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RESEARCH ARTICLE

Antibiotics-mediated intestinal microbiome perturbation aggravates tacrolimus-induced glucose disorders in mice

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Abstract **Both immunosuppressants and antibiotics (ABX) are indispensable for transplant patients. However, the former increases the risk of new-onset diabetes, whereas the latter impacts intestinal microbiota (IM). It is still unclear whether and how the interaction between immunosuppressants and ABX alters the IM and thus leads to glucose metabolism disorders. This study examined the alterations of glucose and lipid metabolism and IM in mice exposed to tacrolimus (TAC) with or without ABX. We found that ABX further aggravated TAC-induced glucose tolerance and increased insulin secretion. Combined treatment resulted in exacerbated lipid accumulation in the liver. TAC-altered microbial community was further amplified by ABX administration, as characterized by reductions in phylum Firmicutes, family Lachnospiraceae, and genus *Coprococcus*. Analyses based on the metagenomic profiles revealed that ABX augmented the effect of TAC on microbial metabolic function mostly related to lipid metabolism. The altered components of gut microbiome and predicted microbial functional profiles showed significant correlation with hepatic lipid accumulation and glucose disorders. In conclusion, ABX aggravated the effect of TAC on the microbiome and its metabolic capacities, which might contribute to hepatic lipid accumulation and glucose disorders. These findings suggest that the ABX-altered microbiome can amplify the diabetogenic effect of TAC and could be a novel therapeutic target for patients.**

Keywords antibiotics; tacrolimus; glucose disorders; microbiome

Introduction

Immunosuppressive agents help many transplant patients to survive. However, immunosuppressive treatment also causes side effects, e.g., new-onset diabetes mellitus (NODM), which seriously impacts the life quality of postoperative patients, and is associated with elevated risks of cardiovascular disease, graft dysfunction, and mortality [1–3]. The use of immunosuppressive agents, especially tacrolimus (TAC), is considered as the primary and controllable risk factor of NODM [4,5]. Recent studies showed that intestinal microbiota (IM) might be involved in the occurrence of NODM relevant to TAC [5,6]. In addition, antibiotics (ABX)-mediated gut dysbiosis increases the incidence of diabetes and accelerates its development in mice [7]. In clinical practice, the use of ABX is unavoidable for transplant patients because of the high incidence of opportunistic infections due to adoption of immunosuppressants [8,9]. Thus, we speculated that the combination of ABX and TAC might alter IM, which lead to glucose disorders and even diabetes.

Commensal bacteria are implicated in human health and disease [10]. They are even considered as an important metabolic organ and a key regulator of multiple metabolic pathways, including those relevant to the host's energy balance and glucose metabolism [11,12]. IM are highly associated with type 2 diabetes (T2DM) [13–15]. Patients

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with T2DM are characterized by decreased abundance of butyrate-producing bacteria and increased opportunistic pathogens, which lead to sulfate reduction and oxidative stress resistance [14]. Our previous study also found the critical role of IM as an important player of NODM in transplant recipients [5]. Modulation of IM by probiotics partially restores intestinal homeostasis, improves microbial metabolic function, attenuates pancreatic inflammation, and maintains glucose homeostasis (our unpublished data). We also found that patients receiving liver transplantation had a significant decrease of butyrate-producing bacteria and an increase of opportunistic pathogens [16], which was similar to patients with diabetes [14,17]. Although these studies indicated that intestinal dysbiosis might contribute to the development of TAC-induced diabetes, it remains unknown whether and how ABX contribute to TAC-induced diabetes.

Here, we performed high-throughput sequencing targeting the 16S rRNA sequences (V3–V4 region) to characterize the fecal microbial communities in TAC-treated mice with or without ABX. The Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was applied to predict function shift of IM. We also tried to identify specific microbial lineages and potential bacterial biomarkers that may play important roles in the promoting process of glucose disorders. This is the first evaluation of the combined effect of ABX and TAC on glucose metabolism mediated by gut microbiota. Our findings provide a more comprehensive understanding of the combined effect of ABX and TAC treatment on the composition and function of IM and its potential influence on the glucose metabolism, and facilitate therapeutic efforts to target the microbiota for patients with such clinical conditions.

Materials and methods

Animal experiments

Wild-type male *C57BL/6* mice (8 weeks old, n = 30; Shanghai, Chinese Academy of Sciences, China) weighing 22–25 g were used in the experiments. Mice were housed in groups of five per cage with a strict 12:12 h light–dark cycle (lights on at 7 AM) at 20 °C and had free access to water and food *ad libitum* in a controlled SPF environment [18]. Body weight (bw) of mice was recorded once a week. All mice were acclimated for 1 week and then were randomly divided into three groups: (1) TAC group, intraperitoneally treated daily with TAC (Astellas Pharma, Inc., Tokyo, Japan) in PBS (2 mg/kg bw per day, TAC, n = 10); (2) TAC + ABX group, treated with TAC and ABX (ampicillin, vancomycin, neomycin, and metronidazole, each at 1 g/L in sterile water [19], TAC + ABX, n = 10); and (3) blank control group, intraperitoneally treated daily with equivalent volume of saline (Control, n = 10). After 4-week treatment, mice were euthanized via intraperitoneal injection of 3% sodium pentobarbital solution at 2.5 mL/kg bw. Blood samples were collected through cardiac puncture immediately, and the plasma was frozen immediately at −80 °C until it was assayed. Stool sample was collected and stored at −80 °C until use.

Glucose tolerance test (GTT) and glucose-stimulated insulin secretion test

After 4-week treatment, we performed GTT in mice. Mice were fasted for 16 h with free access to water overnight. Then, they were intraperitoneally injected with D-glucose (1 g/kg bw) [20]. Glucose from tail vein blood was monitored before (0 min) and after (15, 30, 60, and 120 min) glucose injection using Accu-Chek Go (Roche Diagnostics GmbH, Basel, Switzerland). The plasma insulin levels were assessed at 0, 30, and 120 min after glucose injection by Rat/Mouse Insulin ELISA Kit (Millipore, Watford, UK).

Bacterial genomic DNA extraction and 16S rRNA Illumina sequencing

Total bacterial genomic DNA was extracted from stool samples using QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions with modifications [21]. Then, the bacterial genomic DNA concentration and purity were determined using the Nano-Drop 1000 Spectrophotometer (NanoDrop Technologies, Thermo Scientific, USA). The V3–V4 region of the bacterial 16S rRNA gene was amplified by PCR with the barcoded forward fusion primers (5′-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3′) in combination with the reverse fusion primer (5′-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′). PCR products were gel-purified (AXYGEN Gel Extraction Kit, AXYGEN). Then, PCR products were pooled in equimolar concentrations and sequenced using an Illumina® MiSeq platform according to the manufacturer's recommendations. All reads were clustered into operational taxonomic units (OTUs) at a sequence identity of 97%, and the taxonomic affiliation of the OTUs was determined with QIIME (version 1.8.0) against the Greengenes database version 13.8 [22]. The following downstream data analyses were conducted in R software. Analysis of variance (ANOVA) was performed to evaluate α diversity (Shannon index, Simpson index, Chao index, ACE index) among the different groups in 16S sequencing data. PERMANOVA (Adonis) was used to test for microbial community clustering (PCoA) using unweighted

UniFrac distance matrices and Bray–Curtis distance matrices. The linear discriminant analysis (LDA) effect size (LEfSe) method was performed to characterize the taxa with statistical significance and biological relevance [23]. For LEfSe analysis, the Kruskal–Wallis rank sum test (α value of 0.05) and LDA score of > 2 were used as thresholds. Pairwise comparisons were analyzed with Mann–Whitney U test. Then, a predicted functional composition of the intestinal microbiome was inferred for each sample using PICRUSt, which can accurately predict the abundance of gene families from the 16S rRNA information based on the close link between phylogeny and function [23]. Briefly, metagenome inference was carried out with 16S rRNA gene data clustered at a 97% identity cut-off using close reference of the Greengenes database. The resulting OTU table was normalized by its gene copy number, and finally predicted gene family abundance of each sample was inferred. In addition, the graphical representation of the results was done with STAMP [24], and significant functional differences among mice in different groups were assessed with Welch's *t*-test using a P value < 0.05.

Detection of triglyceride (TG) levels in the liver and blood

Hepatic TG content was measured using the Triglyceride Quantification Kit (Abcam, Cambridge, UK) according to the manufacturer's instructions as previously reported [25]. Briefly, fresh liver sample (100 mg) was suspended and homogenized in 1 mL of 5% Nonidet P-40/ddH_2O solution. The samples were slowly heated to 80 °C–100 °C until the solution became cloudy and cooled down to room temperature. Then, the samples were centrifuged at 2000 *g* for 2 min. The supernatant was collected. Finally, the absorbance of the samples was quantified with a spectrophotometer (BioTek, Vermont, USA) at 570 nm. The TG values were expressed as mg/g of liver. The blood TG was measured using a Hitachi 7600-210 Automatic Analyzer (Hitachi, Tokyo, Japan).

Statistical analysis

To determine the statistical significance for the difference among groups, one-way ANOVA for data with normal distribution or Kruskal–Wallis test for data with abnormal distribution was used. Spearman's or Pearson's correlation tests were employed to address the correlation between microbiomes and metabolic parameters. P values < 0.05 were considered significant. Statistical analysis was performed with GraphPad Prism software (version 6.00; San Diego, California, USA) and SPSS software (version 16.0, Chicago, USA)

Results

ABX exacerbated the effect of TAC on hepatic lipid deposition and glucose disorders in mice

To evaluate the influence of TAC and ABX on glucose metabolism, intraperitoneal GTT was performed on controls and TAC-treated mice with or without ABX. Among the three groups, TAC + ABX mice showed the highest blood glucose levels from 30 min (TAC + ABX vs. Control: 24.74 vs. 15.61, $P < 0.0001$; TAC vs. Control: 21.60 vs. 15.61, $P < 0.0001$; TAC + ABX vs. TAC: 24.74 vs. 21.60, $P < 0.01$) to 60 min (TAC + ABX vs. Control: 19.51 vs. 11.01, $P < 0.0001$; TAC vs. Control: 15.36 vs. 11.01, $P < 0.001$; TAC + ABX vs. TAC: 19.51 vs. 15.36, $P < 0.01$) after receiving glucose and the area under the curve (AUC) (TAC + ABX vs. Control: 2010 vs. 1447, $P < 0.0001$; TAC vs. Control: 1691 vs. 1447, $P < 0.05$; TAC + ABX vs. TAC: 2010 vs. 1691, $P < 0.01$) (Fig. 1A and 1B). The results suggested that ABX significantly improved glucose tolerance induced by TAC treatment. The insulin levels in TAC mice were elevated at fasting state (25.07 vs. 39.39, $P < 0.0001$) and at 120 min (35.41 vs. 60.86, $P < 0.0001$) following glucose treatment but decreased at 30 min (72.67 vs. 52.81, $P < 0.0001$) after glucose injection compared with control mice (Fig. 1C). The results demonstrated that the peak of insulin secretion was delayed and insulin levels were lowered by TAC. Unexpectedly, a significant improvement in insulin secretion was observed in TAC + ABX mice compared with TAC mice ($P < 0.05$) (Fig. 1C).

Given that TAC also disturbs lipid metabolism [26], we further detected TG levels in the liver and blood of mice with different treatments. The blood levels of TG in TAC mice were significantly increased (1.69 vs. 2.17, $P < 0.001$) compared with the controls and slightly decreased after ABX treatment (Fig. 1D). Interestingly, the liver levels of TG in TAC mice were significantly increased compared with the controls (6.30 vs. 16.50, $P < 0.5$) and further increased in TAC + ABX mice (TAC vs. TAC + ABX: 16.50 vs. 23.00, $P < 0.0001$, Fig. 2). These results suggested that ABX increased hepatic lipid deposition in TAC-treated mice.

Impact of ABX on intestinal microbiomes of TAC-treated mice

To examine the effect of ABX on microbial community in TAC-treated mice, we performed high-throughput Illumina® MiSeq sequencing targeting the bacterial 16S rRNA genes. Detailed statistical characteristics of pyrosequencing data are summarized in Supplementary Table S1. Although the Simpson and Shannon index showed no significant difference among the mice with different

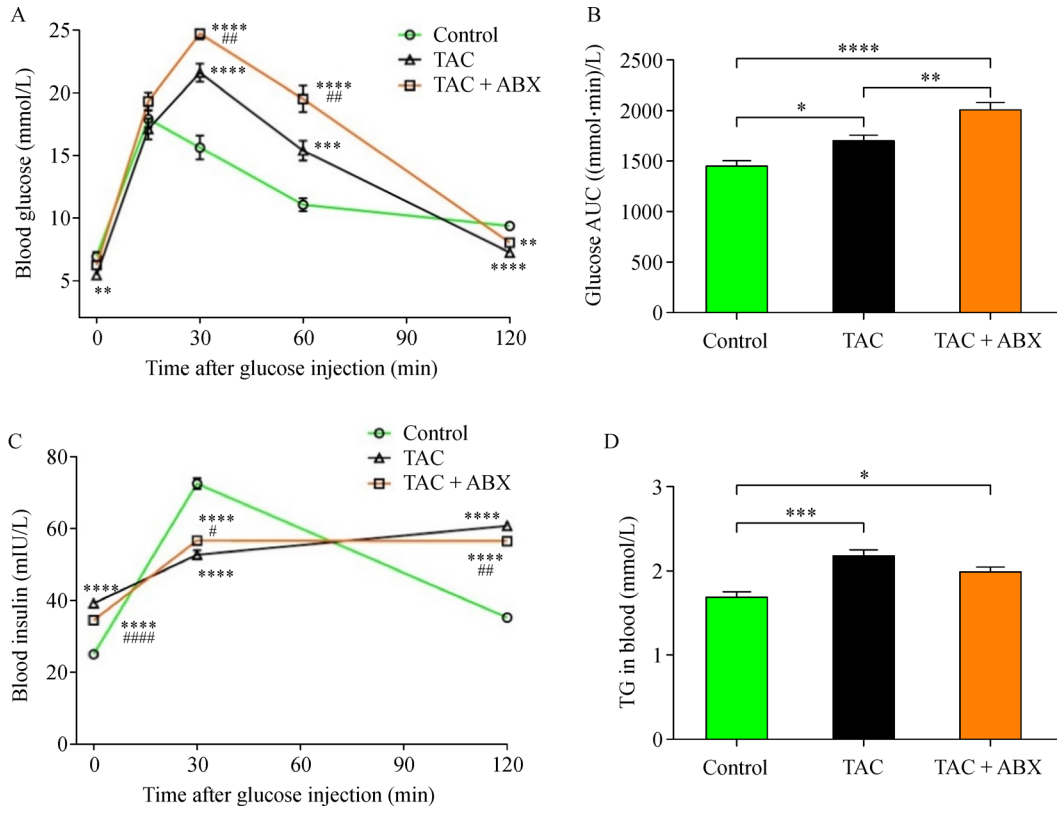

Fig. 1 Impact of ABX and TAC on glucose disorders in mice. (A) Glucose tolerance test (GTT). (B) Area under the curve (AUC) for the GTT curves. (C) Insulin release test. (D) Triglyceride levels in the blood. (A and C) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ compared with the control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#\#}P < 0.0001$ compared with the TAC group. (B and D) $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. Data are expressed as mean ± SEM, n = 10 mice/group. Groups: blank control, Control; tacrolimus (2 mg/kg bw per day), TAC; tacrolimus and antibiotics, TAC + ABX.

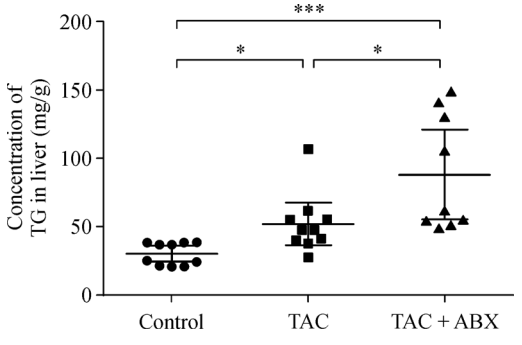

Fig. 2 Oral ABX augmented the effect of TAC on triglyceride accumulation in the liver of mice. Concentration of triglyceride in the liver. $^{*}P < 0.05$ and $^{***}P < 0.001$. Data are expressed as median with interquartile range, n = 10 mice/group. Groups: blank control, Control; tacrolimus (2 mg/kg bw per day), TAC; tacrolimus and antibiotics, TAC + ABX.

treatments, TAC markedly increased commensal diversity as indicated by elevated ACE (Control vs. TAC: 15 100 vs. 18 750, $P < 0.05$) and Chao 1 indexes (Control vs. TAC: 14 026 vs. 17 679, $P < 0.05$), whereas ABX decreased α diversity (ACE indexes, TAC + ABX vs. TAC: 15 209 vs. 18 750, $P < 0.05$, Table S1). IM in different groups of mice were clearly separated by the PCoA plot analysis (PERMANOVA, unweighted UniFrac, TAC vs. Control: $P < 0.01$, $r = 0.509$; TAC + ABX vs. Control: $P < 0.01$, $r = 0.488$; TAC + ABX vs. TAC: $P < 0.05$, $r = 0.435$, Fig. 3A). Similar results were obtained using the Bray–Curtis distance matrices (TAC vs. Control: $P < 0.05$, $r = 0.680$; TAC + ABX vs. Control: $P < 0.01$, $r = 0.652$; TAC + ABX vs. TAC: $P < 0.05$, $r = 0.602$, Figure S1). Administration of ABX altered the relative abundance of predominant microbiota at the phylum level, including Firmicutes, Verrucomicrobia, and Actinobacteria (Fig. 3B).

We identified the distinguished bacteria in mice with different treatment by LEfSe analysis. Compared with controls, the relative abundance of phylum Firmicutes (44.71% vs. 25.78%, $P < 0.01$), family Lachnospiraceae (4.14% vs. 1.38%, $P < 0.05$), and genus *Coprococcus* (9.72% vs. 0.76%, $P < 0.01$) and the ratio of Firmicutes to Bacteroidetes (1.062 vs. 0.4180, $P < 0.05$) were significantly reduced in TAC + ABX-treated mice (Fig. 3C and 3D; Fig. 4A, 4B, 4D, and 4F). However, the abundance of

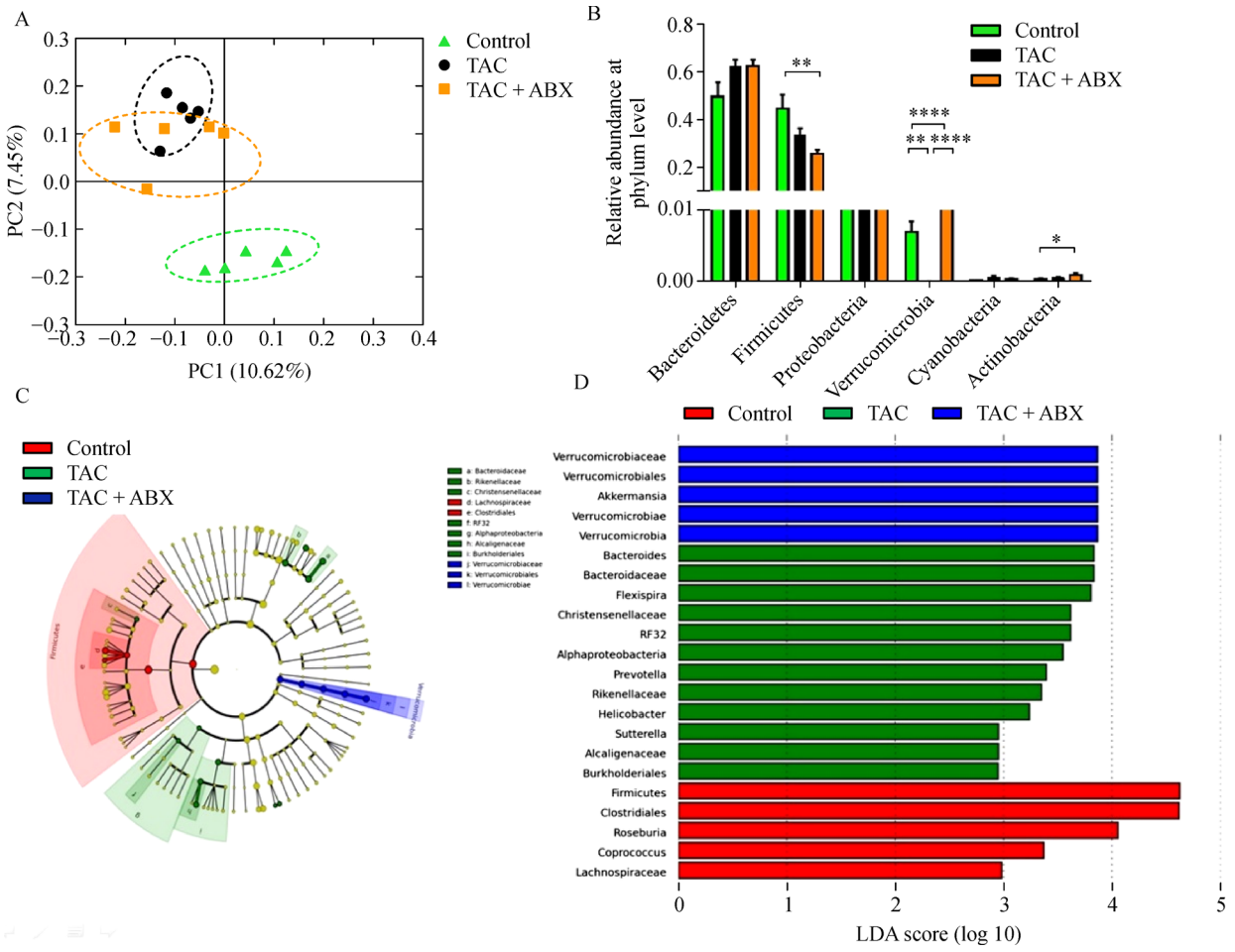

Fig. 3 Impact of ABX and TAC on intestinal microbiome in mice. (A) PCoA plot of the IM based on unweighted UniFrac metric. Each spot represents one sample. (B) Relative abundance of bacteria at the phylum level. (C) LEfSe cladograms represented taxa enriched in each group. Rings from the inside out represented taxonomic levels from phylum to genus. Sizes of circles indicate relative abundance of the taxon. (D) Discriminative biomarkers with an LDA score > 2. $n = 5$ mice/group. Groups: blank control, Control; tacrolimus (2 mg/kg bw per day), TAC; tacrolimus and antibiotics, TAC + ABX.

phylum Verrucomicrobia (0.70% vs. 4.38%, $P < 0.0001$), family Verrucomicrobiaceae (1.15% vs. 4.90%, $P < 0.0001$), and genus *Akkermansia* (5.67% vs. 20.06%, $P < 0.0001$) was enhanced in TAC + ABX-treated mice (Fig. 3C and 3D; Fig. 4C, 4E, and 4G).

Altered key microbiota relevant to ABX and TAC were associated with glucose disorders

We assessed the correlation between ABX- and TAC-altered microbiota and glucose metabolism. Fig. 4 shows that the AUC and peak level of glucose in GTT negatively correlated with the ratio of Firmicutes to Bacteroidetes and the relative abundances of Firmicutes, Lachnospiraceae, and *Coprococcus* and positively correlated with the relative abundances of Bacteroidetes ($P < 0.05$). Notably, those altered microbiota were associated with liver TG levels ($P < 0.05$, Fig. 4H). These findings indicated that ABX-dependent IM dysbiosis might contribute to glucose disorders in TAC mice.

Predicted function pathways of microbiome altered by ABX and TAC significantly correlated with glucose disorders

The potential functional changes of gut microbiome in mice with different treatment were predicted with PICRUSt using Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs. Statistical analysis revealed that compared with those of controls and TAC mice, the gut microbiome of TAC + ABX mice was significantly enriched in functional categories relevant to lipid metabolism, metabolism of cofactors, biosynthesis and biodegradation of secondary metabolites and vitamins, amino acid metabolism, and xenobiotic biodegradation ($P < 0.05$, Fig. 5A). Compared with controls, the gut microbiome in

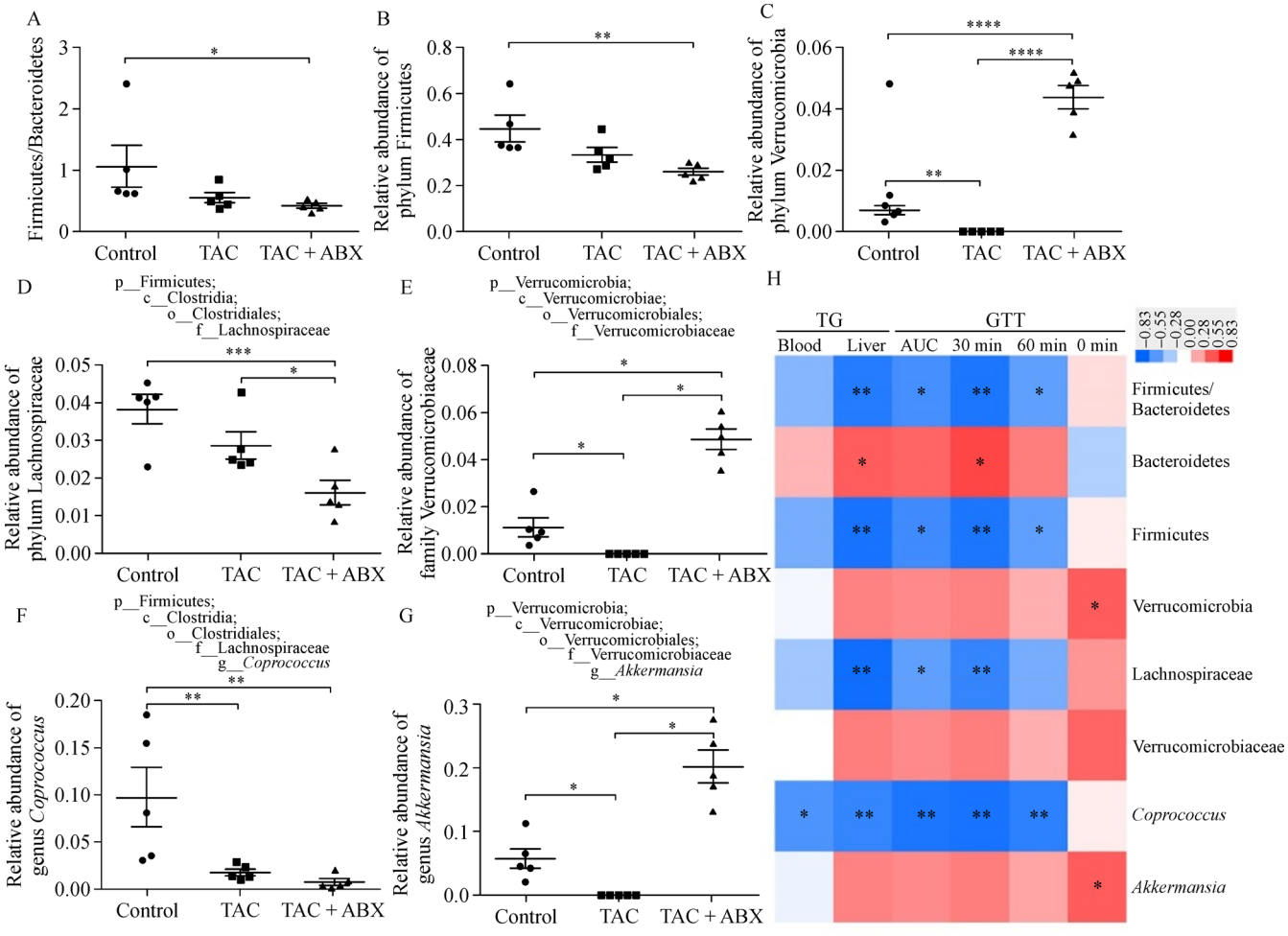

Fig. 4 Differing microbes among groups and its correlation with the parameters of glucose tolerance. (A) Ratio of Firmicutes to Bacteroidetes. Relative abundances of (B) Firmicutes and (C) Verrucomicrobia at the phylum level in each group. Relative abundances of (D) Lachnospiraceae and (E) Verrucomicrobiaceae at the family level in each group. Relative abundances of (F) *Coprococcus* and (G) *Akkermansia* at the genus level in each group. (H) Heatmaps of Pearson correlation analysis between the relative abundance of bacteria and AUC of GTT and lipid deposition in the liver. AUC of GTT indicates the value of the area under the curve in the glucose tolerance test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$. Date are expressed as mean ± SEM, $n = 5$ mice/group. Groups: blank control, Control; tacrolimus (2 mg/kg bw per day), TAC; tacrolimus and antibiotics, TAC + ABX.

TAC + ABX mice had significantly reduced carbohydrate metabolism-related functional categories ($P < 0.05$, Fig. 5A). The altered functional categories in level three KEGG pathways are summarized in Supplementary Table S2.

Further analyses showed that the altered functional genes of inferred metagenome induced by ABX were correlated with glucose metabolism. The AUC of GTT was positively related with microbial genes associated with lipid metabolism and biosynthesis and biodegradation of secondary metabolites genes ($P < 0.05$, Fig. 5C). The levels of blood glucose at 30 min of GTT were positively related with lipid metabolism, biosynthesis and biodegradation of secondary metabolites, amino acid metabolism, and metabolism of cofactors and vitamins genes ($P < 0.05$, Fig. 5C). The values at 15 min of GTT were positively related with metabolism of cofactors and vitamins genes ($P < 0.05$, Fig. 5C).

Moreover, we found that the IM functional categories related to lipid metabolism, biosynthesis and biodegradation of secondary metabolites, amino acid metabolism, and metabolism of cofactors and vitamins genes were significantly associated with liver TG levels ($P < 0.05$, Fig. 5C). These results suggested that the microbial metabolic function altered by ABX and TAC might play a role in hepatic lipid deposition and glucose disorders.

Discussion

Recent studies showed evidence of the diabetogenic IM induced by ABX and TAC in the pathogenesis of

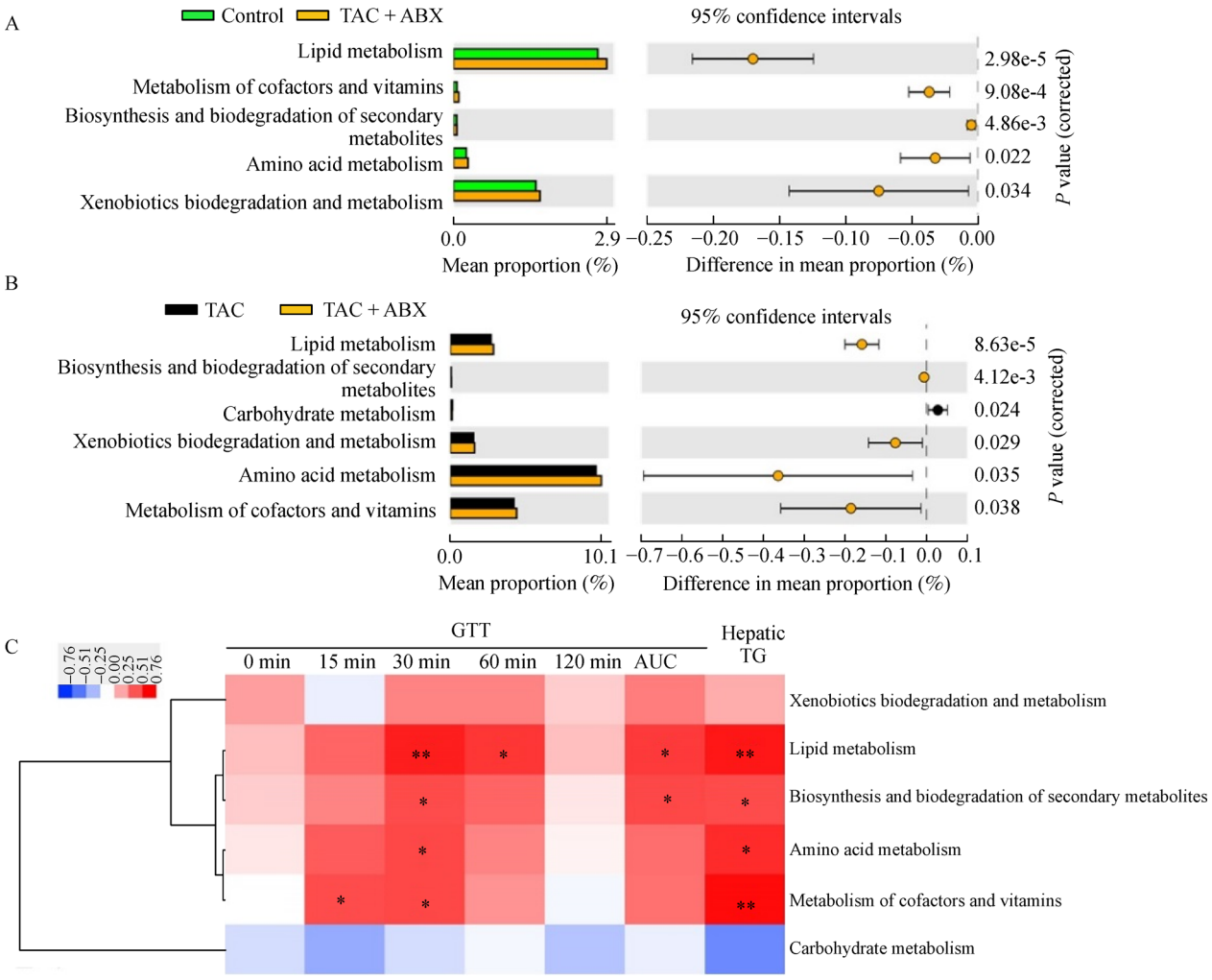

Fig. 5 Functional shifts of IM among groups and their relationship with the parameters of glucose tolerance. (A) KEGG pathway categories were inferred from 16S rRNA gene sequences using PICRUSt. Comparison of the KEGG functional categories for the case-enriched gene markers is shown by percentage. (B) Spearman correlation analysis between changed four functional categories and glycometabolism-related indices. $*P < 0.05$; $**P < 0.01$. $n = 5$ mice/group. Groups: blank control, Control; tacrolimus (2 mg/kg bw per day), TAC; tacrolimus and antibiotics, TAC + ABX. GTT: glucose tolerance test; AUC: the value of the area under the curve in GTT.

metabolic disorders [27–29]. Interest in the effect of the microbiome and ABX-induced dysbiosis on diabetes during treatment had heightened with the widespread use of TAC. However, whether ABX use could impact the diabetogenic effect of TAC on microbiome and glucose homeostasis remains unclear. Here, we found that ABX significantly increased hepatic lipid accumulation and glucose intolerance in TAC-treated mice. ABX further augmented the TAC-induced shift in gut microbiome, which enhanced metabolic capacities in particular for lipid metabolism. Furthermore, the altered microbiome and microbial function pathways were significantly correlated with hepatic lipid accumulation and glucose tolerance. This study provides clear clues pointing out that the combination of ABX and TAC exacerbated gut microbiota, which directly lead to glucose disorder in mice.

The influence of ABX on IM and glucose tolerance is controversial. The altered microbiota induced by ABX affects physiological and pathophysiological status, e.g., obesity and insulin resistance [19,30–32]. In mice fed a high-fat diet, ABX improved glucose homeostasis [33,34]. However, diabetogenic intestinal microbiomes induced by prolonged ABX accelerate diabetes in NOD mice [7,35]. In addition, Livanos *et al*. reported that ABX-mediated altered microbiome accelerates the development of type 1 diabetes in mice [36]. In humans, oral administration of vancomycin significantly reduces fecal microbial diversity and decreases insulin sensitivity [37]. In transplant patients, the administration of ABX and immunosuppressants is unavoidable to prevent immune rejection and

infections related to immune inhibition [38]. Therefore, investigating the effect of combined ABX and TAC on IM is a critical issue to understand the mechanisms underlying transplantation-related diabetes. Before performing human study, we investigated the combined effect of TAC and ABX treatment on IM and its mediated effect on glucose metabolism in a mouse model.

Based on the high-throughput 16S rRNA sequencing analysis, we found that ABX significantly augmented the TAC-induced alterations of microbiotal composition and metabolic capacities in mice. ABX further decreased the relative abundance of Firmicutes and the ratio of Firmicutes to Bacteroidetes and improved glucose tolerance in TAC-treated mice. In both human and animal gut, Firmicutes and Bacteroidetes are the dominant bacteria, accounting for approximately 99% of the whole microbiota, and correlated positively with blood glucose levels [39,40]. Thus, the findings indicated that the decreased Firmicutes in TAC- and ABX-treated mice might be crucial in the pathogenesis of glucose tolerance. In addition, we observed a significant inhibition of ABX on TAC-reduced family Lachnospiraceae and its genus *Coprococcus*. The family Lachnospiraceae is one of the predominant members of Firmicutes in both human and animal gut [41]. These bacteria produce short-chain fatty acids (SCFAs), which have health-promoting functions, including the production of energy substrates for the colonic epithelium (butyrate) [42,43], the maintenance of host immune homeostasis [44,45], and promotion of glucose homeostasis [46]. Reduction of SCFAs results in deterioration of intestinal integrity and increase of intestinal permeability [47,48]. The novelty of our study was the suggestion of a link between Firmicutes and the amplified effect of TAC on glucose disorders. Further studies to assess the impact of ABX and TAC on fecal SCFA are needed.

Akkermansia, Verrucomicrobiaceae, and Verrucomicrobia were the genus, family, and phylum that were significantly decreased by TAC in mice. Interestingly, ABX markedly restored the relative abundance of *Akkermansia* in TAC-treated mice. Moreover, ABX treatment significantly improved insulin secretion in TAC-induced diabetic mice. These findings suggested that *Akkermansia* might play a key role in insulin secretion. Several previous studies support our findings. Hansen *et al*. reported that vancomycin propagated *Akkermansia muciniphila* in NOD mice [49]. In addition, Dubourg *et al*. showed that broad-spectrum ABX treatment increased the proportion of *Akkermansia* in humans [50]. Recently, Shin *et al*. [51] reported that the administration of *Akkermansia* to murines displayed antidiabetic effects. More recently, Hanninen *et al*. found that *Akkermansia muciniphila* could induce IM remodeling and control the islet autoimmunity in NOD mice [52]. Further studies are required to assess how the lack of *Akkermansia* impacts glucose disorders.

To further explore how altered IM by ABX and TAC resulted in glucose disorders, we used PICRUSt to assess the metagenomic profile of IM [23]. We found that ABX reinforced the effect of TAC on four microbial metabolic pathways related to lipid, cofactor, vitamin, and amino acid metabolism, which were closely correlated with the improvement of hepatic lipid accumulation and glucose tolerance. The disorders of these metabolites, including lipid and amino acids, were observed in the onset and progression of diabetes [53,54]. Distinct microbiota and lipid metabolism-related genes were associated with hepatic lipid accumulation. The results strongly suggested the key role of IM in ABX- and TAC-induced glucose disorders.

The current study has several limitations. Although we observed distinct distributions of microbiota and its relationship with glucose metabolism, it remained unclear that how intestinal dysbiosis and its functional changes cause the hepatic lipid metabolic disorders and subsequently glucose disorders after TAC treatment. To date, we have not yet assessed differences in the fecal content of SCFAs and their role in the development of TAC-treated diabetes. As an immunosuppressor, TAC is used by transplant patients, and the currently used mouse models cannot mimic the disease situation in clinical practice. Establishing an ideal animal model is urgently required before initiating a successful translational study.

In summary, this novel study first found that the administration of ABX could amplify the TAC effect on IM profiles and functional categories, which were significantly associated with the hepatic lipid accumulation and glucose tolerance in mice. Our study also provided support for the link between depleted Firmicutes and glucose disorders induced by TAC treatment. The findings might open new avenues for the microbial therapeutic targets in diabetes, in particular for transplant patients receiving treatment of both ABX and TAC. For these patients, manipulation on IM by beneficial microbes, such as *Akkermansia muciniphila*, might overcome the effect of the diabetogenic microbiome.

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Compliance with ethics guidelines

Yuqiu Han, Xiangyang Jiang, Qi Ling, Li Wu, Pin Wu, Ruiqi Tang, Xiaowei Xu, Meifang Yang, Lijiang Zhang, Weiwei Zhu, Baohong Wang, and Lanjuan Li declare that they have no conflicts of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-019-0686-8 and is accessible for authorized users.

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