Gut Microbiota as an Epigenetic Regulator: Pilot Study Based on Whole-Genome Methylation Analysis

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ABSTRACT The core human gut microbiota contributes to the developmental origin of diseases by modifying metabolic pathways. To evaluate the predominant microbiota as an epigenetic modifier, we classified 8 pregnant women into two groups based on their dominant microbiota, i.e., Bacteroidetes, Firmicutes, and Proteobacteria. Deep sequencing of DNA methylomes revealed a clear association between bacterial predominance and epigenetic profiles. The genes with differentially methylated promoters in the group in which Firmicutes was dominant were linked to risk of disease, predominantly to cardiovascular disease and specifically to lipid metabolism, obesity, and the inflammatory response. This is one of the first studies that highlights the association of the predominant bacterial phyla in the gut with methylation patterns. Further longitudinal and in-depth studies targeting individual microbial species or metabolites are recommended to give us a deeper insight into the molecular mechanism of such epigenetic modifications.

IMPORTANTX Epigenetics encompasses genomic modifications that are due to environmental factors and do not affect the nucleotide sequence. The gut microbiota has an important role in human metabolism and could be a significant environmental factor affecting our epigenome. To investigate the association of gut microbiota with epigenetic changes, we assessed pregnant women and selected the participants based on their predominant gut microbiota for a study on their postpartum methylation profile. Intriguingly, we found that blood DNA methylation patterns were associated with gut microbiota profiles. The gut microbiota profiles, with either Firmicutes or Bacteroidetes as a dominant group, correlated with differential methylation status of gene promoters functionally associated with cardiovascular diseases. Furthermore, differential methylation of gene promoters linked to lipid metabolism and obesity was observed. For the first time, we report here a position of the predominant gut microbiota in epigenetic profiling, suggesting one potential mechanism in obesity with comorbidities, if proven in further in-depth studies.
Gut microbiota composition associates with promoter DNA methylation status of genes associated with lipid metabolism, obesity, and inflammation. Pathway analysis revealed that the most significant functional network altered in the HighBact group was linked to cardiovascular diseases, together with gene expression and cell morphology functions (score of 43; Fisher exact test, \( P = 1 \times 10^{-43} \)) (see Table S2 in the supplemental material). In addition, differentially methylated genes were enriched in other functional networks, including the inflammatory response, metabolic pathways, and diseases like cancer, mostly affecting the gastrointestinal system (312 molecules, \( P < 0.05 \)). As the Bacteroidetes/Firmicutes ratio was associated with obesity-related comorbidities, the cardiovascular disease risk network was further expanded, and associations with lipid metabolism (72 genes), inflammatory response (85 altered genes), and obesity (23 altered genes) were found (Fig. 2B). Consistent with these results, the gene SCDS5, which had the greatest difference between the two groups (fold change, 6.239; \( P = 0.00005 \)), encodes primate-specific stearoyl-CoA desaturase, which has a key function in the catalysis of monounsaturated fatty acids from saturated fatty acids. The promoter region of SCDS5 was more methylated in the HighFirm group and had an undetectable methylation in the HighBact group. LPS (\( P = 0.00208 \)) was one of the upstream regulators of genes identified in the network (see Table S3), which further strengthens the role of microbial molecules in epigenetic modifications.

Some of the epigenetically regulated genes include the genes encoding USF1 (\( P = 0.00805 \)), ACOT7 (\( P = 0.035 \)), ASA2H2 (\( P = 0.0367 \)), TAC1 (\( P = 0.00972 \)), and LMNA (\( P = 0.03081 \)). USF1 is one of the key regulators of fatty acid synthase (FAS) and is also a key enzyme in lipogenesis (14). USF1 and LMNA have also been linked with the onset of coronary heart disease (15, 16). The expression of ACOT and microRNAs 103/107 was also found to be upregulated in obese rats and mice, respectively (17, 18). Similarly, gut microbiota or its metabolites are directly linked to obesity and associated metabolic pathways. However, the association with epigenetic regulation of these genes should be further confirmed by quantitative PCR (qPCR) and in vitro experiments.

These findings are consistent with previous studies, which have linked higher levels of Firmicutes to the development of overweight, obesity, higher energy extraction, and metabolic functions, including lipid metabolism. Additionally, deviant gut microbiota composition could also be one of the risk factors which may contribute to metabolic syndrome. Our findings are novel, but due to the small sample size, larger studies and interventions, and possibly animal experiments also, are required to assess the mechanisms. Nonetheless, this approach is intriguing and could offer a new basis for prevention and treatment strategies involving the gut microbiota and its impact on long-term genomic modifications.

**Microbial phylum comparisons.** All the OTU tables were retrieved from our earlier study (10). The percentages of relative abundance for all phyla were used to compare the mothers (divided into two groups). Statistical package SPSS was used for the
test and to make box plots of the percentages of relative abundance.

DNA methylome analysis. The DNA methylome analysis was carried out from 5 μg of genomic DNA that was extracted from EDTA blood with a QIAamp DNA blood maxikit (Qiagen) and fragmented into an average size of 150 bp with a Covaris S2 sonicator. Methylated DNA was enriched with a MethylMiner methylated DNA enrichment kit (Invitrogen) by following the high-salt (2 M NaCl), single-elution workflow. The sequencing libraries were prepared from 500 ng of enriched DNA with a SOLiD fragment library construction kit (Life Technologies), and the SOLiD fragment library barcoding kit module 1-16 (Life Technologies) was used for multiplexing. The libraries were purified (AMPure XP beads; Agencourt) and size selected (150 to 300 bp) from 1% agarose gels (QIAquick gel extraction kit; Qiagen). The bead preparation was carried out according to the SOLiD 4 System Templated Bead Preparation Guide. The SOLiDEZ bead system was used for automated templated bead preparation. The libraries were sequenced with a SOLiD 4 or SOLiD 5500XL sequencer (Life Technologies) by using 50-bp chemistry.

Methylation sequencing data analysis. The raw sequence data were mapped to hg19 reference genome sequences with Life Technologies Bioscope (version 2.0) software using the default parameters, yielding on average 56.7 M mapped reads per sample (standard deviation, 14.26 M reads). The read counts for proximal promoters (region between 1,000 bp upstream and 500 bp downstream from the transcription start site, coordinates derived from RefSeq gene annotations) were calculated using bedtools (version 2.17.0). Statistical analysis for comparing differentially methylated promoters between sample groups was carried out using R/Bioconductor limma package on TMM-normalized and voom-transformed count values as suggested in the limma manual (19, 20). The promoters with absolute fold changes above 2 and \( P \) values below 0.05 were listed as significantly differentially methylated.

SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.02113-14/-/DCSupplemental.

Table S1, DOC file, 0.1 MB.
Table S2, XLS file, 0.3 MB.
Table S3, XLS file, 0.1 MB.
Table S4, PDF file, 2.1 MB.

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