



The Impact of Gut Microbiome on Metabolic Disorders During Catch-Up Growth in Small-for-Gestational-Age

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Objective: Catch-up growth (CUG) in small for gestational age (SGA) leads to increased risk of metabolic syndrome and cardiovascular diseases in adults. It remains unclear if microbiota could play an important role in CUG-SGA independent of genetic or nutritional factors. The present study explored the role of gut microbiota in, and its association with, metabolic disorders during CUG-SGA.

Methods: An SGA rat model was established by restricting food intake during pregnancy, and the rats were divided into catch-up growth (CUG-SGA) and non-catch-up growth (NCUG-SGA) groups based on body weight and length at the fourth postnatal week. High-throughput sequencing of 16S rRNA was conducted to detect the diversity and composition of the gut microbiota. Fecal short-chain fatty acids (SCFAs) were detected by gas chromatography-mass spectrometry. Transcriptome sequencing of liver tissue was performed and verified using real-time PCR. Concentrations of insulin and total cholesterol were determined using enzyme-linked immunosorbent assay.

Results: The composition of gut microbiota in CUG-SGA rats differed from that of NCUG-SGA rats, with reduced abundance of *Lactobacillus* in the CUG-SGA group. The decrease in *Lactobacillus* was significantly associated with increased body weight and upregulated insulin and total cholesterol levels. Five SCFAs and two branched chain fatty acids were significantly higher in the CUG-SGA group than in the NCUG-SGA group. Additionally, SCFAs were positively associated with clinical indices such as weight, body mass index, insulin, and total cholesterol. Transcriptomic data revealed that insulin-like growth factor-2 expression was significantly decreased in CUG-SGA rats and was associated with a decrease in *Lactobacillus* bacteria.

Conclusion: *Lactobacillus* and SCFAs were associated with the metabolic disorders during CUG in SGA. Gut microbiome may play a certain role on metabolic disorders during catch-up growth in small-for-gestational-age.

Keywords: catch-up growth, gut microbiome, *Lactobacillus*, short-chain fatty acids, small for gestational age

INTRODUCTION

Small for gestational age (SGA) refers to newborns whose birth weight and/or length are at least 2 standard deviations (SDs) below the mean for the gestational age (≤ -2 SD) (1). SGA is a common medical problem with a worldwide incidence of 2.3%–10% (2). Newborns with SGA often require additional medical care, including temperature-controlled incubators, tube feeding, and monitoring of blood glucose levels. With advances in medical care, the mortality rate of newborns with SGA has significantly decreased. However, survivors are at increased risks of developing metabolic syndrome and cardiovascular diseases in adulthood compared with infants with normal birth weight (3, 4).

About 85%–90% of infants with SGA demonstrate catch-up growth (CUG) during the first two years of life (5). Worldwide epidemiological data have revealed that CUG of SGA infants may contribute to insulin resistance (6). Eriksson et al found that some SGA infants caught up rapidly after birth, reaching or exceeded the normal body mass index level of children of the same age by 7 years, but with a high death rate associated with cardiovascular disease (7). A cohort study in India found similar results. They found that insulin resistance indicators, total lipoproteins, and low-density lipoproteins were higher in such group of infants (8). However, the relevant mechanism remains unclear.

In the last two decades, our understanding of the causes of SGA has significantly improved, although it still remains limited. For example, fetal genetic deficiencies in the growth hormone/insulin-like growth factor-1 axis have been discovered in SGA (9, 10). Several single nucleotide polymorphisms have been associated with diabetes or obesity in patients born with SGA (9, 11) and maternal placental gene mutations can also cause SGA (12). In addition to genetic factors, other factors such as environmental factors can cause SGA, although the mechanism remains elusive. Whether the intestinal microbiota can impact the host's metabolic activity and contribute to SGA also remains unclear.

In recent years, gut microbiota has been found to be an important environmental factor in the regulation of host metabolism and to significantly impact body weight. Studies have found that the fat content of conventionally raised mice is 40% higher than that of Germ free (GF) mice, independent of food intake (13). When the intestinal microbiota of normal mice is transplanted into GF mice, it changes the body fat composition of the latter (13, 14), suggesting that the intestinal microbiota is involved in regulating the host's ability to obtain energy from the diet. Another study found that transplanting the intestinal microbiota of lean donors into patients with metabolic syndrome improved their insulin sensitivity, implying that the intestinal microbiota is involved in the control of body weight and insulin resistance (15). The intestinal microbiota is involved in the metabolism and transformation of a variety of nutrients, including carbohydrates, proteins, and fats. Studies have found that the intestinal microbiota transforms the indigestible carbohydrates into monosaccharides and short-chain fatty acids (SCFAs). Meanwhile, SCFAs can be used for de novo

synthesis of lipids and glucose, thereby affecting the host's glucose and lipid metabolism (16). A cross-sectional study found that the fecal SCFA concentration of overweight or obese subjects is significantly higher than that of thinner controls, and higher SCFA levels have a good correlation with metabolic syndrome indicators (17–20). Studies on genetically obese mice have shown that the intestinal microbiota increases energy utilization by producing excessive SCFAs, thereby affecting the host's metabolism (14). Therefore, we hypothesized that the intestinal microbiota and its metabolites play an important role in host metabolism during the CUG stage in SGA.

We established an SGA rat model and divided these rats into two groups based on their body weight and length—catch-up growth (CUG-SGA) and without catch-up growth (non-catch-up growth, NCUG-SGA). Further, we compared the composition of gut microbiota in these groups. Since the liver is the major metabolic organ, to further verify our hypothesis, we sequenced the transcriptome of liver tissues to identify metabolism-related genes in CUG- and NCUG-SGA rats. The findings of our study will further our understanding of SGA in humans.

MATERIALS AND METHODS

Animal Model

A rat model of SGA was established in animals under maternal undernutrition during pregnancy as previously described (21, 22). In brief, adult SPF-grade Sprague-Dawley female ($n=15$) and male ($n=5$) rats were mated overnight. The pregnant rats were randomly divided into SGA group ($n=10$) and normal control group ($n=5$). Pregnant rats in the normal control group had standard food diet available *ad libitum* throughout pregnancy, while pregnant rats in the SGA group were under food restriction, with 50% reduction of food intake (about 10 g) from the first day after conception. Rats in both groups gave birth naturally. All newborns were breastfed for the first 3 weeks after birth, and then fed with standard chow. The litter size was culled to five pups per litter at birth in the SGA group and the normal control group, to ensure the catch-up growth of the offspring with SGA. Newborns were defined as SGA rats when the birth weight and length were -2 SD below those of the newborns in the normal control. Body weight and length were monitored weekly. NCUG-SGA rats were identified when the body weight and body length were -2 SD lower than those in the normal group at week 4 following parturition, and the rest were labelled as CUG-SGA rats. All procedures in this study were approved by the animal experiment ethics committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

Enzyme-Linked Immunosorbent Assay (ELISA)

Commercial ELISA kits were used to measure fasting insulin (Crystal Chem, Illinois, USA), IGF-1 (Crystal Chem, Illinois, USA), and total cholesterol (Zhuocai Biological, Shanghai, China), following the manufacturers' protocols.

Fecal Genomic DNA Extraction and 16S rRNA High-Throughput Sequencing

Five 4-week-old pups were randomly selected from each of the two groups (10 in total, all from separate cages), and their feces collected for 16S rRNA sequencing and SCFA quantification.

Fecal genomic DNA was extracted using TIA Namp Stool DNA Kit (Invitrogen, California, USA) according to the manufacturer's protocol. DNA concentration and integrity were determined using gel electrophoresis. PCR amplification of the bacterial 16S rRNA genes V3–V4 region was performed using the forward primer 520F (5'- AYTGGGYDTAAAGNG -3') and the reverse primer 802R (5'- TACNVGGGTATCTAATCC -3'). Thermal cycling consisted of initial denaturation at 98°C for 5 min, followed by 25 cycles consisting of denaturation at 98°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 45 s, with a final extension of 5 min at 72°C. PCR products were purified with AmpureXp beads (AGENCOURT), diluted, denatured into single-strands in NaOH buffer, and sequenced on an Illumina HiSeq2500 platform. The raw sequence reads were then processed based on sequence quality. After quality control, the raw reads were classified based on index and barcode, and clustered into operational taxonomic units using QIIME2 dada2 or Vsearch software (<https://docs.qiime2.org/2019.7/citation/>). Sequencing data analyses were mainly performed using QIIME2 and R packages (v3.2.0). ASV-level alpha diversity indices, such as Chao1 richness estimator, were calculated using the ASV table in QIIME2, by Kruskal-Wallis test, and visualized as box plots. Wilcoxon rank-sum test was utilized to detect differentially enriched microbiota at genus between NCUG-SGA and CUG-SGA groups ($P < 0.05$). Beta diversity analysis was performed to investigate the structural variation of microbial communities among different samples using Bray-Curtis metrics and visualized *via* principal coordinate analysis (PCoA). Non-parametric permutational multivariate analysis of variance (PERMANOVA) was conducted for analyzing significant difference of microbiota structure between the two groups. LEfSe (Linear discriminant analysis effect size) was performed to detect differentially abundant taxa in both groups (23). Microbial functions were predicted by PICRUSt2 (Phylogenetic investigation of communities by reconstruction of unobserved states) upon KEGG (<https://www.kegg.jp/>) databases (24).

Fecal SCFA Detection

Feces from the 4-week-old rats were thawed on ice, and approximately 10 mg of feces were homogenized in 50 μ l of 15% phosphoric acid. The suspensions were homogenized with a vortex and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatants were processed for gas chromatography-mass spectrometry on an Agilent 7890A/5975C mass spectrometer (Agilent Technologies, California, USA). The injection volume was 1 μ l, and the split ratio was 10:1. Samples were separated with an Agilent HP-INNOWAX capillary GC column (30 m \times 0.25 mm ID \times 0.25 μ m). The initial temperature was 90°C and was increased to 120°C at 10°C/min, after which the

temperature was increased to 150°C at 5°C/min and then to 250°C at 25°C/min, where it remained for 2 min. The carrier gas was helium (1.0 ml/min). The temperatures of the injection port and ion source were 250°C and 230°C respectively under SIM model. Agilent MSD ChemStation software was used for quantitative analysis of chromatographic peak area and retention time (25).

RNA Extraction, Library Construction, Transcriptome Sequencing and Bioinformatics Analysis

Rat liver tissues were stored in liquid nitrogen prior to homogenization with a pestle and total RNA was extracted using Trizol reagent (Invitrogen, California, USA) according to the manufacturer's instructions. RNA integrity was analyzed *via* gel electrophoresis using 1% agarose gel. RNA concentration and quality were measured using a Nanodrop 2000 spectrophotometer (Thermo, Massachusetts, USA). RNA samples (3 μ g) were used for library construction using a TruSeq RNA kit (Illumina, California, USA), following the manufacturer's protocol. Clone library was quality-checked using an Agilent 2100 Bioanalyzer (Agilent Technologies, California, USA) and processed for Paired-end sequencing using Next-Generation Sequencing technology on the Illumina MiSeq platform (Illumina, California, USA).

After sequencing, the images were first converted into raw data in FASTQ format and filtered using Cutadapt (v1.15) software to obtain high-quality reads (clean reads) and analyzed for Q20, Q30 and GC. The filtered clean reads were then mapped to the reference genome using the HISAT2 software (<http://ccb.jhu.edu/software/hisat2/index.shtml>) to obtain CDS (coding region), Intron (intron), Intergenic (intergenic region) and UTR (5' and 3' untranslated regions) sequences. Gene expression was calculated using the HTSeq Read Count and those with a normalized FPKM value > 1 were considered to be expressed. DESeq analysis of RNA-Seq data was set as \log_2 fold change > 1 or < -1 and P value < 0.05 . The volcano map of DEG was generated using the "ggplots2" package in R. DAVID software (6.8) was used for GO and KEGG pathway analysis of the differentially expressed genes. A protein-protein interaction (PPI) network was constructed *via* STRING (Search3 Tool for the Retrieval of Interacting Genes/Proteins), and visualized using Cytoscape software.

RNA-Seq Validation by qRT-PCR

To verify the accuracy of RNA-Seq, Igf2, Mmp14, and Hgf were selected from among the differentially expressed genes, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference. After being transcribed into cDNA, PCR was performed on a TIB8600 system (Taipu Biosciences, China) using a 2 \times SYBR real-time PCR premixture reagent. The primers were designed using Primer Premier 5.0 software and synthesized by Personalbio, Shanghai, China. The relative expression of each gene was calculated using the $2^{-\Delta\Delta C_t}$ method and data were expressed as the mean \pm SD. The specific primers used are shown in S1.

Statistical Analysis

GraphPad Prism 8.0 (GraphPad Software, California, USA) and SPSS 23.0 (IBM, Chicago, USA) were used for analyzing data and drawing charts. Normally distributed data was expressed as the mean \pm standard deviation ($X \pm SD$). Pairwise differences between groups were analyzed using *t* test. Correlation analysis was performed using Spearman correlation analysis. Correlation coefficient (*r*) values > 0.6 were considered to represent strong correlations. *P* values lower than 0.05 were considered significant: **p* ≤ 0.05 , ***p* ≤ 0.01 , ****p* ≤ 0.001 .

RESULTS

Characteristics of Rats With NCUG-SGA and CUG-SGA

We first characterized the SGA model in our study. Normal pups are born with normal body weight and length. As described in the methods above, SGA rats are defined as body weight and body length $-2SD$ below the rats from control group at birth, i.e. rats appropriate for gestational age at birth. There were 70 offspring of pregnant rats in the SGA group with restricted diet, including 8 normal weight pups and 62 SGA pups. To ensure catch-up growth of the SGA pups, the litter size was culled to five pups per litter at birth, a total of 50 SGA rats were left. All newborns were breastfed for the first 3 weeks after birth, and then fed standard food. Although given the same food, 26 SGA rats exhibited CUG while other 24 did not. NCUG-SGA rats were identified by week 4, when their body weights were $-2SD$ lower than those in the normal group, and the rest of the SGA rats were defined as CUG-SGA. There were significant differences in the body weight, length, and BMI between rats

with CUG-SGA and NCUG-SGA (Figures 1A–C). Thus, 4-week-old rats were used in further investigations.

Since CUG in SGA has been associated with increased insulin resistance and metabolic syndrome in humans, we measured if this is consistent with our rat model. Serological analysis showed that both fasting insulin (INS) and total cholesterol concentrations in CUG-SGA rats were higher than those in the NCUG-SGA rats (Figures 1D, E).

Differences in the Gut Microbiota Between Rats With NCUG-SGA and CUG-SGA as Revealed by 16S rRNA Sequencing

To compare the microbiota profiles between the NCUG-SGA and CUG-SGA rats, we performed 16S rRNA high-throughput sequencing on rat feces. We first compared the microbiota diversity of individual samples, as indicated by the α diversity index (Figure 2A). In both groups, we observed relatively diverse microbiota and variations within each group, without significant difference between NCUG-SGA and CUG-SGA. When we compared the microbiota between the two groups, as indicated by the β diversity index, gut microbiota clearly clustered separately in the NCUG-SGA and CUG-SGA groups, indicating that despite individual variations within the same group, the composition of the gut microbiota differed in these two groups (Figure 2B). We further ran the linear discriminant analysis (LDA) effect size algorithm (LEfSe) analysis to evaluate the classification difference of the microbiota between the NCUG-SGA and CUG-SGA groups (Figures 2D–F).

It is possible that the difference in microbiota could be the driving force for the differences observed in host growth and metabolism. Therefore, we used the relative abundance results to identify the genera of gut microbiota that are associated with

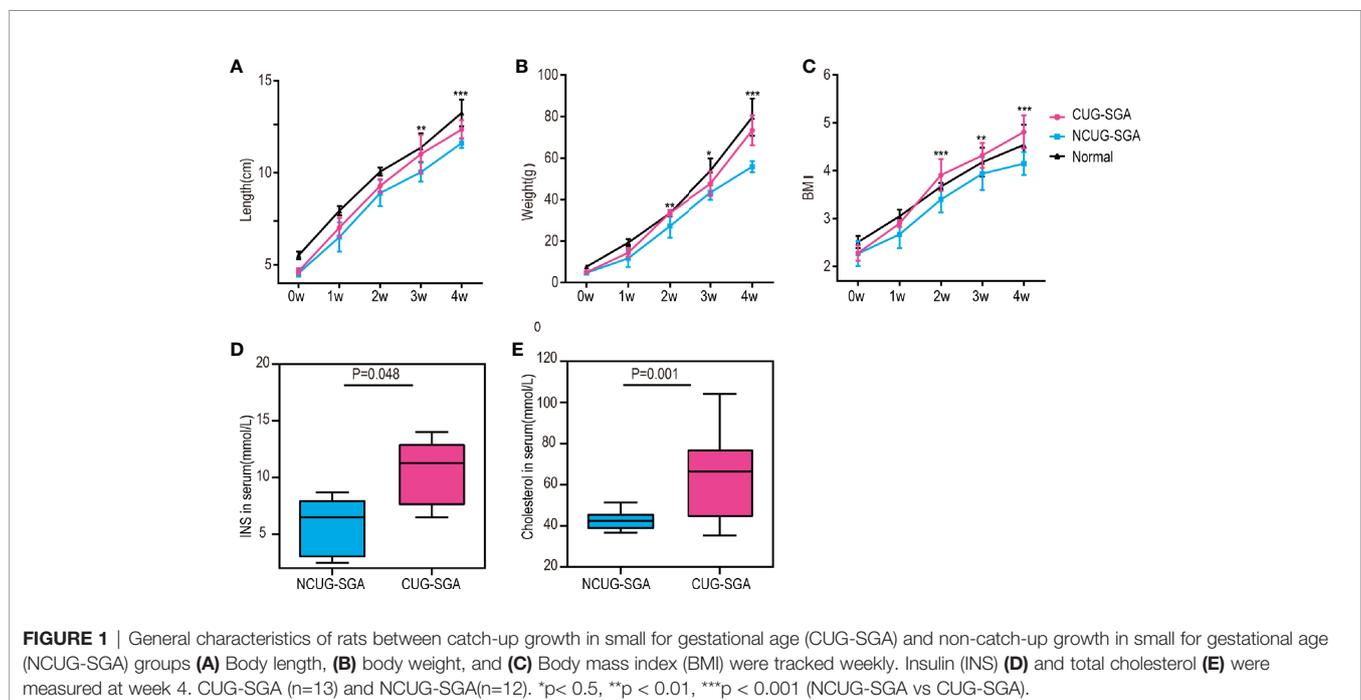
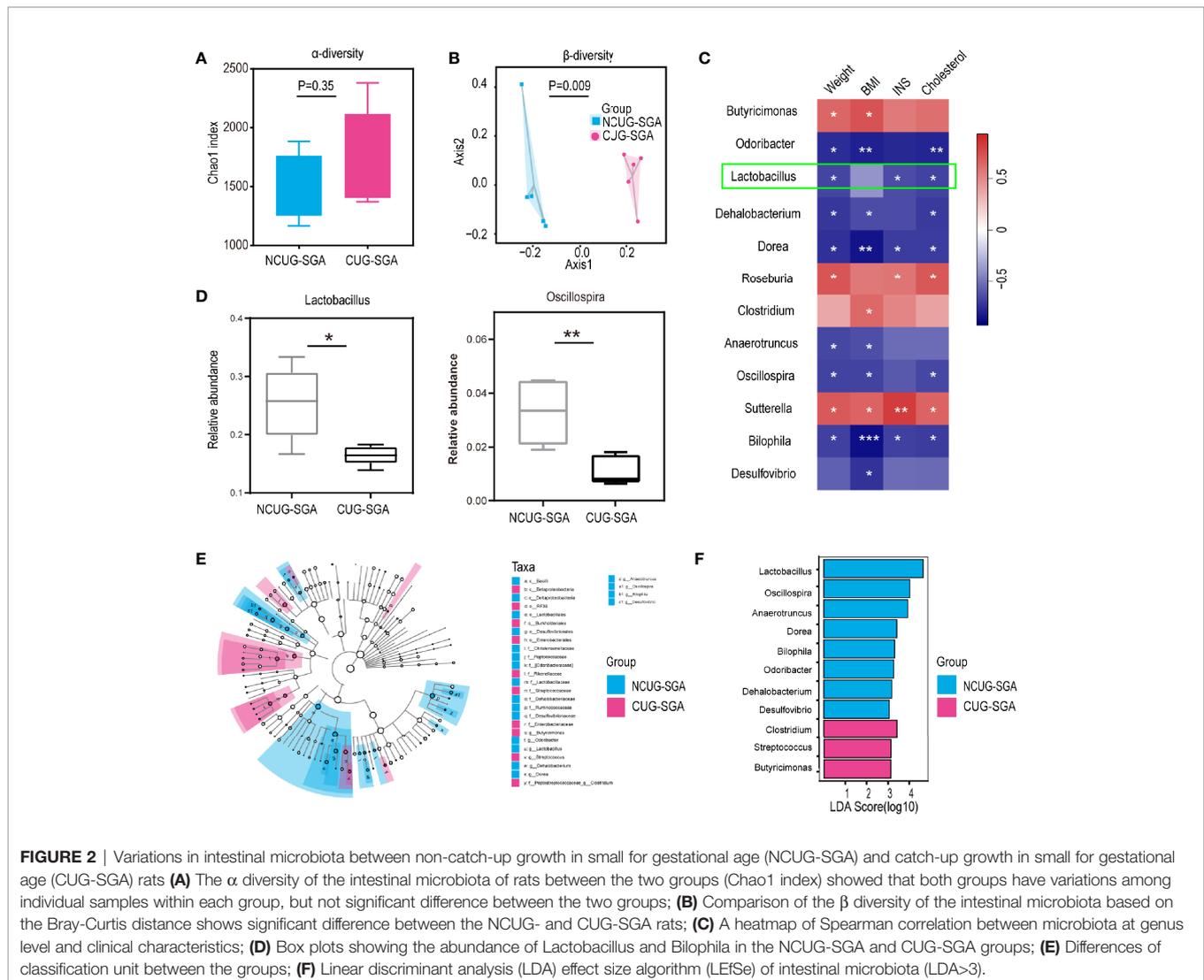


FIGURE 1 | General characteristics of rats between catch-up growth in small for gestational age (CUG-SGA) and non-catch-up growth in small for gestational age (NCUG-SGA) groups (A) Body length, (B) body weight, and (C) Body mass index (BMI) were tracked weekly. Insulin (INS) (D) and total cholesterol (E) were measured at week 4. CUG-SGA (n=13) and NCUG-SGA (n=12). **p* < 0.5, ***p* < 0.01, ****p* < 0.001 (NCUG-SGA vs CUG-SGA).



body weight, BMI, INS, and total cholesterol. As shown in **Figure 2C**, *Butyricimonas*, *Roseburia*, *Clostridium*, and *Sutterella* were positively correlated with body weight, BMI, INS level, and/or total cholesterol level respectively, while *Odoribacter*, *Lactobacillus*, *Dehalobacterium*, *Dorea*, *Anaerotruncus*, *Oscillospira*, *Bilophila*, and *Desulfovibrio* were negatively associated with body weight, BMI, INS and/or cholesterol level, suggesting that among the thousands of bacteria detected, these 12 different genera may play an important role in shaping the host body weight and metabolic activity.

Fecal SCFA Content Is Higher in the CUG-SGA Group Than in the NCUG-SGA Group

Gut microbiota and their metabolic products play a critical role in shaping host metabolism and growth, as shown by the defects observed in GF animals (13, 14). However, the 16S rRNA sequencing data does not provide functional profile directly. To further understand the potential effect of the microbiota on host in SGA, we used PICRUSt2 for functional prediction. We

found that the abundance of 30 KEGG pathways was significantly different between the NCUG-SGA and CUG-SGA groups. The pathways enriched in the CUG-SGA group include the pathways of fatty acid biosynthesis, propionate metabolism, and butanoate metabolism, which implied that SCFAs may play an important role in the mechanism of CUG and its metabolic change (**Figure 3A**).

SCFAs, one of the main metabolites of the gut microbiota, play a role in energy regulation and metabolism by acting as intermediaries between the gut microbiota and the host. To examine the potential role of SCFAs in regulating host metabolism during CUG, we first measured SCFA concentrations in the feces of the rats at four weeks of age. As shown in **Figure 3B**, there were five SCFAs (acetic acid, propionic acid, butyric acid, valeric acid and caproic acid) and two branched chain fatty acids (iso butyric acid, isovaleric acid), that were significantly higher in the feces of rats in the CUG-SGA group than in the NCUG-SGA group (all p values < 0.05), which was consistent with the prediction results of intestinal microbiota function.

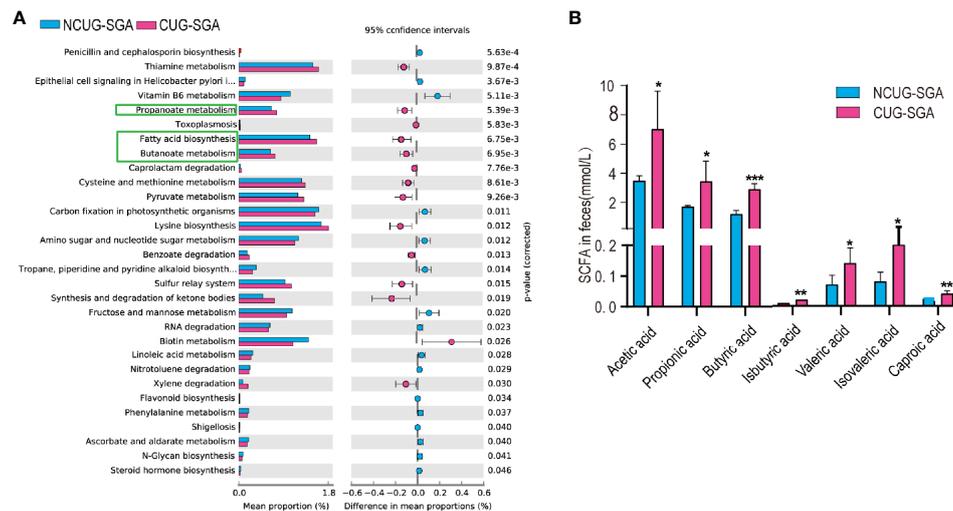


FIGURE 3 | Intestinal microbiota functional prediction and short-chain fatty acid (SCFA) detection. **(A)** KEGG pathway analysis based on 16S rRNA between NCUG-SGA and CUG-SGA groups in rats, among them, fatty acid biosynthesis, propionate metabolism, and butanoate metabolism are significantly upregulated in the CUG-SGA group rats. **(B)** Detection of SCFA from fecal samples in NCUG-SGA and CUG-SGA groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. CUG-SGA, catch-up growth in small for gestational age; NCUG-SGA, non-catch-up growth in small for gestational age.

SCFAs Are Associated With the Gut Microbiota and Clinical Features in SGA

The function prediction of gut microbiota and SCFA levels suggested that the gut microbiota may play an important role in metabolic syndrome in CUG in SGA through SCFA. First, we established a correlation matrix based on the Spearman rank correlation coefficient to explore the relationship between intestinal microbiota and fecal SCFA levels. Eleven different bacterial genera were significantly related to at least one SCFA ($P < 0.05$, $|r| > 0.6$), indicating that SCFA levels were closely related to the intestinal microbiota. Among them, *Lactobacillus*, *Bilophila*, *Oscillospira*, and *Desulfovibrio* had a significant negative correlation with different SCFAs ($P < 0.05$, $r < -0.6$). The concentration of butyric acid was positively correlated with the abundance of butyric acid-producing *Butyrivibrio* ($P < 0.05$, $r > 0.6$) (Figure 4A).

Spearman correlation analysis was performed to determine the correlation between the clinical indicators of NCUG-SGA and CUG-SGA rats and SCFAs. The results showed that five SCFAs and two branched chain fatty acids had a significant positive correlation with at least one clinical index (weight, BMI, insulin, and total cholesterol), indicating that SCFAs may affect the metabolism of the host (Figure 4B). These findings suggest that gut microbiota and SCFAs may play a role in the metabolic syndrome associated with the catch-up growth of SGA.

Changes in Metabolism-Related Pathways in the Liver Transcriptome in CUG-SGA Rats With Decreased Igf2 Expression

The study on the gut microbiota of SGA rats indicated that SCFAs may be involved in the metabolic disorders in the process of catch-up growth. The liver is an important metabolic organ in

mammals, and it establishes connections with other tissues of the body through its metabolic function. To clarify the correlation between SCFAs and the metabolic disorders associated with CUG in SGA, we sequenced the transcriptome of liver tissues in NCUG-SGA and CUG-SGA rats. Results revealed 2416 differentially expressed genes (DEGs), of which 1403 were upregulated and 1013 were downregulated (Figure 5A). To clarify the biochemical metabolism and signal transduction pathways involved in DEGs, we conducted KEGG pathway analysis and found significant differences in 53 metabolic pathways ($P < 0.05$). Figure 5B shows the top 34 metabolic pathways, including protein synthesis, fatty acid metabolism, AMPK signaling, insulin signaling, PPAR signaling, MAPK signaling, and insulin resistance. These results provide important clues for studying the metabolic syndrome in CUG in SGA.

To systematically analyze the functions of DEGs in the liver tissues of the NCUG-SGA and CUG-SGA groups, we mapped the DEGs to the protein-protein interaction (PPI) (<https://string-db.org/>) database and generated the PPI network diagram (S2). Igf2, Mmp14, and Hgf were significantly different between the two groups, and showed interactions with each other (Figure 5C). Igf2 was very close to the gene encoding insulin on human chromosome 11p, which may contribute to regulate the body weight and obesity in children and adults (26); Mmp14 and Hgf were also associated with insulin resistance (27, 28), and these three genes are members of MAPK pathway mentioned above. Igf-2 was significantly downregulated in the CUG-SGA group. The expression of Hgf and Mmp14 significantly increased in the CUG-SGA group. These results were verified using qRT-PCR, indicating that transcriptome sequencing can reliably reflect the changes in gene expression (Figure 5D).

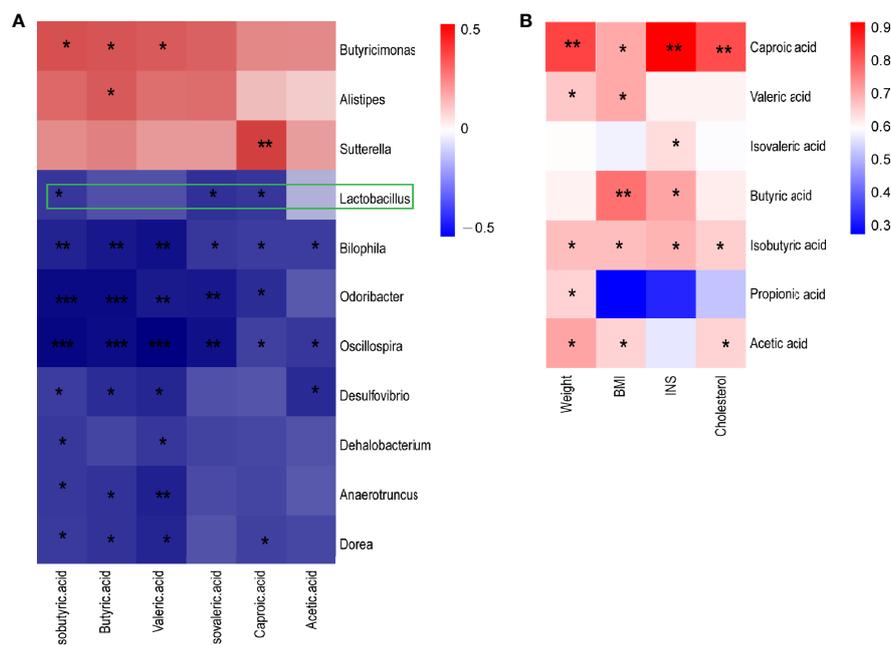


FIGURE 4 | A heatmap of Spearman correlation between intestinal microbiota at genus level and metabolism index and SCFA **(A)** A heatmap of Spearman correlation between microbiota at genus level and SCFAs. **(B)** A heatmap of Spearman correlation between clinical characteristics and SCFAs. Red: positive correlation; blue: negative correlation; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. SCFAs, short-chain fatty acids.

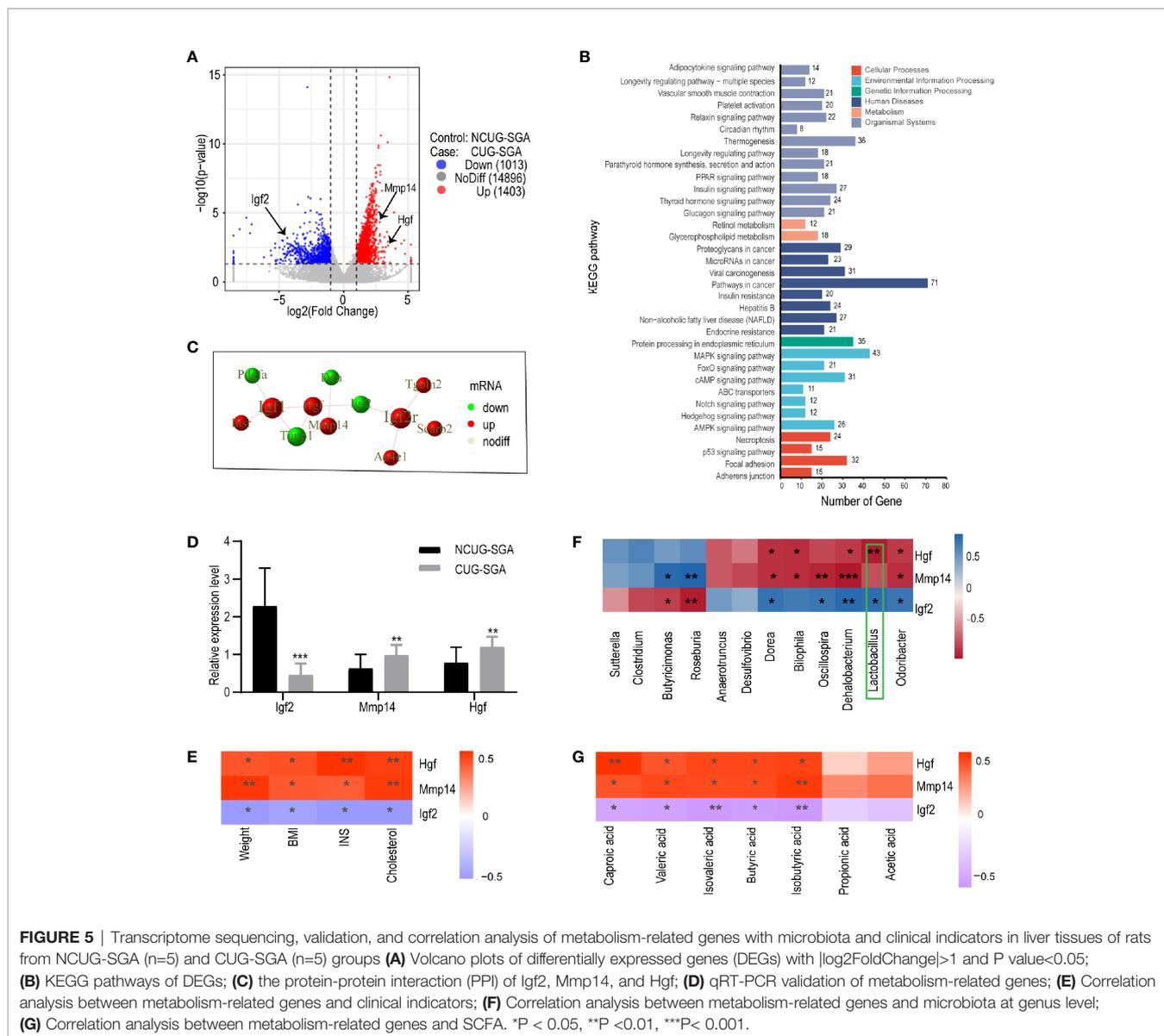
Correlation analysis showed that *Igf2*, *Hgf*, and *Mmp14* were significantly correlated with clinical metabolic indicators (weight, BMI, INS, and TC) ($P < 0.05$, $|r| > 0.6$) (**Figure 5E**), indicating that these three genes may be involved in metabolic changes during SGA growth catch-up. To clarify the correlation between DEGs and intestinal microbiota, we analyzed the correlation between *Igf2*, *Hgf*, and *Mmp14* and the gut microbiota and SCFA. The results showed that *Igf2* and *Hgf* were significantly related to the abundance of *Lactobacillus* ($P < 0.05$, $|r| > 0.7$) (**Figure 5F**); the expression levels of *Igf2*, *Hgf*, and *Mmp14* were related to the changes in SCFA ($P < 0.05$, $|r| > 0.6$) (**Figure 5G**).

DISCUSSION

Insulin resistance and metabolic syndrome greatly affect human life expectancy and quality of life. A large number of studies have found that the risk of insulin resistance and obesity is significantly increased in the growth of SGA individuals, and the metabolic disorders are related to CUG. In this study, we aimed to evaluate the role of gut microbiota independent of genetic factors in the process of CUG in SGA. Pregnant rats were given restricted food (reduced by 50% from the first day of gestation until delivery as compared to normal diet) to give birth to SGA pups. At 4 weeks, the length and weight of CUG-SGA rats were significantly higher than those in the NCUG-SGA group, with elevated levels of insulin and total cholesterol. This was associated with significant differences in the diversity and

composition of gut microbiota between these two groups, including *Lactobacillus*, whose relative abundance was most significantly decreased in the CUG-SGA group. SCFA levels in the CUG-SGA group were significantly increased, with significant changes in the mRNA level of IGF-2 as shown by the liver transcriptome analysis. Our findings suggest that the gut microbiota and its metabolites may relate to the metabolic process in the liver and may be associated with increased body weight and the systemic metabolic dysfunction in the host.

To study the relationship between gut microbiota and host metabolism, we used different techniques to capture and present a complete and unbiased picture, including 16s rRNA sequencing, SCFA detection, and liver tissue RNA sequencing. This allowed us to identify the decrease in *Lactobacillus* as the most significant event across different analyses in CUG-SGA as compared to the NCUG-SGA group. The gram-positive *Lactobacillus* is the dominant bacteria in the gastrointestinal microbiota of mammals and can affect the composition and metabolism of gut microbiota by producing metabolites such as lactic acid which lowers pH (29, 30). The impact of *Lactobacillus* on weight change varied according to the species: some species were associated with weight gain, while others were linked to weight loss. Meta-analyses have shown that *Lactobacillus* can promote loss of weight and fat in overweight adults and children (31, 32). *Lactobacillus* strains isolated from the feces of super-longevity people reduced serum cholesterol levels in rats with a high-cholesterol diet (33). *Lactobacillus* selected from fermented cow milk also reduced serum cholesterol levels in rats (34). Moreover, the effect of the same strain of *Lactobacillus* could be



different depending on the species of the host (35). In our current study, the increase in body weight, insulin, and total cholesterol in the CUG group was significantly associated with decreased abundance of *Lactobacillus*. Therefore, the metabolic disorder of catch-up of SGA may be related to the decrease in *Lactobacillus* and other bacteria.

Furthermore, our study explored the changes of metabolites such as SCFAs. PICRUSt2s functional prediction based on 16S rRNA sequencing data showed that CUG-SGA rats microbiome were associated with metabolic pathways including fatty acid, propionate, butyrate, etc. In consistency, the contents of fecal SCFAs were significantly higher in the CUG-SGA group than in the NCUG-SGA group. Correlation analysis showed that the increase in SCFAs was related to the decrease in *Lactobacillus*, *Bilophila*, *Odoribacter*, and *Oscillospira*. As the main metabolite of the intestinal microbiota, SCFAs act as intermediaries

between the gut microbiota and the host, and their composition is closely related to the intestinal microbiota. Numerous studies have demonstrated that SCFAs play a positive role in the energy metabolism of mammals. After absorption, SCFAs could be used as energy sources for the host and for regulating energy metabolism (36). In the human body, SCFAs provide about 10% of the daily caloric requirement (37). SCFAs are transported to the liver through the portal vein where butyric acid could stimulate the expression of genes associated with gluconeogenesis (38). Butyric acid has also been shown to be metabolized by acetyl CoA for the synthesis of fatty acids, cholesterol and ketone body through acetyl-CoA metabolism, thereby providing specific substrates for lipid biosynthesis (39). Butyric acid and propionic acid also act as ligands for G protein-coupled receptors Gpr41 and Gpr43 and affect the body's metabolism (40–42). Moreover, changes in

SCFA levels may contribute to metabolic disorders such as insulin resistance and obesity. In our study, the increase in SCFA levels may be associated with the metabolic disorders during CUG in SGA. It has to be mentioned that the fecal SCFAs are only a fraction of the total SCFAs and thus changes of fecal SCFAs may not accurately reflect the changes in total SCFAs (37). In addition, it has been proposed that increased SCFAs contributes to the development of obesity, this remains controversial (43).

The metabolism of SCFAs depend on the enterohepatic circulation. By comparing the transcriptome sequences of rat livers in the NCUG-SGA and CUG-SGA groups, we found that the DEGs were mainly involved in insulin response, and carbohydrate, protein, and fatty acid metabolism. Among them, *Igf2* was significantly reduced in the CUG group. Studies have found that lower *Igf2* concentration is associated with weight gain and obesity (44). Murphy et al. have reported a case of a patient harboring an *Igf2* defect combined with insulin resistance and lipid metabolism disorders (45). The *Igf2* imprinting gene is very close to the gene encoding insulin on human chromosome 11p, which may play an important role in regulating the body weight and fat in children and adults (26, 46–50). *In vivo* assay showed that decreased *Igf2* expression occurred with fat deposition and obesity, which may not be caused by an increase in food intake, but more likely by changes in energy homeostasis (51).

PPI networks suggested that *Igf2* may interact with *Hgf* and *Mmp14*. *Hgf* stimulates glucose uptake and metabolism in mouse skeletal muscles and plays an important role in insulin resistance (27). In humans, serum *Hgf* is significantly higher in patients with obesity, metabolic syndrome, and diabetes compared to healthy subjects (52–54). A prospective study revealed that elevation of serum *Hgf* is significantly correlated with insulin resistance (27). *Mmp14*, *Igf2*, and *Hgf* are members of MAPK pathway. The circulating glucose and triglyceride levels in *Mmp14*-knockout mice decreased by 50% compared to levels in wild type mice, as demonstrated by reduced tissue glycogen and lipid levels, and plasma glucose and triglycerides (28). Therefore, we speculated that *Igf2*, *Hgf*, and *Mmp14* may be involved in the metabolic changes in CUG in SGA. Correlation analysis showed that the expression levels of *Igf2*, *Hgf* and *Mmp14* were associated with the abundance of *Lactobacillus* and SCFA contents. It is possible that the metabolites of *Lactobacillus* and other SCFAs are detected by the host, leading to altered expressions of *Igf2*, *Hgf* and *Mmp14*, which then regulate the host blood glucose and triglyceride levels and insulin sensitivity in SGA. The detailed mechanism needs further research.

In the present study, we found that *Lactobacillus* may affect the host's body weight and insulin and cholesterol levels by

regulating SCFAs during the catch-up process in SGA rats. Changes in *Lactobacillus* levels were associated with increased levels of SCFAs. Following transportation into the liver *via* the enterohepatic circulation, SCFA may be related to the decreasing *Igf2*, associated with insulin resistance and lipid metabolism disorder. Although the relationships between the microbiota, SCFAs and liver transcriptomes are based on computational analysis and predictions, the consistent findings combining three different profiles of the SGA rats strengthened our study. These findings provide a theoretical basis for elucidating the metabolism and developing novel therapies in SGA patients, especially those with CUG.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are publicly available. This data can be found here: NCBI PRJNA679705.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Experimental Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine.

AUTHOR CONTRIBUTIONS

ZD and XW proposed the initial experimental ideas. JA, ZD, YX, WL, and XW developed the experimental design. JA, JW, LL, and LC conducted the experiments. JA, ZD, XW, and LG wrote the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fendo.2021.630526/full#supplementary-material>

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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