome and their biological effects. Furthermore, we included the participators in the same hospital with a large sample size and adjusted a large number of covariates in data analysis, which makes the results more convinced. However, the following limitations should be also paid attention to. First, the present study was only a case-control study and cannot validate the causal relationship between intrauterine microbiome diversity and incidence of IUGR. Second, we did not connect the microbiome from oropharyngeal aspirates to other intrauterine samples, such as umbilical cord, placenta and fetal membranes. We cannot completely eliminate the pollution of the external environment, although prior study showed that the microbial communities in the amniotic fluid were very consistent with that in neonatal oropharyngeal aspirate samples.

In conclusion, the present study demonstrated that carriage of *Afipia*, *Hydrogenophaga*, and *Perlucidibaca* in the intrauterine environment is associated with a decreased risk of IUGR, while carriage of *Holdemanella*, *Parvimonas*, *Sandaracinus*, and *Streptococcus*, and increase of relative abundance of *Catenibacterium* and *Senegalimassilia* are associated with an increased risk of IUGR. The study provides evidence that the intrauterine microbiome may play a role in the etiology of IUGR.

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Conflict of Interest

The authors declare no conflict of interest.

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