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Maternal gut microbiota is associated with newborn anthropometrics in a sex-specific manner

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Original Article

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Abstract

Maternal gut microbiota is thought to be one of the important factors in the developmental origins of health and disease (DOHaD) concept, but the effects of maternal gut microbiota on foetal growth are not well known. In this study, the association between maternal gut microbiota and foetal growth was investigated. Maternal and newborn information, as well as stool samples at the third trimester of pregnancy, were obtained from 51 mother–newborn pairs from the Chiba study of Mother and Child Health (C-MACH). Gut microbiota was analysed by 16S rRNA sequencing of stool samples and short-chain fatty acids (SCFAs) in stool were analysed by gas chromatography-tandem mass spectrometry. After adjustment for covariates, it was found that maternal gut microbial diversity had a positive association with head circumference in newborn males (Chao 1: adjusted $r = 0.515$, $p = 0.029$). Genus *Parabacteroides* and genus *Eggerthella* showed negative associations with newborn head circumference and weight, respectively in males (genus *Parabacteroides*: adjusted $r = -0.598$, $p = 0.009$, genus *Eggerthella*: adjusted $r = -0.481$, $p = 0.043$). On the other hand, genus *Streptococcus* showed a negative association with newborn height in females (adjusted $r = -0.413$, $p = 0.040$). In addition, hexanoate was involved in the association between maternal gut microbiota and newborn anthropometrics in the univariate analysis, but not in the multivariate analysis. These data suggest that maternal gut microbiota has sex-specific effects on foetal growth. Maternal gut microbiota is an important factor for optimal intrauterine growth.

Introduction

Known as the developmental origins of health and disease (DOHaD) concept, exposure to environmental factors during the foetal period to early childhood affects the risk of noncommunicable diseases (NCDs) in adulthood.[1–3] In other words, according to this concept, foetal growth is a good predictor of the risk of NCDs in adulthood.[3] A wide range of maternal factors, including body composition, nutrition, stress, and smoking, have been reported to affect foetal growth and the subsequent risk of NCDs.[4,5]

Recently, in addition to these factors, there has been great interest in the role of maternal gut microbiota in the DOHaD concept. Disruption of the maternal gut microbiota balance (dysbiosis) alters the child's gut microbiota and immunity through vertical transmission from leaky gut and/or placenta, delivery, or breastfeeding, and this dysbiosis affects the risk of NCDs in adulthood.[6,7] Thus, the maternal gut microbiota is thought to be an important factor contributing to the child's health in later life, but studies investigating the effects of maternal gut microbiota on foetal growth are limited.

A few studies have reported the associations between maternal gut microbiota and foetal growth.[8,9] Santacruz *et al.* reported that some genera of maternal gut microbiota were associated with increased newborn weight. Priyadarshini *et al.* reported that maternal serum levels of short-chain fatty acids (SCFAs), partly originated from microbiota fermentation in the gut, were associated with newborn height and weight. These studies suggest the importance of further research on the effects of maternal gut microbiota on foetal growth.

Additionally, there is emerging indirect evidence on the importance of sex dimorphism on the association between maternal gut microbiota and foetal growth. It has been found that prenatal exposures that are linked to the composition of the maternal gut microbiota, such as maternal smoking[10,11] and antibiotic use,[12] have sex-specific effects on foetal growth. However, to our knowledge, there is no study that has investigated the direct association between maternal gut microbiota and foetal growth with respect to sex dimorphism. It was

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hypothesized that the maternal microbiota has sex-specific effects on foetal growth. Therefore, this study aimed to clarify the associations between maternal gut microbiota and foetal growth in both males and females.

Methods

Study subjects

The Chiba study of Mother and Child Health (C-MACH) is an ongoing prospective birth cohort study comprising approximately 400 mother–child pairs. This study investigates the association of genetic and environmental factors with child health using multi-omics analysis.[13] Of the participants in C-MACH, 58 women consented to provide their stool samples. Of those, six were excluded from the analysis due to missing maternal or newborn information and one due to maternal antibiotic use. Therefore, a total of 51 mother–newborn pairs were included in this analysis.

This study was conducted according to the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Japanese Ministry of Health, Labour and Welfare. The study protocol was approved by the Biomedical Research Ethic Committee of the Graduate School of Medicine, Chiba University. Prior to the start of the study, all participants provided written informed consent.

Maternal and newborn information

Maternal information, including age, parity, anthropometrics and antibiotic use during pregnancy, were obtained from self-administered questionnaires at the first trimester (around 12 weeks) or third trimester (around 32 weeks). The other maternal and newborn information, including delivery mode, gestational age, newborn gender and anthropometrics, were obtained from medical records. Gestational weight gain was calculated by subtracting pre-pregnancy weight from weight at the third trimester. To adjust for variations in parity, gestational age, and newborn gender, newborn anthropometrics were converted to standard deviation scores (SDSs) based on Japanese growth reference curves.[14]

Analysis of gut microbiota and SCFAs in stool

Sample collection and DNA extraction

Stool samples were collected at the third trimester of pregnancy for analysis of the gut microbiota and SCFAs. They were frozen at −18 °C following collection at home, and transferred to the Chiba University Center for Preventive Medical Sciences (CPMS) Biobank and stored at −80 °C until ready for use.

Microbial DNA extraction was performed according to previous studies with minor modifications.[15] Stool samples with a mass of 5.0 g were suspended in 30.0 ml of methanol, and then filtered using Steriflip filters (Merck, Darmstadt, Germany) with a pore size of 100 µm. The filtered suspension was centrifuged and separated into the supernatant and pellet. The supernatant and the pellet in methanol were stored at −80 °C until metabolomic analysis and DNA extraction, respectively. The 50.0 mg pellet was re-suspended in 450 µl of 10× Tris-EDTA buffer and incubated with 15 mg/ml lysozyme (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) at 37 °C for 1 h. Purified achromopeptidase (FUJIFILM Wako Pure Chemical Corporation) at a final concentration of 2000 units/ml was added and then incubated at 37 °C for 30 min. The suspension was added to 1% (wt/vol) sodium dodecyl sulphate and 1 mg/ml proteinase K (Merck) and incubated at 55 °C for 1 h. The microbial DNA was purified using a phenol/chloroform/isoamyl alcohol (25:24:1) solution (Nacalai Tesque, Kyoto, Japan). The DNA was precipitated by adding ethanol and sodium acetate. RNase (NIPPON GENE, Tokyo, Japan) treatment and polyethylene glycol precipitation were performed. The DNA was cleaned using the Wizard SV Gel and PCR Clean-Up System (Promega, WI, USA).

16 S rRNA sequencing V1-2 region

The V1-2 variable region (27Fmod – 338R[15]) was sequenced on an Illumina Miseq (Illumina, San Diego, CA, USA). The 16 S rRNA V1–V2 amplicon was amplified using KAPA HiFi Hot Start Ready Mix (2x) (TaKaRa Bio, Shiga, Japan). Two Universal bacterial 16 S rRNA gene primers were used: (1) the forward primer (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGRGTTTGATYMTGGCTCAG) and (2) reverse primer (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTACCGCGGCTGCTGG). The first reaction mixture contained 6 pmol of each primer, 12.5 ng of microbial DNA, 12.5 µl of KAPA HiFi Hot Start Ready Mix (2x), and sterilised water to reach a final volume of 30 µl. PCR conditions were as follows: 95 °C for 2 min, 20 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, followed by 72 °C for 3 min. The PCR product was purified through the use of AMPure XP (Beckman Coulter, Brea, CA, USA) and confirmed using electrophoresis in 3% (w/v) agarose gels.

Dual indexes and Illumina sequencing adapters were attached to PCR products using the Nextera XT Index Kit (Illumina). After purification of the amplicon using AMPure XP beads, these samples were quantified using a Quant-iT PicoGreen ds DNA Assay Kit (Life Technologies Japan, Tokyo, Japan).

Mixed samples were prepared by pooling approximately equal amounts of PCR amplicons from each sample. The pooled library was analysed with an Agilent High Sensitivity DNA Kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Real-time PCR for quantification of the pooled library was performed using a KAPA Library Quantification Kit (Kapa Biosystems, Boston, MA, USA) for Illumina following the manufacturer's protocols. Based on the quantifications, the sample library was denatured and diluted. A sample library with 20% denatured PhiX spike-in (SeqMatic LLC, Fremont, CA, USA) was sequenced on the Miseq using a 500 cycle kit.

Taxonomic assignments and estimation of relative abundance of sequencing data were performed using the analysis pipeline of the QIIME software package.[16] Chimera checking was performed using UCHIME.[17] An operational taxonomic unit (OTU) was defined at 97% similarity. The OTU was assigned a taxonomy based on a comparison with the Greengenes database using RDP classifier.[18,19] The proportions of identified taxa in each sample were summarised and the amount of bacterial diversity was calculated. The Shannon index, a measurement of within-sample (alpha-diversity) community diversity, as well as Chao 1 (estimates richness), and observed OTUs were used to evaluate the alpha diversity.

Measurement of SCFAs

Extraction and measurement of SCFAs was performed according to previous studies with some modifications.[20] A methanol extract with a volume of 25.0 µl was added to 5.0 µl of Milli-Q water containing internal standards (2.2 mM [1,2-$^{13}C_2$] acetate, 2.2 mM [$^{2}H_7$] butyrate and 2.2 mM crotonate), and then, centrifugally concentrated at 40 °C and reconstituted with 100.0 µl of Milli-Q water. Next, 50.0 µl of hydrochloric acid and 200.0 µl of diethyl ether were added and the solution was mixed well. After centrifugation at

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Table 1. Maternal and newborn characteristics

Variables	Total ($n = 51$)	Males ($n = 22$)	Females ($n = 29$)	p value
Maternal				
Age (year)	34 (5)	34 (6)	34 (6)	0.491
Height (cm)	159.0 (8.0)	157.0 (10.2)	160.0 (6.3)	0.614
Pre-pregnancy weight (kg)	52.0 (7.9)	52.5 (9.4)	51.0 (7.5)	0.932
Pre-pregnancy BMI (kg/m^2)	20.3 (3.4)	20.2 (3.9)	20.3 (3.3)	0.746
Weight at the third trimester (kg)	60.2 (10.6)	60.4 (12.2)	59.6 (9.7)	0.739
BMI at the third trimester (kg/m^2)	24.1 (3.5)	24.4 (4.2)	23.0 (3.8)	0.323
Gestational weight gain (kg)	8.5 (5.1)	8.5 (4.2)	7.2 (5.9)	0.254
Delivery mode (% vaginal)	86.3	81.8	89.7	0.447
Parity (% first)	29.4	40.9	20.7	0.117
Newborn				
Gender (% females)	56.9	–	–	
Gestational age (weeks)	39 (2)	39 (2)	39 (2)	0.849
Height (cm)	49.5 (1.0)	49.8 (1.4)	49.5 (1.0)	0.614
Weight (g)	3054 (322)	3040 (481)	3062 (247)	0.739
Head circumference (cm)	33.0 (1.5)	33.5 (1.1)	33.0 (2.0)	0.412
Height SDS	0.12 (0.88)	0.22 (0.80)	0.12 (0.88)	0.493
Weight SDS	−0.13 (0.97)	−0.20 (0.91)	0.05 (0.99)	0.077
Head circumference SDS	−0.14 (0.89)	0.04 (0.79)	−0.21 (1.23)	0.351

Values are presented as median (interquartile range) or percentage of subjects.
Mann–Whitney *U* tests, Chi-square tests or Fisher's exact tests were performed to compare newborn characteristics between males and females.
BMI, body mass index; SDS, standard deviation score.

3000 g for 10 min, 80.0 µl of the organic layer was transferred to a glass vial and 16.0 µl of *N*-tert-butyldimethylsilyl-*N*-trifluoroacetamide (MTBSTFA) (Sigma-Aldrich, St. Louis, MO, USA) was added to derivatise the samples. The vials were incubated at 80 °C for 20 min and were allowed to stand for 48 h before injection. The analysis was performed using gas chromatography-tandem mass spectrometry (GC/MS/MS) platforms on a Shimadzu GCMS-TQ8030 triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) with a capillary column (BPX5) (SGE Analytical Science Pty. Ltd., Melbourne, Australia). The GC oven was programmed as follows: 60 °C held for 3 min, increased to 130 °C (at a rate of 8 °C/min), increased to 330 °C (at a rate of 30 °C/min), and held at 330 °C for 3 min. The detector and injector temperatures were 230 °C and 250 °C, respectively. GC was performed in constant linear velocity mode set to 40 cm/sec. Injection volume was set at 1.0 µl with a split ratio of 1:30.

Statistical analyses

Baseline characteristics are presented as median (interquartile range; IQR) or percentage of subjects. Mann–Whitney *U* tests, Chi-square tests or Fisher's exact tests were performed to compare newborn characteristics between males and females.

Maternal microbial diversity, the proportion of microbiota at the phylum and genus levels, and concentrations of SCFAs in stool are presented as median (IQR). Only the microbiota or SCFAs detected in more than half of the subjects were used for analysis.

Spearman's rank correlation coefficient analysis was performed to assess the association of maternal gut microbial diversity or composition with newborn anthropometrics. Then, Spearman's partial correlation coefficient analysis was performed to adjust the basic models for covariates including maternal height, pre-pregnancy body mass index (BMI), gestational weight gain and delivery mode. The same analyses were performed to assess the association of maternal SCFAs in stool with newborn anthropometrics. In addition, Spearman's rank correlation coefficient analysis was also performed to assess the association of maternal SCFAs in stool with the maternal gut microbiota related to newborn anthropometrics.

All statistical analyses were conducted using SPSS version 24 (IBM Corp., Armonk NY, USA) and R version 3.4.0 (R Core Team, 2017).[21] Partial correlation coefficients were calculated using package "ppcor".[22] Statistical significance was defined by a value of $p < 0.05$.

Results

Maternal and newborn characteristics

Characteristics of the mothers and newborns are presented in Table 1. The median (IQR) maternal age, pre-pregnancy BMI and gestational weight gain was 34 (5) years, 20.3 (3.4) kg/m^2 and 8.5 (5.1) kg, respectively. Of the 51 mothers, only 8% ($n = 4$) were overweight prior to pregnancy. Of the 51 newborns, 56.9%

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Table 2. Correlation between maternal gut microbial diversity and newborn anthropometrics

	Height SDS		Weight SDS		Head circumference SDS	
Variables	*r*	*p* value	*r*	*p* value	*r*	*p* value
Males						
Observed OTUs	−0.082	0.717	0.015	0.948	0.526	**0.012**
Chao 1	0.070	0.757	0.127	0.573	0.591	**0.004**
Shannon index	−0.218	0.330	0.057	0.801	0.477	**0.025**
Females						
Observed OTUs	−0.020	0.917	−0.182	0.345	−0.217	0.258
Chao 1	0.065	0.738	−0.155	0.422	−0.327	0.083
Shannon index	0.129	0.506	−0.122	0.528	−0.237	0.217

r Values indicate Spearman's rank correlation coefficients.
Significant *p* values ($p < 0.05$) are shown in bold.
SDS, standard deviation score; OTUs, operational taxonomic units.

were females ($n = 29$), and the remaining 44.1% were males ($n = 22$). The median (IQR) newborn height, weight and head circumference at birth was 49.5 (1.0) cm, 3054 (322) g and 33.0 (1.5) cm, respectively. All newborn heights and weights were within the standard ranges. Only 1 male newborn's head circumference deviated from the range of ±2.0 standard deviations (2.21), while all others were within the standard ranges.

Association between maternal gut microbial diversity and newborn anthropometrics

Maternal gut microbial diversity is presented in Supplemental Table S1. To assess the association of maternal gut microbial diversity with newborn anthropometrics, Spearman's rank correlation coefficient analysis was performed. There were significant positive correlations between all alpha-diversity indexes of maternal gut microbiota and newborn head circumference SDS in males (observed OTUs: $r = 0.526$, $p = 0.012$, Chao 1: $r = 0.591$, $p = 0.004$, Shannon index: $r = 0.477$, $p = 0.025$; Table 2); this was not found in females. Newborn height SDS and weight SDS were not significantly correlated with maternal gut microbial diversity, regardless of gender.

To evaluate the association of maternal gut microbial diversity with newborn head circumference in detail, adjusted correlation coefficient was calculated with maternal factors as covariates (Table 3). Chao 1 and newborn head circumference in males were significantly positively correlated, even after adjustment for the covariates (adjusted $r = 0.515$, $p = 0.029$).

Table 3. Adjusted correlation between each alpha-diversity index of maternal microbiota and newborn head circumference in males

Variables	Adjusted *r*	*p* value
Observed OTUs	0.430	0.075
Chao 1	0.515	**0.029**
Shannon index	0.335	0.174

Adjusted *r* values indicate Spearman's partial correlation coefficients with covariates including maternal height, pre-pregnancy BMI, gestational weight gain, and delivery mode.
Significant *p* value ($p < 0.05$) is shown in bold.
OTUs, operational taxonomic units.

Association between maternal gut microbiota composition and newborn anthropometrics

The proportions of maternal gut microbiota at the phylum and genus levels are presented in Supplemental Table S2. To assess the association of maternal gut microbiota composition with newborn anthropometrics, Spearman's rank correlation coefficient analysis was performed. There were no significant correlations between the phyla of the maternal gut microbiota and newborn anthropometrics, regardless of gender (Supplemental Table S3). In contrast, there were significant correlations between three genera of maternal gut microbiota and newborn weight SDS in males, as well as between seven genera of maternal gut microbiota and newborn head circumference SDS (Table 4). Only one genus of maternal gut microbiota was significantly correlated with newborn height SDS and weight SDS in females.

To evaluate the association of these genera of maternal gut microbiota with newborn anthropometrics in detail, adjusted correlation coefficient was calculated with maternal factors as covariates (Table 4). Genus *Parabacteroides* and genus *Eggerthella* showed significant negative correlations with newborn head circumference SDS and weight SDS, respectively, in males, even after adjustment for the covariates (genus *Parabacteroides*: adjusted $r = -0.598$, $p = 0.009$, genus *Eggerthella*: adjusted $r = -0.481$, $p = 0.043$). On the other hand, genus *Streptococcus* showed a significant negative correlation with newborn height SDS in females, even after adjustment for the covariates (adjusted $r = -0.413$, $p = 0.040$).

Association between maternal SCFAs in stool and newborn anthropometrics

The concentrations of SCFAs in maternal stool are presented in Supplemental Table S4. As the mechanism by which gut microbiota affects the host's physiology could be, in part, mediated by SCFAs, the association of SCFAs in stool with newborn anthropometrics was assessed. There was a significant positive correlation between hexanoate and newborn head circumference SDS in males, as well as newborn height SDS in females (Supplemental Table S5).

To evaluate the association of hexanoate with newborn anthropometrics in detail, adjusted correlation coefficient analysis was calculated with maternal factors as covariates (Table 5). There were

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Table 4. Adjusted correlation between genus of maternal microbiota and newborn anthropometrics

	Height SDS			
Variables	*r*	*p* value	Adjusted *r*	*p* value
Females				
Streptococcus	−0.371	**0.047**	−0.413	**0.040**
	Weight SDS			
	r	*p* value	Adjusted *r*	*p* value
Males				
Eggerthella	−0.530	**0.011**	−0.481	**0.043**
Collinsella	0.479	**0.024**	0.356	0.148
Anaerostipes	−0.450	**0.036**	−0.452	0.060
Females				
Streptococcus	−0.404	**0.030**	−0.232	0.265
	Head circumference SDS			
	r	*p* value	Adjusted *r*	*p* value
Males				
Lachnospiraceae Ruminococcus	−0.546	**0.009**	−0.328	0.183
Parabacteroides	−0.489	**0.021**	−0.598	**0.009**
Lachnobacterium	0.484	**0.022**	0.392	0.107
Bacteroides	−0.451	**0.035**	−0.336	0.172
Eggerthella	−0.438	**0.042**	−0.408	0.093
Dorea	−0.426	**0.048**	−0.457	0.057
Coriobacteriaceae unclassified	0.424	**0.049**	0.297	0.232

The data with significant *p* values in non-adjusted correlation are shown.
r values indicate Spearman's rank correlation coefficients.
Adjusted *r* values indicate Spearman's partial correlation coefficients with covariates including maternal height, pre-pregnancy BMI, gestational weight gain and delivery mode.
Significant *p* values ($p < 0.05$) are shown in bold; SDS, standard deviation score.

Table 5. Adjusted correlation between maternal SCFAs in stool and newborn anthropometrics

	Height SDS			
Variables	*r*	*p* value	Adjusted *r*	*p* value
Females				
Hexanoate	0.448	**0.015**	0.377	0.063
	Head circumference SDS			
	r	*p* value	Adjusted *r*	*p* value
Males				
Hexanoate	0.463	**0.030**	0.373	0.127

The data with significant *p* values in non-adjusted correlation are shown.
r values indicate Spearman's rank correlation coefficients.
Adjusted *r* values indicate Spearman's partial correlation coefficients with covariates including maternal height, pre-pregnancy BMI, gestational weight gain and delivery mode.
Significant *p* values ($p < 0.05$) are shown in bold.
SCFAs, short-chain fatty acids.

no significant associations between hexanoate and newborn anthropometrics after adjustment for the covariates.

In addition, to evaluate the involvement of SCFAs in the associations between maternal gut microbiota and newborn anthropometrics, we attempted to explore the correlations between the maternal SCFAs in stool and the maternal gut microbiota related to newborn anthropometrics (Fig. 1). There were stronger correlations between hexanoate and the maternal gut microbiota, when compared to other major SCFAs including acetate, propionate and butyrate.

Discussion

In this study, the associations between maternal gut microbiota and foetal growth in both males and females were investigated. It was found that lower maternal gut microbial diversity as well

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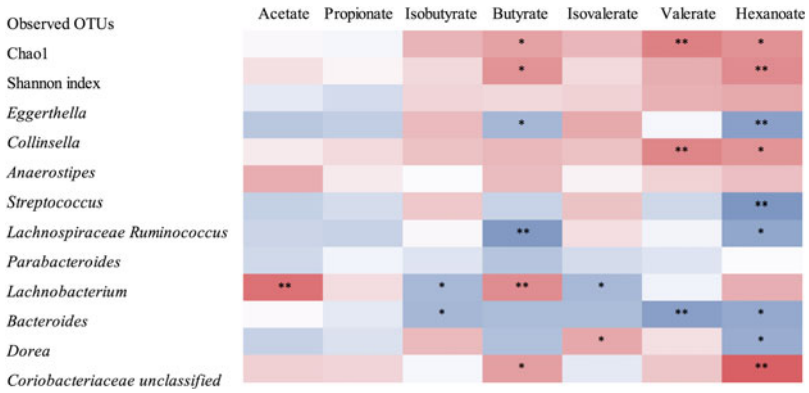

Fig. 1. (Colour online) Correlation between maternal gut microbiota and SCFAs in stool in all study subjects ($n = 51$). Heatmap shows correlations between maternal gut microbiota related to newborn anthropometrics and SCFAs in stool. Colour intensity represents magnitude of correlation evaluated using Spearman's rank correlation coefficients. Blue colour means negative correlation; Red colour means positive correlation. SCFAs, short-chain fatty acids; OTUs, operational taxonomic units.
$^{*}p < 0.05$, $^{**}p < 0.01$.

as higher proportions of genera *Parabacteroides* and *Eggerthella* were associated with smaller newborn head circumference or weight in males. On the other hand, a higher proportion of genus *Streptococcus* was associated with smaller newborn height in females. Moreover, SCFAs in stool, especially hexanoate, were involved in the association between maternal gut microbiota and newborn anthropometrics in the univariate analysis, but not in the multivariate analysis. These findings suggest that maternal gut microbiota possibly have sex-specific effects on foetal growth. To our knowledge, this is the first study to show a sex-specific relationship between maternal gut microbiota and newborn anthropometrics.

The findings observed predominately in males in this study are similar to those of a previous study that showed male-specific effects of prenatal exposures, which were linked to the composition of the gut microbiota.[12] Indeed, maternal use of antibiotics during pregnancy was associated with lower birth weight in males but not in females. While the previous study provided indirect evidence for the male-specific effect of the gut microbiota, the results of this study provide direct evidence of a male-dominant association between maternal gut microbiota and foetal growth. Although the mechanism underlying the sex-specific effects on foetal growth remains unclear, maternal exposure during pregnancy could be transmitted from the maternal to the foetal compartment via the placenta in a sex-specific manner and subsequently affect foetal tissue development.[23]

Unlike in previous studies,[12] we found that maternal gut microbiota (genus *Streptococcus*) was associated with newborn anthropometrics in females as well. This difference may be due to the different distribution of the microbiota in mothers with male and female foetuses. Although the cause was unclear, all mothers with a male foetus had a proportion of genus *Streptococcus* ≤5%, while approximately a quarter of mothers with a female foetus had higher proportions of *Streptococcus* than the mothers with a male foetus (data not shown). *Streptococci* are pathobionts in both mothers and newborns, and the vertical transmission from mother to foetus has been shown to cause poor outcomes in newborns.[24,25] The high rates of *Streptococcus* in some mothers with a female foetus in our cohort might have a large impact on the female-specific results. In other words, when these mothers were excluded, there was no significant association between maternal *Streptococcus* and newborn height in females (data not shown).

Many studies have demonstrated that smaller birth size is associated with worse outcomes in later life. For instance, several studies have reported that smaller birth size, especially head circumference, is strongly associated with increased risk of death from coronary heart disease in adulthood.[26,27] In addition, a recent study showed that small head circumference at birth was associated with early adiposity rebound, which is a predictor of obesity and type 2 diabetes, and hence coronary heart disease in later life.[28] Moreover, smaller birth size has been reported to be associated with poor intellectual ability in childhood[29] or adulthood.[30] Together with the present results, further studies investigating adiposity rebound or intellectual ability in childhood are needed to clarify the effects of maternal gut microbiota and small birth size on health and disease in later life.

The alterations of gut microbiota observed in this study could be associated with the host's systemic inflammatory condition. Indeed, lower maternal gut microbial diversity increases translocation of bacteria-derived products across the intestinal mucosa, which could contribute to systemic and placental inflammation.[7,31] Genus *Parabacteroides* was reported to be present at a higher abundance in non-alcoholic steatohepatitis (NASH) patients, whose gut dysbiosis induced pro-inflammatory activity and immune imbalances through hepatotoxic effects.[32] Genus *Eggerthella* has been known as a cause of severe bacteraemia from a gastrointestinal tract source.[33,34] Gastrointestinal colonisation by genus *Streptococcus* is a risk factor for vaginal colonization, which induces inflammatory cytokine release in placental tissues or the amniotic fluid.[25] Based on these studies, it could be hypothesized that such systemic inflammatory conditions caused by maternal gut microbiota might contribute to foetal growth. Many studies have reported that prenatal inflammation contributes to foetal growth restriction and neurodevelopmental impairments through pro-inflammatory cytokines and chemokines.[35,36] Additionally, such prenatal inflammation is reportedly of greater magnitude,[37,38] and produces a larger effect[39] in the male foetus. In other words, this hypothesis could explain the male-dominant effects of maternal gut microbiota on foetal growth. It is important to analyse these various cytokines and chemokines in maternal stool and serum samples, as well as cord serum samples, to clarify the mechanism by which maternal gut microbiota affects newborn anthropometrics in a sex-specific manner.

In this study, it was not clear whether the SCFAs contributed to the association between maternal microbiota and foetal growth. Hexanoate was involved in the association between maternal gut microbiota and newborn anthropometrics in the univariate analysis, but not in the multivariate analysis. However, decreased

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hexanoate has been reported to indicate gut dysbiosis and subsequent gut systemic inflammation in patients with metabolic syndrome[40] or psoriatic arthritis.[41] Assuming that the decreased hexanoate and altered microbiota mentioned above are related to the host systemic inflammatory condition, the sample size might have been insufficient to show a significant association between maternal hexanoate in stool and foetal anthropometrics.

This study had several limitations. First, the sample size was relatively small; therefore, *p* values could not be adjusted sufficiently to control for multiple testing. Studies with a large number of subjects are necessary to perform more robust statistical analyses and further validate the findings presented here. Furthermore, all the study subjects were Japanese, and therefore, some of the taxonomic findings may not necessarily conform to other ethnic or racial groups, or individuals from other geographic regions. To generalise these findings, the association between maternal gut microbiota and foetal growth in other ethnic or regional groups would need to be investigated.

Conclusion

In conclusion, it was found that lower maternal gut microbial diversity and higher proportions of genera *Parabacteroides* and *Eggerthella* were associated with smaller newborn anthropometrics in males, while a higher proportion of genus *Streptococcus* was associated with smaller newborn anthropometrics in females. These findings suggest that maternal gut microbiota is an important factor for optimal intrauterine growth. Further studies are needed to confirm the mechanism by which maternal gut microbiota affects foetal growth in a sex-specific manner, and its effects on health and disease in later life.

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Conflicts of Interest. None.

Ethical Standards. This study was conducted according to the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Japanese Ministry of Health, Labor and Welfare. Study protocol was approved by the Biomedical Research Ethic Committee of the Graduate School of Medicine, Chiba University. At the start of the study, all participants signed an informed consent.

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