Basic Study

Correlation between gut microbiota and glucagon-like peptide-1 in patients with gestational diabetes mellitus

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Background

Gestational diabetes mellitus (GDM) places both the mother and offspring at high risk of complications. Increasing evidence suggests that the gut microbiota plays a role in the pathogenesis of GDM. However, it is still unclear whether the gut microbiota is related to blood biochemical traits, particularly glucagon-like peptide-1 (GLP-1), in GDM patients.

Objective

To explore the correlation between the gut microbiota and blood biochemical traits, particularly GLP-1, in GDM patients.

Methods

The V4 region of the 16S ribosomal ribonucleic acid (rRNA) gene was sequenced...
based on the fecal samples of 35 pregnant women with GDM and was compared to that of 25 pregnant women with normal glucose tolerance (NGT).

RESULTS
The results showed that Ruminococcaceae_UCG-002, Ruminococcaceae_UCG-005, Clostridium_sensu_stricto_1, and Streptococcus were more abundant in the NGT group than in the GDM group. Bacteroides and Lachnoclostridium were more abundant in the GDM group than in the NGT group. Spearman’s correlation analysis was performed to identify the relationships between microbiota genera and blood biochemical traits. Paraprevotella, Roseburia, Faecalibacterium, and Ruminococcaceae_UCG-002 were significantly negatively correlated with glucose. Ruminococcaceae_UCG-002 was significantly negatively correlated with hemoglobin A1c. Bacteroides was significantly positively correlated with glucose. Sutterella, Oscillibacter, and Bifidobacterium were significantly positively correlated with GLP-1. A random forest model showed that 20 specific genera plus glucose provided the best discriminatory power, as indicated by the area under the receiver operating characteristic curve (0.94).

CONCLUSION
The results of this study reveal novel relationships between the gut microbiome, blood biochemical traits, particularly GLP-1, and GDM status. These findings suggest that some genera are crucial for controlling blood glucose-related indices and may be beneficial for GDM treatment. Alteration in the microbial composition of the gut may potentially serve as a marker for identifying individuals at risk of GDM.

Key Words: Gut microbiome; Glucagon-like peptide-1; Gestational diabetes mellitus; Glucose

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Evidence indicates that the gut microbiota is closely related to GDM[4]. The mechanism underlying the effect of probiotics in diabetes has not yet been fully elucidated, but it may be related to reductions in oxidative stress, regulation of the immune response, reductions in inflammation, and regulation of the gut microbiota[5,6]. In addition, probiotics can also reduce postprandial blood lipid levels and improve the absorption of antioxidants, which are related to oxidative stress[7]. Numerous studies have demonstrated that GLP-1 has insulin tropic and antioxidant effects[8-10]. Since GLP-1 and the gut microbiota each play roles in GDM, is there a correlation between the two?

Both clinical and animal studies have reported correlations between changes in the GLP-1 level and changes in the gut microbiota after gastrointestinal bypass surgery in type 2 diabetes mellitus (T2DM) patients or mice[11]. Therefore, it is speculated that GLP-1 may regulate blood glucose by regulating the number and structure of gut microbiota. Several authors have argued that GLP-1 may play a role in regulating blood glucose by increasing the diversity of the gut microbiota[12] and increasing the proportion of probiotics. Together, the current literature provides a comprehensive explanation of the hypoglycemic mechanism of GLP-1 and a reliable experimental basis for the study of GDM therapeutic targets and therapeutic drugs based on GLP-1. Accordingly, one study found that bifidobacteria improved insulin sensitivity by increasing the production of GLP-1[13].

The rapid increase in the prevalence of GDM in recent years cannot be easily explained by genetic factors; thus, it has been hypothesized that environmental factors may play a more important role. The gut microbiota constitutes an important environmental factor. GLP-1, as the most important representative of gastrointestinal hormones, may also be involved in the pathogenesis of GDM. The gastrointestinal microbiota and gastrointestinal hormones share the same root, are inseparable, influence and restrict each other, and jointly participate in the occurrence, development, and prognosis of GDM. Thus, a comprehensive study of the correlations between changes in the gut microbiota and GLP-1 will help to further clarify the pathogenesis of GDM. This is of great significance for the prevention, treatment, and prognosis of GDM, and may provide a novel and sensitive index for the clinical evaluation of GDM.

**MATERIALS AND METHODS**

**Subjects**

Patients were screened for GDM in the obstetric outpatient department according to the GDM diagnostic criteria (2014). Thirty-five patients with GDM were randomly selected from the patients who met the diagnostic criteria for GDM; these patients formed the GDM group. A further 25 pregnant women with normal glucose tolerance (NGT) were selected as the NGT group. Each subject provided written informed consent before inclusion in the study. The study was approved and carried out in accordance with the guidelines of the Ethics Committee of Nanhai District People’s Hospital of Foshan.

The inclusion criteria were as follows: 18-45 years old, being female, and any education level. Based on the diagnostic criteria for gestational diabetes (2014), during the 24-28th week of gestation, the 75 g oral glucose tolerance test (OGTT) was used to measure each patient’s blood glucose levels before, 1 h after, and 2 h after consuming sugar. If the patient’s blood glucose level reached or exceeded 5.1, 10.0, or 8.5 mmol/L, respectively, they were deemed to have GDM and were eligible for inclusion in the GDM group.

The exclusion criteria were as follows: (1) History of chronic digestive system disorder; (2) history of treatment with GLP-1 analogues or GLP-1 receptor agonists; (3) history of cardiac, renal, or liver dysfunction; (4) multiple pregnancy; (5) pregnancy-induced hypertension syndrome, placental insufficiency, placenta previa, placental abruption, pelvic or soft birth canal abnormalities, or other pregnancy complications; (6) history of mental disorders; (7) exposure to a large amount of radiation, chemical poisons, or drugs that can affect the fetus during pregnancy; (8) tumor history or history of radiotherapy and chemotherapy within the past 6 mo; (9) participation in other research studies; (10) patients lost to follow-up due to various reasons, including the occurrence of other serious diseases during the study; and (11) consumption of antibiotics or probiotics within 1 mo prior to admission.

**Sample collection and testing**

Fresh fecal samples were collected from the participants and immediately frozen in a refrigerator at -80 °C. After collection of all samples, they were sent to the Treat Gut company for 16S rDNA sequencing.

Blood samples were collected after fasting and then 1 h and 2 h following consumption of sugar. Plasma glucose (Glu), glycosylated serum protein (GSP), low-density lipoprotein (LDL), uric acid (UA), hemoglobin (HB), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) were determined with a Beckman AU5800 fully automatic biochemical analyzer. Glycosylated hemoglobin A1c (HbA1c) was determined using an ADAMS™ A1c HA-8180 automatic glycosylated hemoglobin analyzer. Insulin (INS), thyroid-stimulating hormone (TSH), and free tetraiodothyronine (FT4) were measured with a Maglumi2000plus automatic chemiluminescence immunoanalyzer.
The active forms of GLP-1 in the plasma samples of patients with and without GDM were measured using a GLP-1 (active) ELISA kit (ELabscience, Wuhan, Hubei Province, China).

**Bacterial 16S rRNA gene sequencing**
The total genomic DNA of each sample was extracted using a fecal genomic DNA extraction kit (Tiangen Company). Sixteen S rDNA sequencing was performed by PCR amplification of V4 variable regions (39 to 297 base pairs), and a purified product library was established. The library construction steps followed the library construction method of the Illumina sequencing platform. The sequencing analysis was as follows. First, the Illumina Miseq 2 × 300bp paired-end sequencing data were analyzed. According to the barcode information, the samples were distinguished. Then, the data were merged, spliced, and filtered, and quality control analysis was conducted, including Q20 and Q30 scores. The final clean data were analyzed by operational taxonomic unit (OTU) cluster analysis and species taxonomy.

**Microbiome data**
The data were filtered using Mothur software and clustered into OTUs (species) at a similarity level of 97% using Quantitative Insights into Microbial Ecology (QIIME) software version 1.80[14]. Based on the OTU analysis, the Ace, Shannon, observed species, Simpson, Chao1, and J indices were calculated as alpha diversity metrics. To compare the microbial composition between the samples, beta diversity analysis was performed using principal component analysis (PCA) and principal coordinate analysis (PCoA). Analysis of similarities (ANOSIM) was applied to evaluate the statistical significance of differences between the groups. A linear discriminant analysis (LDA) effect size (LEfSe) method was employed to evaluate any differences in the gut microbe between the groups.

**Statistical analysis**
GraphPad Prism (version 7.0) and R version 3.0.2 (R Foundation for Statistical Computing) were using for statistical analyses. The measurement data are expressed as the mean ± SD. Differences between groups were analyzed using oneway ANOVA. The differences were considered statistically significant at \( P < 0.05 \). Random-forest classification was performed for discriminating the samples from different groups using the R package “random forest”. The model was employed for five-fold cross-validation of the relative species abundance profile. Case probabilities were calculated by drawing receiver operating characteristic (ROC) curves.

**RESULTS**

**Characteristics of the study population**
GDM was diagnosed in 35 women based on fasting or oral glucose-stimulated hyperglycemia, or a combination of the two. Markers of glucose and insulin homeostasis were higher in the GDM group compared with the NGT group (Table 1). Individuals with GDM also had higher hemoglobin A1C (\( P = 0.003 \)) and fasting blood glucose levels (\( P < 0.001 \)). There were no significant differences in pre-pregnancy body weight, BMI, UA, TCH, TG, HDL, LDL, TSH, or FT4 between the two groups.

**OTU distributions**
In this study, the OTUs annotated included 14 phyla, 62 families, and 214 genera of gut microbiota; the similarity among samples was 97% (Figure 1A). The total number of OTUs of the NGT group (at the 97% similarity level) was 652, and for the GDM group, it was 619. Venn diagram shows that 560 OTUs were shared by the NGT and GDM groups (Figure 1B).

**Alpha and beta diversities**
The observed species index of the GDM group was significantly difference from that of the NGT group (25; \( P = 0.044 \)). The Chao1 richness index of the GDM group was significantly different from that of the NGT group (43; \( P = 0.004 \)). The ACE index of the GDM group differed significantly from that of the NGT group (25; \( P = 0.0055 \)) (Figure 2). There were no significant differences in the Shannon, Simpson, or J indices between the GDM group and NGT group (Shannon, \( P = 0.65 \); Simpson, \( P = 0.9 \); J, \( P = 0.91 \); Figure 2A). PCA and PCoA indicated that the gut microbiota in GDM patients differed significantly from that of the NGT subjects. There was no difference in the gut microbiota structure between the groups (ANOSIM, \( r = 0.019 \), \( P = 0.2232 \)). NMDS cluster analysis indicated marked differences between the GDM patients and NGT subjects (Figure 2B).

**Taxonomy**
The composition of gut microbiota was different between the groups at the phylum, family, and genus levels. At the phylum level, **Bacteroidetes**, **Proteobacteria**, **Firmicutes**, **Verrucomicrobia**, **Actinobacteria**, **Fusobacteria**, **Synergistetes**, and **Tenericutes** were common phyla in both the GDM group and NGT group,
Table 1 Clinical variables of gestational diabetes mellitus patients and healthy controls

| Variable          | Control (25) | GDM (31) | P value |
|-------------------|--------------|----------|---------|
| Age (yr)          | 28.42 (3.11) | 30.18 (3.26) | 0.055   |
| Pre-body weight (kg) | 52.42 (7.68) | 55.18 (6.48) | 0.165   |
| Height (cm)       | 160.13 (5.44) | 157.88 (4.10) | 0.063   |
| Pre-BMI           | 20.73 (2.85) | 22.2 (2.37) | 0.054   |
| GLU (mmol/L)      | 4.49 (0.38) | 5.77 (0.95) | 3.53 × 10⁻⁻⁷ |
| GLP-1 0 h (ug/L)  | 67.72 (22.89) | 75.45 (23.23) | 0.223   |
| GLP-1 1 h (ug/L)  | 75.33 (26.14) | 84.34 (19.84) | 0.099   |
| GLP-1 2 h (ug/L)  | 71.75 (23.83) | 79.21 (24.20) | 0.312   |
| OGTT 0 h (mmol/L) | 4.47 (0.39) | 5.74 (0.99) | 3.53 × 10⁻⁻⁷ |
| OGTT 1 h (mmol/L) | 7.77 (1.55) | 11.23 (2.95) | 3.00 × 10⁻⁶ |
| OGTT 2 h (mmol/L) | 6.26 (1.06) | 9.64 (3.12) | 3.96 × 10⁻⁶ |
| Insulin 0 h (uIU/mL) | 9.78 (3.41) | 14.03 (15.93) | 0.239   |
| Insulin 1 h (uIU/mL) | 85.85 (43.99) | 64.52 (39.67) | 0.061   |
| Insulin 2 h (uIU/mL) | 57.51 (39.36) | 66.52 (45.21) | 0.675   |
| GSP (mmol/L)      | 1.68 (0.35) | 1.97 (0.62) | 0.054   |
| HbA1c (%)         | 5.04 (0.30) | 5.79 (1.12) | 0.003   |
| UA (umol/L)       | 279.52 (68.47) | 263.80 (81.27) | 0.463   |
| TCH (mmol/L)      | 5.40 (1.06) | 5.35 (1.08) | 0.993   |
| TG (mmol/L)       | 1.82 (0.72) | 2.45 (1.54) | 0.051   |
| HDL (mmol/L)      | 2.11 (0.52) | 2.08 (0.63) | 0.946   |
| LDL (mmol/L)      | 2.41 (0.89) | 2.34 (0.82) | 0.739   |
| TSH (uIU/mL)      | 1.82 (3.43) | 1.22 (1.24) | 0.337   |
| FT4 (pg/mL)       | 11.39 (3.37) | 11.77 (1.96) | 0.598   |

OGTT: Oral glucose tolerance test; GDM: Gestational diabetes mellitus; GLP-1: Glucagon-like peptide-1; Glu: Glucose; GSP: Glycosylated serum protein; HbA1c: Hemoglobin A1c; UA: Uric acid; TCH: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TSH: Thyroid-stimulating hormone; FT4: Free tetraiodothyronine.

accounting for 98.81% and 98.58% of the gut bacteria of each group, respectively (Figure 3). The GDM group had a lower abundance of Firmicutes (31.4% vs 33.2%), Verrucomicrobia (0.18% vs 1.01%, P < 0.05), Synergistetes (0.003% vs 0.110%, P < 0.01), and Tenericutes (0.05% vs 0.08%), and a higher abundance of Bacteroidetes (63.50% vs 60.81%), Proteobacteria (3.03% vs 2.56%), and Fusobacteria (0.33% vs 0.29%), compared to the NGT group. The ratio of Firmicutes to Bacteroidetes was decreased in the GDM group compared to the NGT group (0.49 vs 0.54).

At the family level, a greater number of different families were identified between the two groups (Figure 4). Fifty-five and 54 of the dominant families were detected in the GDM group and NGT group, respectively. Bacteroidaceae (phylum Bacteroidetes), Prevotellaceae (phylum Bacteroidetes), Acidaminococcaceae (phylum Firmicutes), Veillonellaceae (phylum Firmicutes), Lachnospiraceae (phylum Firmicutes), Ruminococcaceae (phylum Firmicutes), Enterobacteriaceae (phylum Proteobacteria), and Tannerellaceae (phylum Bacteroidetes) had the highest relative abundance in the GDM group, while Bacteroidaceae, Prevotellaceae, Acidaminococcaceae, Veillonellaceae, Lachnospiraceae, Enterobacteriaceae, Ruminococcaceae, and Rikenellaceae were the eight most abundant families in the NGT group.

The bacterial taxa whose levels differed significantly between the two groups were identified by LEfSE analysis (Figure 4). At the family level, Atopobiaceae, Eggerthellaceae, Streptococcaceae, Christensenellaceae, Clostridiaceae, Bifidobacteriaceae, Lachnospiraceae, and Ruminococcaceae were significantly more abundant in the NGT group than in the GDM group.

At the genus level, bacterial genera exhibited significant differences between the two groups (Figure 5). In the NGT group, Bacteroides (phylum Bacteroidetes), Prevotella_9 (phylum Bacteroidetes), Phascolarctobacterium (phylum Firmicutes), Megasphaera (phylum Firmicutes), Meganuma (phylum Firmicutes), Lachnospiraceae (phylum Firmicutes), Escherichia-Shigella (phylum Proteobacteria), and
Prevotella_2 (phylum Bacteroidetes) were the eight most dominant genera. The eight most dominant genera in the GDM group were Bacteroides (phylum Bacteroidetes), Prevotella_9 (phylum Bacteroidetes), Megamonas (phylum Firmicutes), Phascolarctobacterium (phylum Firmicutes), Lachnospiraceae (phylum Firmicutes), Megasphaera (phylum Firmicutes), Prevotella_2 (phylum Bacteroidetes), and Parabacteroides (phylum Bacteroidetes).

Ruminococcaceae_UCG-002, Ruminococcaceae_UCG-005, Clostridium_sensu_stricto_1, and Streptococcus were more abundant in the NGT group than in the GDM group ($P < 0.05$). Bacteroides and Lachnobacterium were more abundant in the GDM group than in the NGT group ($P < 0.05$). Prevotella_9, Oscillibacter, Roseburia, and Faecalibacterium were slightly more abundant in the NGT group than in the GDM group.

Functional profiling of the gut microbiome

The cluster of orthologous groups (COG) categories and KEGG pathways were compared between the GDM and NGT groups. Figure 6A shows that three functional KEGG pathways differed between the GDM group and NGT group, including the glycosphingolipid biosynthesis-globo series, synthesis and degradation of ketone bodies, and renal cell carcinoma pathways. Figure 6B shows that 20 COG categories differed between the GDM group and NGT group, including the phosphotransferase system, galactitol-specific IIC component, metal-dependent proteases with possible chaperone activity, uncharacterized protein, homolog of phage Mu protein gp30, uncharacterized protein conserved in bacteria, Acyl-CoA dehydrogenases, putative virion core protein (lumpy skin disease virus), predicted phosphohydrolase, large-conductance mechanosensitive channel, uncharacterized conserved protein, uncharacterized protein predicted to be involved in DNA repair, predicted permease, DMT superfamily, nicotinic acid mononucleotide adenylyltransferase, amidases related to nicotinamidase, histone acetyltransferase, plasmid maintenance system antidote protein, uncharacterized conserved protein, DNA polymerase III, alpha subunit, uncharacterized protein conserved in bacteria, antirestriction protein, NA polymerase III, and alpha subunit (Gram-positive type).

Correlations between blood biochemical traits and gut composition

Spearman’s correlation analysis was performed to identify whether the different dominant genera were associated with blood biochemical traits in the second trimester of pregnancy (Figure 7). Paraprevotella, Roseburia, Faecalibacterium, and Ruminococcaceae_UCG-002 were negatively correlated with Glu ($P < 0.05$). Ruminococcaceae_UCG-002 was negatively correlated with HbA1c ($P < 0.05$). Clostridium_sensu_stricto_1, Desulfovibrio, and (Ruminococcus)_torques_group were negatively correlated with pre-pregnancy body weight ($P < 0.05$). Phascolarctobacterium was negatively correlated with HDL ($P < 0.05$). Ruminococ-
Figure 2 Gut microbiota alpha and beta diversity indices in patients with gestational diabetes mellitus. A: Gut microbiota alpha diversity indices in patients with gestational diabetes mellitus. The Observed_species, ACE, Chao1, Simpson, Shannon, and J values are shown; B: Principal component analysis score plot based on the relative abundance of operational taxonomic units (97% similarity levels); C: Principal coordinate analysis; D: Similarities analysis; E: Non-metric multidimensional scaling. NGT: Normal glucose tolerance; GDM: Gestational diabetes mellitus; PCoA: Principal coordinate analysis; PCA: Principal component analysis.

cocae, UCG-003 and Faecalibacterium were negatively correlated with height (P < 0.05). Lachnoclostridium and Lachnospiraceae_NK4A136_group were positively correlated with age (P < 0.05). Bacteroides was significantly positively correlated with Glu (P < 0.01). Sutterella, Oscillibacter, and Bifidobacterium were positively correlated with GLP-1 (P < 0.05).
Liang YY et al. Correlation between gut microbiota and GLP-1

**Figure 3** Taxonomy. A: Top eight abundant species at the phylum level; B: Different bacteria were compared between each group at the phylum level. NGT: Normal glucose tolerance; GDM: Gestational diabetes mellitus.

*Roseburia* was negatively correlated with OGTT (0 h), OGTT (1 h), and OGTT (2 h) ($P < 0.05$). *Faecalibacterium* was negatively correlated with OGTT (0 h) and OGTT (1 h) ($P < 0.05$). *Bacteroides* was positively correlated with OGTT (0 h), OGTT (1 h), OGTT (2 h), and GSP ($P < 0.05$). *Lachnoclostridium* was positively correlated with OGTT (1 h) and OGTT (2 h) ($P < 0.05$). *Sutterella* was positively correlated with GLP-1(0 h), GLP-1(1 h), GLP-1(2 h), and pre-pregnancy BMI ($P < 0.05$). *Oscillibacter* was positively correlated with GLP-1(0 h), GLP-1(1 h), and GLP-1(2 h) ($P < 0.05$). *Bifidobacterium* was positively correlated with GLP-1(0 h), GLP-1(1 h), OGTT (2 h), TG, and TCH ($P < 0.05$).

**Gut microbiota–based prediction of GDM**

Finally, random forest models were used to assess the ability of the genera abundance profiles to predict GDM status (**Figure 8A**). Twenty genera plus Glu provided the best discriminatory power, as indicated by the area under the receiver operating characteristic (AUROCC) value of 0.94. The value was higher than that achieved with a model including just 20 genera (the best AUC was 0.828) (**Figure 8B**). Further, models with 20 genera plus GLP-1, INS, or HbA1c had lower AUROCC values than the model with 20 genera plus Glu. The AUROCC values were 0.81, 0.8288, and 0.8502, respectively.

**DISCUSSION**

In recent years, the relationships between the gut microbiota and diabetes as well as other endocrine diseases have become research hotspots. Similarly, the characteristics of the gut microbiota among pregnant women with GDM have received widespread research attention. To date, research on GDM has focused on the correlation between the gut microbiota and blood glucose or insulin, but there is still a lack of research on the relationship between the gut microbiota and GLP-1. Many studies have reported that GLP-1 is closely related to the gut microbiota and short-chain fatty acids (SCFAs)[15-17], and changes in the gut microbiota directly affect the secretion of GLP-1, which, in turn, affects insulin and blood glucose. These are closely related to the occurrence of GDM. Therefore, we focused on the relationship between GLP-1 and the gut microbiota in GDM patients. To the best of our knowledge, it is the first report on the relationship between GLP-1 and the gut microbiota in patients with GDM.
Figure 4 LEfSE analysis to determine which bacterial taxa differ significantly between the groups. A: At the family level, a greater number of different families were identified between the normal glucose tolerance (NGT) and gestational diabetes mellitus groups (GDM); B: The bacterial taxa whose levels differed significantly between the NGT and GDM groups were identified by LEfSE analysis. NGT: Normal glucose tolerance; GDM: Gestational diabetes mellitus; LDA: Linear discriminant analysis.
Figure 5 Bacterial genera exhibit significant differences between the two groups. A: Heatmap showing the relative total abundance of the first 30 genera; B: Microbial community at the genus level between groups. *P < 0.05, GDM group compared with NGT group. NGT: Normal glucose tolerance; GDM: Gestational diabetes mellitus.

At the phylum level, the abundance of *Firmicutes* in the gut microbiota of the GDM group was lower than that in NGT group. *Firmicutes* are known to transform carbohydrates and undigested proteins into SCFAs, producing energy for the host organism. As a crucial SCFA, butyrate participates in the activation of multiple physiological signal pathways, including the proliferation and differentiation of regulatory T cells and anti-inflammatory activities[18,19]. Moreover, the GDM group exhibited reduced phylum levels of *Verrucomicrobia*, *Synergistetes*, and *Tenericutes* compared to the NGT group. Mucin-degrading bacteria *Verrucomicrobia* contribute to glucose homeostasis and intestinal health, and play a
key role in the interaction between the host tissues and gut microbiome[20]. The gut microbiota in the GDM patients also exhibited a higher abundance of Bacteroidetes, Fusobacteria, and Proteobacteria compared to healthy subjects. Proteobacteria is an opportunistic pathogen that creates a major structural imbalance in the gut microbiota of GDM patients. The ratio of Firmicutes to Bacteroidetes in GDM patients is lower than that of NGT individuals.

At the family level, Atopobiaceae, Eggerthellaceae, Streptococcaceae, Christensenellaceae, Clostridiaceae, Bifidobacteriaceae, Lachnospiraceae, and Ruminococcaceae were more abundant in the NGT subjects than in the GDM patients. Zhang et al[21] reported that Ruminococcaceae, Bifidobacteriaceae, Christensenellaceae, Erysipelotrichaceae, Peptostreptococcaceae, and Eggerthellaceae were more abundant in the NGT subjects, which is consistent with the current study. In line with the study of Ma et al[22], the current results revealed that Ruminococcaceae were more abundant in the NGT group than in the GDM group. However, other studies have observed the opposite result[23,24]. The mechanisms remain unclear.

At the genus level, Ruminococcaceae_UCG-002, Ruminococcaceae_UCG-005, Clostridium_sensu_stricto_1, and Streptococcus were more abundant in the NGT group than in the GDM group. Bacteroides and Lachnoclostridium were more abundant in the GDM group than in the NGT group. Kuang et al[25] found that the proportion of Bifidobacterium in the gut microbiota of GDM pregnant women was significantly reduced, while the proportions of Bacteroides and Klebsiella were significantly increased. Liu et al[26] found that compared with normal pregnant women, the proportion of Faecalis in GDM patients was significantly lower, while the proportion of Prevotella was significantly higher. In the present study, there were no significant differences in Bifidobacterium, Klebsiella, or Prevotella between the GDM group and NGT group. Ma et al found that Ruminococcaceae_UCG-002 and Ruminococcaceae_UCG-005 were reduced in women with GDM[22].

Figure 6 KEGG pathways and COG categories between the GDM and NGT groups. A: KEGG pathway; B: COG categories. NGT: Normal glucose tolerance; GDM: Gestational diabetes mellitus.
Figure 7 Spearman’s correlations between different dominant genera and blood biochemical traits. *P < 0.05, **P < 0.01. GDM: Gestational diabetes mellitus; GLP-1: Glucagon-like peptide-1; GSP: Glycosylated serum protein; Glu: Glucose; HbA1c: Hemoglobin A1c; UA: Uric acid; TCH: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; INS: Insulin; OGTT: Oral glucose tolerance test; TSH: Thyroid-stimulating hormone; FT4: Free tetraiodothyronine.

One study found that supplementation with *Lactobacillus rhamnosus* in pregnant women may reduce the prevalence of GDM[27]. Another study showed that additional probiotic supplementation from pregnancy to 12 months post-delivery can reduce insulin levels and improve insulin sensitivity[28]. In this study, to identify beneficial bacteria for pregnant women, Spearman’s correlation analysis was performed to identify the relationships between bacterial genera and blood biochemical traits. *Paraprevotella*, *Roseburia*, *Faecalibacterium*, and *Ruminococcaceae_UCG-002* were negatively correlated with Glu. *Ruminococcaceae_UCG-002* was negatively correlated with HbA1c. *Clostridium_sensu_stricto_1*, *Desulfovibrio*, and *Ruminococcus_torques_group* were negatively correlated with pre-pregnancy body weight. *Phascolarctobacterium* was negatively correlated with HDL. *Ruminococcaceae_UCG-003* and *Faecalibacterium* were negatively correlated with height. *Lachnoclostridium* and *Lachnospiraceae_NK4A136_group* were positively correlated with age. Zhang *et al* [21] found that *Ruminococcaceae_UCG-002* was negatively correlated with fasting blood glucose levels. In the study of Crusell, *Clostridium (sensu stricto)* was positively correlated with gestational weight[29]. To the best of our knowledge, no studies have yet reported on the relationships between *Paraprevotella*, *Roseburia*, and *Faecalibacterium* and Glu in GDM. The current findings suggest that these genera are crucial for controlling blood glucose-related indices and may be beneficial for GDM treatment.

GLP-1 and its receptor agonist can promote insulin secretion only when the blood glucose level is elevated[30]. This safety feature makes GLP-1 and its agonist suitable for the treatment of GDM, which requires strict maintenance of blood glucose levels and stable, safe blood glucose regulation. Thus, the current study aimed to examine the correlations between the gut microbiota and GLP-1 levels, and...
Liang YY et al. Correlation between gut microbiota and GLP-1

Figure 8 Gut microbiota-based prediction of gestational diabetes mellitus. A: Identification of gestational diabetes mellitus (GDM) markers by random forest models; B: Receiver operating characteristic (ROC) curves of operational taxonomic units-based diagnostic biomarkers for GDM. NGT: Normal glucose tolerance; GDM: Gestational diabetes mellitus; INS: Insulin; Glu: Glucose; HbA1c: Hemoglobin A1c; GLP-1: Glucagon-like peptide-1.

identify beneficial bacteria that can improve the expression of GLP-1 in patients with GDM, so as to more safely control blood glucose. In the present study, Sutterella was significantly positively correlated with GLP-1 (0 h), GLP-1 (1 h), GLP-1 (2 h), and pre-pregnancy BMI. Wang et al.[31] reported that subjects taking metformin exhibited a significantly increased relative abundance of Sutterella, whereas liraglutide dosing was associated with a significant increase in the genus Akkermansia. Another study showed that Sutterella was associated with C-reactive protein levels[32]. In the current study, Oscillibacter was significantly positively correlated with GLP-1 (0 h), GLP-1 (1 h), and GLP-1 (2 h). One study reported that Cyclocarya paliurus polysaccharides alleviated type 2 diabetic symptoms by increasing eleven SCFA-producing species, including Oscillibacter_valericigenes and Oscillibacter_ruminantium[33]. Oscillibacter belongs to the Clostridia class of Firmicutes, and in the human gut microbiota, this bacterium grows fermentatively, predominantly producing valerate when grown using glucose as a carbon source[34]. In the current study, Bifidobacterium was significantly positively correlated with GLP-1 (0 h), GLP-1 (1 h), TG, TCH, and OGTT (2 h). In the study of Zhao et al[35], Bifidobacterium longum DD98 improved the serum and intestinal cell GLP-1 levels, which protected pancreatic β-islet cells from damage induced by type 2 diabetes. To the best of our knowledge, this is the first report on the associations between GLP-1 and genera such as Sutterella, Oscillibacter, and Bifidobacterium in GDM.

CONCLUSION

In summary, this study contributes to a better understanding of the relationships between the gut microbiota and blood biochemical traits, particularly GLP-1, in individuals with GDM. The current findings suggest that some genera are crucial for controlling blood glucose-related indices and may be beneficial for GDM treatment. Alteration in the microbial composition of the gut may potentially serve as a marker for identifying individuals at risk of GDM. Future studies combining metagenomics and metabolomics would be of value for improving our understanding of the roles of specific strains and metabolites in patients with GDM and supporting precise prevention and intervention strategies for GDM.

ARTICLE HIGHLIGHTS

Research background

Gestational diabetes mellitus (GDM) places both the mother and offspring at high risk of complications. Increasing evidence suggests that the gut microbiota plays a role in the pathogenesis of GDM.
Research motivation
To confirm whether the gut microbiota is related to blood biochemical traits, particularly glucagon-like peptide-1 (GLP-1), in GDM patients.

Research objectives
To explore the correlation between the gut microbiota and blood biochemical traits.

Research methods
The V4 region of the 16S ribosomal ribonucleic acid (rRNA) gene was sequenced based on the fecal samples of 35 pregnant women with GDM and was compared to that of 25 pregnant women with normal glucose tolerance (NGT).

Research results
The results showed that Ruminococcaceae_UCG-002, Ruminococcaceae_UCG-005, Clostridium_sen-su_stricto_1, and Streptococcus were more abundant in the NGT group than in the GDM group. Bacteroides and Lachnoclostridium were more abundant in the GDM group than in the NGT group. Spearman’s correlation analysis was performed to identify the relationships between bacterial genera and blood biochemical traits. Paraprevotella, Roseburia, Faecalibacterium, and Ruminococcaceae_UCG-002 were significantly negatively correlated with glucose. Ruminococcaceae_UCG-002 was significantly negatively correlated with HbA1c. Bacteroides was significantly positively correlated with glucose. Sutterella, Oscillibacter, and Bifidobacterium were significantly positively correlated with GLP-1. A random forest model showed that 20 specific genera plus glucose provided the best discriminatory power, as indicated by the area under the receiver operating characteristic curve (0.94).

Research conclusions
The results of this study reveal novel relationships between the gut microbiome, blood biochemical traits, particularly GLP-1, and GDM status.

Research perspectives
These findings suggest some genera are crucial for controlling blood glucose-related indices and may be beneficial for GDM treatment. Alteration in the microbial composition of the gut may potentially serve as a marker for identifying individuals at risk of GDM.

FOOTNOTES
Author contributions: Liang YY, Liu LY, and Jia Y designed the study, collected the samples, compiled the data, and drafted and critically revised the manuscript; Li Y, Cai JN, and Shu Y compiled the data and performed the statistical analysis and data interpretation; Tan JY collected the samples and compiled the data; Chen PY critically revised the manuscript; Cai HH and Cai XS designed this study, collected the samples, and drafted and critically revised the manuscript; all authors contributed to the article and approved the submitted version.

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Conflict-of-interest statement: The authors declare that no competing interest exists.

Data sharing statement: Illumina sequencing reads were uploaded to the SRA under accession number PRJNA11381. The rest of the data that support the conclusions of this study are available from the corresponding author upon request.

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