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SHORT REPORT

Associations between changes in the maternal gut microbiome and differentially methylated regions of diabetes-associated genes in fetuses: A pilot study from a birth cohort study

Kaori Tachibana, Kenichi Sakurai, Masahiro Watanabe, Hidenobu Miyaso, Chisato Mori*

Center for Preventive Medical Sciences, Chiba University, Chiba, Japan

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***Correspondence**
Chisato Mori
Tel.: +81-43-226-2017
Fax: +81-43-226-2018
E-mail address: cmori@faculty.chiba-u.jp

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ABSTRACT

Several intrauterine environmental factors can increase the future risk of type 2 diabetes. The microbiome can influence the balance between health and disease. However, the influence of the maternal gut microbiome on the future risk of diabetes in the fetus is unknown. The present study investigated the associations between maternal gut microbiome and differentially methylated regions of diabetes-associated genes in umbilical cord samples. The present study included 10 pregnant participants from a birth cohort study. 16S ribosomal ribonucleic acid metagenome analysis of maternal stool samples and deoxyribonucleic acid methylation assays of umbilical cord samples were carried out. The present study found that changes in the *UBE2E2* and *KCNQ1* methylation rates in umbilical cord samples were associated with the proportion of *Firmicutes* in the maternal gut, albeit with marginal correlations after adjustment for age and body mass index. These findings suggest a link between the methylation of diabetes-associated genes in fetuses and maternal microbiota components during pregnancy.

INTRODUCTION

Epigenetic alternations of diabetes-associated genes have been reported to increase the risk of diabetes[1,2]. Although, low birthweight and maternal malnutrition, which are considered as fetal environmental factors, can cause epigenetic alternations of diabetes-associated genes[3], few studies have investigated the epigenetic alternations in type 2 diabetes-associated genes of fetal tissues.

The gut microbiome has been shown to be a novel environmental factor that directly affects metabolism[4]. Perturbations in the gut microbiome have been implicated to cause metabolic syndrome[5], and the role of the gut microbiome in pregnancy has become the subject of considerable interest[6].

Although a change in the exposure to maternal microbial diversity in fetuses remains one of the leading explanations for epigenetic changes and an increase in the risk of non-communicable diseases[7], the association between the maternal gut microbiome and fetal epigenetic changes of diabetes-associated genes has not been elucidated.

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The umbilical cord is fetal tissue, and it can be obtained from infants non-invasively. Several studies have reported that alternations of umbilical cord deoxyribonucleic acid (DNA) methylation are associated with some phenotypes of children[8]. In the present study, we investigated associations between changes in the maternal gut microbiome and differentially methylated regions of diabetes-associated genes in umbilical cord samples.

MATERIALS AND METHODS

Participants

We recruited 10 pregnant women who were participants in the Chiba Study of Mother and Children's Health, which included collection of stool, blood and umbilical cord samples; assessment of dietary records; and administration of questionnaires[9]. This observational study was carried out according to the guidelines of the Declaration of Helsinki, and study protocol was approved by the Biomedical Research Ethics Committee of the Graduate School of Medicine, Chiba University. Additionally, written informed consent was obtained from the participants.

Clinical data were obtained from the medical records, and food consumption data were obtained using a brief

self-administered diet history questionnaire[10]. The serum levels of biochemical parameters were measured using enzyme-colorimetric automated methods (SRL Inc., Tokyo, Japan).

16S ribosomal ribonucleic acid metagenome analysis

Stool samples were collected for analysis of the gut microbiome composition at the third trimester of pregnancy. Stool samples were transferred to Chiba University CPMS Biobank and kept frozen at −80°C.

Total DNA from the stool samples was extracted, sequenced and analyzed by quantitative metagenomics at Hokkaido System Science (Sapporo, Japan). A detailed description of the metagenomic analysis has been reported previously[11]. In each sample, bacterial 16S ribosomal ribonucleic acid gene sequences were polymerase chain reaction-amplified using a primer for the V3-V4 hypervariable region of the 16S ribosomal ribonucleic acid gene with overhang adapters attached. Pair-ended sequencing with read lengths of 300 bp was carried out using an Illumina sequencing system (Illumina, San Diego, CA, USA). Analysis was carried out using the open-source software package Quantitative Insights Into Microbial Ecology (QIIME, qiime.org). Sequences were clustered as operational taxonomic units based on 0.97 similarity using UCLUST (drive5.com).

DNA methylation assays

Umbilical cord samples were obtained at delivery and stored at −80°C until analysis. Genomic DNA was extracted from each sample using the NucleoSpin Tissue Kit (TaKaRa Bio, Shiga, Japan). The DNA methylation profiles of the umbilical cords were determined using the Infinium HumanMethylation450 BeadChip (Illumina). The methylation levels at each cytosine-phosphate-guanine dinucleotide quantified with average β-values were calculated using GenomeStudio 2011.1 (Module M Version 1.9.0; Illumina). We selected the candidate region using following criteria: (i) standard deviation of β-values >0.05; (ii) presence of a differentially methylated regions tag, according to the data sheet provided by the manufacturer[12]; and (iii) location near or in diabetes-associated genes[13,14].

Table 1 | Characteristics of the participants at the first trimester of pregnancy

Characteristic		
Age (years)	34.1 ± 3.0	
Prepregnancy body mass index (kg/m^2)	21.2 ± 1.9	
Prepregnancy body weight (kg)	52.3 ± 5.8	
Bodyweight gain during pregnancy (kg)	9.75 ± 2.47	
Food consumption		
Energy intake (kJ/day)	6532.4 ± 1854.7	
Carbohydrate intake (%energy)	57.0 ± 4.6	
Fat intake (%energy)	27.0 ± 4.7	
Protein intake (%energy)	15.1 ± 2.1	
Fiber (g/10 MJ)	16.5 ± 5.2	
Biochemical parameters		Reference value
Glycoalbumin (%)	13.8 ± 0.9	(12.4–16.3)
Total cholesterol (mg/dL)	186.2 ± 18.0	(150–219)
HDL-cholesterol (mg/dL)	76.0 ± 8.2	(40–96)
Triglyceride (mg/dL)	114.4 ± 42.8	(50–149)
Folic acid (ng/mL)	15.2 ± 11.8	(≥4.0)
Vitamin B_{12} (pg/mL)	280.7 ± 75.7	(180–914)
Total homocysteine (nmol/mL)	4.69 ± 0.88	(3.7–13.5)

Values are presented as mean ± SD. Food consumption data were obtained using a brief self-administered diet history questionnaire. The density method was used to compute the amount of each nutrient consumed daily, as a percentage of daily energy intakes for energy-containing nutrients or per 10 MJ of daily energy intake for non-energy-containing nutrient. HDL, high-density lipoprotein.

Statistical analysis

Clinical baseline characteristics are presented as mean ± SD. Spearman's rank correlation coefficient analysis was carried out. All statistical analyses were carried out using SPSS version 22 (IBM Corp., Armonk NY, USA). Statistical significance was defined as a *P*-value <0.05.

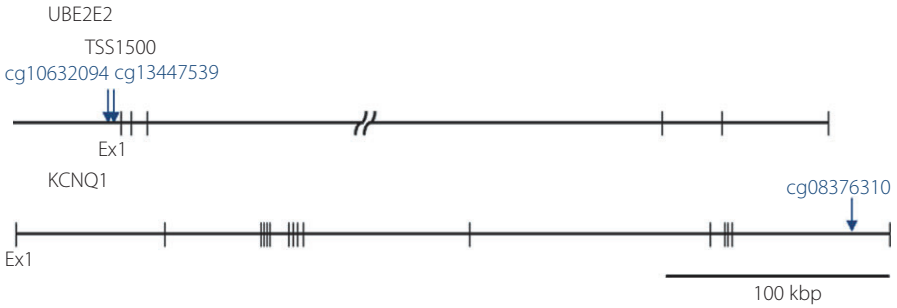

Figure 1 | The location of the identified cytosine-phosphate-guanine dinucleotide sites in the *UBE2E2* and *KCNQ1* genes. The three regions that fulfilled our criteria were *UBE2E2* (cg13447539 and cg10632094) and *KCNQ1* (cg08376310). Each arrow indicates the position of each probe in the BeadChip.

RESULTS

Health characteristics and diets of the participants

The clinical baseline characteristics of the participants are presented in Table 1. None of the participants had gestational diabetes.

Relative abundances of gut bacterial phyla

The four major bacterial phyla in the third trimester were *Firmicutes* (71.8 ± 7.8%), *Actinobacteria* (16.7 ± 8.0%), *Bacteroidetes* (7.3 ± 4.8%) and *Proteobacteria* (1.7 ± 2.5%).

Correlation between the microbiome composition and host parameters

According to previous reports, we selected *Firmicutes* to assess the correlation between the microbiome composition and host parameters[15]. We found no significant correlation between the proportion of *Firmicutes* and their anthropometric or nutritional parameters.

Umbilical cord DNA methylation of the diabetes-associated genes

The number of genes with standard deviation of the β-value >0.05 was 72,848 in the chip without sex chromosomes. Of these genes, 3,097 had differentially methylated regions tags. The candidates that fulfilled our criteria were the following three regions: *UBE2E2* (cg13447539, cg10632094) and *KCNQ1* (cg08376310). The locations of the identified regions are shown in Figure 1. We investigated the relationships of these three regions with the proportion of *Firmicutes*. There was a significant positive correlation between the *UBE2E2* (cg13447539) β-value and the proportion of *Firmicutes*, and a significant negative correlation between the *KCNQ1* β-value and the proportion of *Firmicutes* in the third trimester (Rs = 0.685, $P = 0.040$; Figure 2a and Rs = –0.661, $P = 0.048$; Figure 2b, respectively). We also carried out multiple regression analysis using age and body mass index. After adjustment, the *UBE2E2* (cg13447539) and *KCNQ1* β-values, and the proportion of *Firmicutes* tended to show a marginal correlation (Rs = 0.641, $P = 0.087$; Rs = –0.606, $P = 0.111$, respectively). Additionally, there was a moderate positive correlation between the β-value of the *UBE2E2* cytosine-phosphate-guanine dinucleotide site (cg10632094) and the proportion of *Firmicutes* (Rs = 0.617, $P = 0.080$).

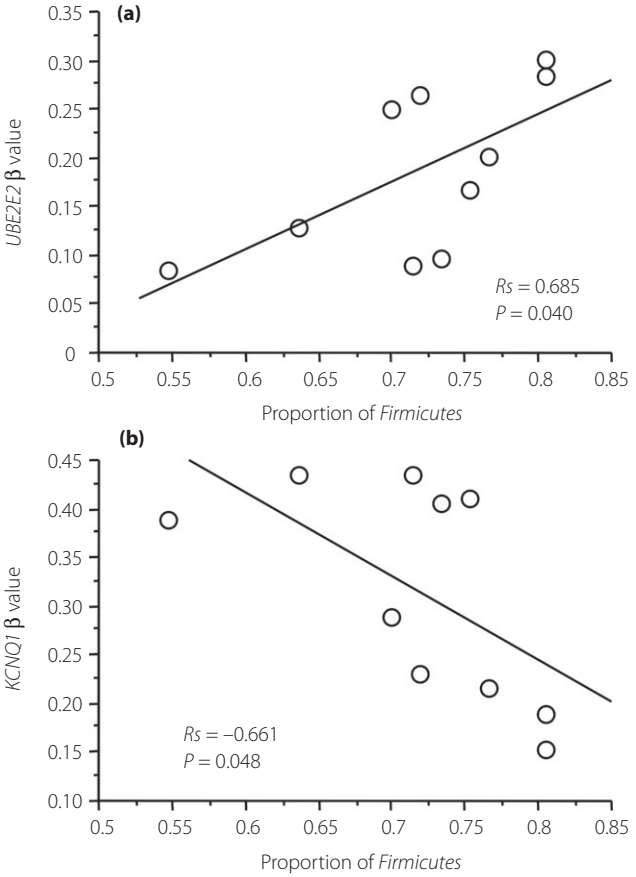

Figure 2 | Correlation between maternal gut microbiome and deoxyribonucleic acid methylation of diabetes-associated genes in the umbilical cord. (a) Scatter plot of the proportion of *Firmicutes* in the maternal gut (*x*-axis) and the umbilical cord *UBE2E2* (cg13447539) β-value (*y*-axis) in the third trimester among the 10 participants. (b) Scatter plot of the proportion of *Firmicutes* in the maternal gut (*x*-axis) and the umbilical cord *KCNQ1* (cg08376310) β-value (*y*-axis) in the third trimester among the 10 participants. The methylation levels at each cytosine-phosphate-guanine dinucleotide that was quantified with average β-values, where 1 corresponds to complete methylation and 0 to no methylation, are shown.

DISCUSSION

Alternations in the levels of DNA methylation have been shown to be present in numerous candidate genes for type 2 diabetes[16]. Furthermore, epigenetic variations were shown to be present before disease development and were found to be risk factors[2]. *UBE2E2* encodes the ubiquitin-conjugating enzyme E2-E2, and has been reported to play an important role in insulin secretion. Single-nucleotide polymorphisms in *UBE2E2* were shown to be associated with type 2 diabetes in individuals of East Asian descent[14]. Similarly, the association of *KCNQ1* with type 2 diabetes was shown in non-European populations[17]. Furthermore, epigenetic regulation of the *KCNQ1* gene has been reported to contribute to the onset of type 2 diabetes in mice[18] and humans[19]. We found that the *UBE2E2* and *KCNQ1* methylation rates in umbilical cord samples were associated with the maternal gut microbiome composition. DNA methylation is known to regulate gene expression in a tissue-specific manner[20]. Further studies are required to investigate whether the methylation rates in umbilical cord samples reflect the methylation rates in other tissues.

Changes in the gut microbiome in late pregnancy have been shown to be associated with known changes in insulin resistance and inflammation[6]. However little is known about the impact of this dysbiosis on maternal and fetal metabolism[4]. It

has been reported that *Firmicutes*-dominant microbiota is associated with the development of obesity and metabolic syndrome[21]. Furthermore, *Firmicutes* might contribute to epigenetic changes by producing folate and butylate[15]. The present study provides possibilities for the interactions between the maternal gut microbiome and the epigenetic changes of diabetes-associated genes in fetal tissue.

The results of the present study are limited by the small sample size. Further studies with a larger sample size and long-term follow up are required to confirm the present findings.

In conclusion, a link is assumed to be present between changes in the methylation of type 2 diabetes-associated genes in fetuses and the microbiota components in mothers during pregnancy.

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DISCLOSURE

The authors declare no conflict of interest.

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