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CFD protein deficiency induce slow transit constipation is correlated with gut microbial dysbiosis

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Abstract

Dysregulation of complement factor D (CFD) has been associated with various diseases, such as metabolic, cardiovascular, and renal disorders. However, its role in intestinal function has been less studied. Our research found that CFD-deficient (*Cfd*$^{-/-}$) mice exhibited spontaneous slow transit constipation(STC) compared to wild-type (WT) mice. Moreover, the fecal

weight and water content in *Cfd*$^{-/-}$ mice were significantly reduced, despite no specific changes in feeding behavior. Reduced C-kit protein expression, colon injury, complement dysfunction and dysbiosis of the fecal microbiota are also observed in *Cfd*$^{-/-}$mice. After microbiota transplantation from WT mice into *Cfd*$^{-/-}$ mice, reduce stool output, water content, decreased C-kit protein expression and colon injury were significantly improved. These results indicate that CFD is critical for controlling microbial homeostasis in the colon and, in its absence, leads to colon injury and promotes slow transit constipation.

Keywords: complement factor D, slow transit constipation, microbiota, colon injury, C-kit

Introduction

The key rate-limiting enzyme for the alternative pathway (AP) of the three main complement activation pathways is complement factor D (CFD), a 24 kDa serine protease[1,2]. Its primary job is to cleave complement B factor (CFB) in the AP, which starts the complement initiation and amplification phases, eliminating pathogens (Figure 1A). The complement cascade and amplification effects start when Bb binds to C3b after CFD cleaves CFB to produce Ba and Bb[3]. Continuous activation of the complement AP allows the body to maintain proper homeostasis; over or under activation of this

system can result in a variety of illnesses, including atypical hemolytic uremic syndrome[4], glomerular diseases[5], and aging-related diseases[6].

Constipation has increased in frequency and steadily decreased people's quality of life in recent years as a result of dietary and lifestyle changes[7]. The two main categories of constipation are functional constipation and secondary constipation. Among functional constipation, slow transit constipation (STC) is the most prevalent kind[8]. STC is caused by a combination of slow peristalsis and decreased intestinal motility, which results in fewer feces, but with no alteration in bowel diameter[9]. Reduced gut motility results from both a decrease in the number and activity of interstitial Cajal cells (ICCs), mostly from a decrease in the expression of C-Kit receptors on their cell surface[10,11]; in other words, STC is caused by damage to the C-kit protein. Moreover, there is increasing evidence that changes in the gut microbiota accompany constipation [12-15], although these alterations in the intestinal microbiota are not consistent among patients with constipation. The intestinal microbiota can decrease the 12.4-hour colon transit time, increase the frequency of bowel movements, and alleviate symptoms associated with constipation, according to existing meta-analyses[16]. Clinically, patients with constipation will have their intestinal microbes returned to normal by the isolation and transplantation of fecal bacteria from healthy individuals. This has been shown to be a safe and effective way to accomplish therapeutic goals[17,18].

However, the majority of research on constipation has focused on the development of therapeutic medications and their mechanisms of action, with only a small number of recent studies investigating the causes of constipation and yielding inconsistent results[18-20]. Therefore, understanding the mechanisms by which constipation occurs is important.

Recent studies have shown that there is no change in the feeding behavior of complement *C3*$^{-/-}$ mice compared to wild-type(WT) mice, but they have reduced fecal water content, decreased intestinal transfer rates, and altered colon histology and mucin secretion, which is consistent with a constipation phenotype, suggesting that C3 deficiency may play an important role in the constipation phenotype in mice[21]. Further studies revealed that intestinal microbial disorders in mice caused by C3 deficiency are ameliorated by transplanting fecal microbes from WT mice to *C3*$^{-/-}$ mice, suggesting that microbial disorders may be a significant factor in constipation in *C3*$^{-/-}$ mice. Additionally, a lack of C3 causes the colonic neural system to become dysregulated, which includes aberrant Cajal mesenchymal stromal cells. Constipation in *C3*$^{-/-}$ mice is also partially caused by the 5-hydroxytryptamine (5-HT) concentration, acetylcholine (ACh) concentration, acetylcholinesterase (AChE) activity, and suppression of 5-HT receptor expression[22,23]. From the above studies, we know that C3 deficiency leads to chronic constipation, which is partly due to intestinal microbial disorders, and that AP in the complement system

plays a crucial role in killing pathogenic bacteria; thus, the relationship between the complement system and constipation production needs to be further explored.

In this study, we generated *Cfd* gene knockout mice, the key rate-limiting enzyme in the complement alternative pathway. The results showed that the mice developed constipation and intestinal microbiota dysbiosis, providing a new perspective for studying the relationship between the complement system and intestinal function.

Results

1. Impaired intestinal motility in *Cfd*$^{-/-}$ mice

We first generated *Cfd*$^{-/-}$ mice using the CRISPR/Cas9-mediated method (Figure 1B), and then, we utilized ELISA to measure the amount of CFD protein in the mouse serum. The successful production of *Cfd*$^{-/-}$ mice was confirmed by the very low expression of CFD protein in the serum of *Cfd*$^{-/-}$ mice compared with that of WT mice (Figure 1C). During feeding, we found that compared with those of WT mice, number of fecal pellets of *Cfd*$^{-/-}$ mice were significantly lower (Figure 1D). After that, we evaluated the fecal characteristics of the mice. The stool output and water content decreased in *Cfd*$^{-/-}$ mice (Figure 1E), and the intestinal transit time increased (Figure 1F). These findings suggest that *Cfd*$^{-/-}$ mice have chronic constipation. We measured the length of the intestines of WT and *Cfd*$^{-/-}$

mice and tested the C-kit expression level to determine which type of constipation occurred in these mice. The intestines of the two species of mice did not differ in length (Figure 1G), but the intestines of *Cfd*$^{-/-}$ mice presented significantly lower levels of C-kit protein expression (Figure 1H), this protein has been shown to be associated with STC[24-26]. These findings suggest that the CFD protein plays an important role in constipation.

2. Colonic injury in *Cfd*$^{-/-}$ mice

To further understand the cause of the constipation phenotype in *Cfd*$^{-/-}$ mice, small intestine, and colon histological structures were compared in WT and *Cfd*$^{-/-}$ mice to determine whether organ abnormalities resulted from CFD protein deficits. The histological anatomy of the small intestine did not differ much, but we did find some inflammatory infiltration in the colon of *Cfd*$^{-/-}$ mice (Figure 2A). Subsequently, we detected the mRNA expression of inflammation factors IL-17, and IL-6, and observed a significant increase in IL-17 and IL-6 mRNA expression levels in *Cfd*$^{-/-}$ mice (Figure 2B) , which is consistent with what other researchers have observed in constipation model colon[27,28]. We detected the MUC2 protein associated with the mucosal barrier, found that the staining of MUC2 in the colon of *Cfd*$^{-/-}$ mice was weaker than that of WT mice by immunohistochemistry (IHC) (Figure 2C). Last, because of the role of CFD protein in the complement alternative pathway is to initiate and

amplify complement activation by acting on C3 cleavage products, we evaluated the expression of intestinal C3 cleavage product (C3b, iC3b and C3c). The results indicate increased deposition of C3b, iC3b, and C3c in the intestines of *Cfd*$^{-/-}$ mice (Figure 2D). These phenomena indicate that CFD protein deficiency can cause colon injury and colon complement dysfunction.

3. Alterations in the profile of the fecal microbiota of *Cfd*$^{-/-}$ mice

Colon injury and complement dysfunction usually causes microbial dysbiosis, and numerous studies have demonstrated that intestinal microbiota dysbiosis is the main cause of STC[17]. To determine whether constipation in *Cfd*$^{-/-}$ mice is associated with gut microbes, we cultured microbes from the feces of *Cfd*$^{-/-}$ and WT mice under aerobic and anaerobic conditions. The number of colonies in the feces of WT mice was significantly greater than that in the feces of *Cfd*$^{-/-}$ mice under both aerobic and anaerobic conditions (Figure 3A). To further demonstrate the disruption of gut microbes in *Cfd*$^{-/-}$ mice, mice fecal microbes were analyzed by 16S rDNA sequencing. We then analyzed the α and β diversity, which revealed that the α and β diversity of *Cfd*$^{-/-}$ mice were significantly different from those of WT mice and that the α diversity of *Cfd*$^{-/-}$ mice was significantly lower than that of WT mice. Compared to the WT mice, the

indexes of ACE (P=0.0376) and Chao (P=0.0296) otu were reduced significantly in *Cfd*$^{-/-}$ mice, and the indexes of Sobs (P=0.0657) otu were reduced even if it not significance(Figures 3B). The principal component analysis (PCA) , principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS) results revealed significant differences in gut microbiota composition at the genus level between *Cfd*$^{-/-}$ mice and WT mice (Figure 3C). At the phylum level, the relative abundances of *Verrucomicrobiota* and *Fimicutes* increased, whereas the relative abundance of *Bacteroidota* decreased in *Cfd*$^{-/-}$ mice (Figure 3D). At the genus level, *Lachnospiraceae_NK4A136_group*, *Rikenellaceae_RC9_gut_group*, *Alistipes*, and *Akkermansia* were increased, whereas *norrank_f_Murbaculaceae, Allprevotella* and *unclassified_f_Lachnospiraceae* were decreased in *Cfd*$^{-/-}$ mice (Figure 3E). Finally, we performed Spearman correlation analyses to investigate the relationship between the abundance of significantly altered microbial taxa and key physiological parameters, including intestinal transit rate, IL-17 and IL-6 mRNA levels, and C-kit protein expression. As shown in Figure 3F, *Akkermansia* and *Rikenellaceae_RC9_gut_group* abundances were positively correlated with intestinal transit rate and IL-17/IL-6 mRNA expression, whereas *Prevotellaceae_NK3B31_group* was positively correlated only with IL-17 and IL-6 mRNA levels. *Norank_f__Muribaculaceae* abundance was specifically positively

correlated with IL-17 mRNA, and *Alloprevotella* abundance was negatively correlated with intestinal transit rate and IL-17/IL-6 mRNA expression. In contrast, *Akkermansia*, *Rikenellaceae_RC9_gut_group*, and *Prevotellaceae_NK3B31_group* were negatively correlated with C-kit protein expression, whereas *Alloprevotella* showed a positive correlation. These findings indicate that CFD deficiency reshapes gut microbiota composition, which in turn is associated with altered intestinal transit, inflammatory cytokine expression, and C-kit protein levels.

4. Effects of FMT on fecal parameters in *Cfd*$^{-/-}$ mice

To investigate the role of the commensal microbiota in the occurrence of slow transit constipation in *Cfd*$^{-/-}$ mice, *Cfd*$^{-/-}$ mice were treated with antibiotics to deplete their microbiota once a day for 5 days before FMT, collect mice feces and colon tissue 6 hours after the last gavage of fecal bacteria (Figure 4A). After which the number of bacterial colonies and the fecal parameters were analyzed. The number of bacterial colonies was lower after antibiotics treated , and higher when Abx-*Cfd* $^{-/-}$ mice receiving fecal microbiota from WT mice (Figure 4B). The stool output, fecal water content and number of fecal pellets were significantly greater in the FMT-*Cfd*$^{-/-}$ mice than in the *Cfd*$^{-/-}$ mice before treatment (Figure 4C). Compared to *Cfd*$^{-/-}$ mice, no inflammatory infiltration was observed in the colon of FMT-*Cfd*$^{-/-}$ mice after HE staining (Figure 4D), and IL-17, IL-6

mRNA, MUC2 protein expression return to normal level (Date not show). Finally, the expression levels of the C-kit protein in the colon were measured in FMT-*Cfd*$^{-/-}$ mice, and the expression of the C-kit protein returned to normal levels (Figure 4E). The above results indicate that the intestinal constipation in *Cfd*$^{-/-}$ mice can be improved by FMT with a normal microbiota.

Discussion

To date, the associations between CFD proteins and several disorders have been investigated in *Cfd*$^{-/-}$ mice. On the one hand, compared with those in WT mice, the number of development and metastasis of breast cancer cells in *Cfd*$^{-/-}$ mice is lower[29,30]. On the other hand, CFD proteins play a significant role in preventing kidney and liver damage[31,32]. However, no reports have demonstrated an association between CFD proteins and the development of constipation. Currently, in the predominantly loperamide hydrochloride-induced mouse model of constipation[33-35], few studies of constipation have been conducted using knockout mice; thus, the current study is significant because it represents a new effort to use the KO mouse model to determine the etiology of constipation, with an emphasis on the intestinal microbiota and complement activation. Our results offer the first scientific evidence that the production of constipation-related phenotypes may be intimately linked to CFD deficit.

Our study shows that *Cfd*$^{-/-}$ mice exhibited slowed intestinal transit rates, elevated mRNA expression of inflammatory factors IL-17 and IL-6, the expression of C-kit and MUC2 protein in the intestine decreases, complement dysfunction and gut microbiota changes occur. Several studies have revealed that intestinal microbes are disrupted in individuals with constipation, but there is no consensus on exactly which microbiota plays a major role in the development of constipation[36,37]. Longer intestinal transit times are associated with specific gut microbiota, such as *Akkermansia*, *Bacteroides*, *Alistipes*[38]. An increase in Firmicutes and a decrease in Bacteroidetes reduces intestinal IL-17 and IL-6 expression[39], meanwhile in the presence of *Salmonella enterica Typhimurium*, *Akkermansia* can promote the production of inflammatory factors, including IL-17 and IL-6[40]. In a rat model of constipation, treatment with fenchone alleviated the constipation phenotype while simultaneously increasing intestinal C-kit protein expression and the abundance of *Bacteroides*, *Enterococcus*, *Alistipes*, and *Escherichia-Shigella* bacteria[41]. Other researchers have also reported that abnormal expression of C-kit protein in constipation models is often accompanied by gut microbiota disorders[41-43]. In previous studies, the genus *Akkermansia* has generally been regarded as a beneficial bacterium, showing a positive correlation with MUC2 expression[44], however, we observed a negative correlation in our study. We believe the reason for this phenomenon may be MUC2

expression express is not influenced by a single bacterium but rather by the interactions among multiple microbial communities. As reported in other studies, MUC2 expression increased along with enhanced abundance of *norank_f__Muribaculaceae* and *Alloprevotella* [45,46], In our *Cfd*$^{-/-}$ mice, the abundance of *norank_f__Muribaculaceae* and *Alloprevotella* was decreased, which may account for the reduced MUC2 expression despite increased *Akkermansia*.

In clinical trials, CFD haploinsufficiency has also been reported to result in a reduced rate and a weakened ability of C3 to bind and kill pathogens[47]. Consistent with previous studies, we found that, in *Cfd*$^{-/-}$ mice, their gut microbes were disorganized with decreased diversity[48]. The abundance of *Akkermensia*, *Lachnospiraceae_NK4A136_group*, *Alistipes*, and *Rikenellaceae_RC9_gut_group* in the feces of *Cfd*$^{-/-}$ mice was significantly higher and *norrank_f_Murbaculaceae*, and *Prevotellaceae_UCG-001* lower than that of WT mice in the current study. This is in line with other researchers' earlier findings in the feces of patients who were constipated[49-54].

To demonstrate that constipation in *Cfd*$^{-/-}$ mice is caused by gut microbiota, we conducted FMT experiments. Following FMT, the constipation phenotype remission, and colon C-kit proteins were restored to normal levels in *Cfd*$^{-/-}$ mice, which is consistent with the findings of fecal transplantation in *C3*$^{-/-}$ mice reported by other researchers[22]. In our study,

we found that compared to WT mice, *Cfd*$^{-/-}$ mice exhibited increased levels of C3 degradation products C3b, iC3b, and C3c in the gut, C3 dysfunction. Owing to the AP function and upstream-downstream relationship in the complement cascade reaction[55], we hypothesized that CFD protein deficiency leads to C3 dysfunction and reduced complement bactericidal function, which causes gut microbial disorders, decreased intestinal motility, and constipation production.

In conclusion, we observed a constipation phenotype in *Cfd*$^{-/-}$ mice. Our research revealed that *Cfd*$^{-/-}$ mice have reduced intestinal motility, aberrant intestinal complement activation, and disrupted intestinal microbes. Based on our experimental results, we believe that the deficiency of intestinal CFD protein may lead to a decrease in the ability of intestinal complement to kill pathogens, causing disruption of intestinal microbiota, inflammation, damage to C-kit protein, and appearance of constipation phenotype. However, our research still has limitations, for example, in this experiment we used whole-body KO mice rather than mice with gut-specific CFD KO. Whether gut-specific CFD deficiency alone can cause the constipation phenotype and C-kit protein damage in mice remains unconfirmed. Additionally, in our FMT experiments, although FMT from WT mice improved the constipation phenotype in *Cfd*$^{-/-}$ mice, we have not yet confirmed whether the fecal microbiota of FMT-treated mice resembles that of WT mice. The basis for future study on the precise mechanisms

underlying gut microbiota disorder and C-kit protein damage has been established by our preliminary findings. Notably, while our results demonstrate that FMT from WT donors can effectively rescue the constipation phenotype and alleviate C-kit damage in *Cfd*$^{-/-}$ mice, this finding primarily underscores the therapeutic potential of a healthy microbiota. It does not definitively prove that the *Cfd*$^{-/-}$ associated dysbiosis is the primary requirement for disease onset. The observed rescue could potentially stem from general anti-inflammatory signals or metabolic compensations provided by the WT microbiota that mask the intrinsic defects of the KO host, leaving the causal necessity of the *Cfd*$^{-/-}$ disrupted microbiota to be further elucidated through reciprocal transplantation studies. In subsequent experiments, we will employ high-throughput sequencing to characterize the microbiota of WT, *Cfd*$^{-/-}$ mice, and FMT-treated *Cfd*$^{-/-}$ mice. We aim to identify the microbial communities most strongly associated with the constipation phenotype and validate whether they induce increased intestinal transit rates, intestinal inflammation, and C-kit protein damage. This will help elucidate and validate the specific mechanisms by which CFD protein deficiency leads to constipation.

Materials and methods

Mice. *Cfd*$^{-/-}$ mice were purchased from Nanjing University Model Animal Research Center (Nanjing, China), and WT mice (without CFD mutation)

were littermates of CFD-mutated mice. All the mice had a C57BL/6J background and were maintained under SPF and controlled conditions (22°C, 55% humidity, and 12-hour day/night cycles). After breeding and maintenance for up to 16 weeks, the WT and *Cfd*$^{-/-}$ mice were used in the analysis of constipation, and only male mice were used in all the mouse experiments. All animal care and experimental procedures were performed in accordance with the institutional guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Shanghai Tenth People's Hospital (Approval number: [SHDSYY-2025-6786]), and conducted in accordance with the standards of the International Association for the Study of Pain (IASP). The work is also reported in line with the ARRIVE guidelines 2.0. Following fecal sample collection, mice were returned to their original cages and maintained normally. Prior to collection of intestinal tissues, mice were sacrificed by cervical dislocation. All mice were finally euthanized by cervical dislocation after the completion of all experiments.

Hematoxylin & eosin (H&E) staining. For histological examination, mouse tissue samples were fixed in 10% neutral buffer formalin and embedded in paraffin. Four-micron-thick sections were stained with hematoxylin and eosin.

Immunohistochemistry (IHC). For IHC staining, sections were incubated with 2% hydrogen peroxide to block endogenous peroxidase for 15 min, Sodium citrate repair boil for 20-25min, blocking with 10% goat serum for 30min, incubated with rabbit anti-MUC2 (1:500; Abcam, ab134119) at 4℃ overnight. After rinsing in PBS, the sections were incubated with HRP-conju-gated goat anti-rabbit IgG H&L (1:200; Abcam, Cambridge, MA) at room temperature (RT) for 1 h. The detection of immunoreactivity was performed using a GTVision III immunohistochemical detection kit (GK500705; Gene Tech, Shanghai, China).

Immunofluorescence(IF). For IF staining, the previous steps are the same as IHC. Then, incubated with rabbit anti-C3b, iC3b, C3c (1:300; Hycult, HM1065) at 4℃ overnight. After rinsing in PBS, the sections were incubated with AF488-conju-gated goat anti-rabbit IgG H&L (1:500; Beyotime, A0423) at room temperature (RT) for 1 h. Subsequently, DAPI (Beyotime, C1005)stained the cell nucleus for 15 minutes, and after elution, it was observed under a fluorescence microscope.

Quantitative real-time polymerase chain reaction. Total RNA was extracted from the colon tissue using the TransZol Up Plus RNA Kit (TransGen Biotech, Beijing, China) and reverse-transcribed as previously described[56]. The gene expression levels were measured by q-PCR using the

commercially available SYBR Green Premix (Accurate Biology, Changsha, China) according to the manufacturer's instructions. Gene expression levels were quantified using the ΔΔCt method. The following primers were synthesized by Tsingke Biology (Nanjing, China): *β-actin* (forward 5′-CCACTGTCGAGTCGCGTCC-3′, reverse 5′-ATTCCCACCATCACACCCTGG-3′), *IL-17* (forward 5′-TCAGCGTGTCCAAACACTGAG-3′, reverse 5′-CGCCAAGGGAGTTAAAGACTT -3′), *IL-6* (forward 5′-CTGCAAGAGACTTCCATCCAG-3′, reverse 5′-AGTGGTATAGACAGGTCTGTTGG-3′).

Fecal water content analyses. The feces of different groups of mice were collected, weighed to determine the wet weight, and subsequently dried in an oven at 80°C to measure the dry weight, moisture content = (wet weight - dry weight)/wet weight × 100%.

Intestinal transit rate analyses. The mice in both groups were gavaged with 0.2 mL of ink and fed normal water, the color of the stools was observed, and the time of the first black stool was recorded.

Fecal microbiota culture. The feces of different groups of mice were collected in sterile PBS. The feces were subsequently crushed, filtered and

centrifuged, and the supernatant was inoculated into a bacterial culture plate (containing tryptone (10 g/L), yeast powder (5 g/L), NaCl (10 g/L), and agar powder (15 g/L)) and then cultured under aerobic and anaerobic conditions at 37°C for 72 h.

Analysis of fecal microbiota. Fresh feces pellets were collected from WT mice and *Cfd*$^{-/-}$ mice using sterile tools, ensuring minimal contamination, and total microbial genomic DNA was extracted by the E.Z.N.A. The DNA Kit (Omega Bio-Tek, Norcross, GA, USA) was used according to the manufacturer's instructions. DNA concentrations were measured using the Nanodrop-1000 instrument (Thermo Fisher Scientific), and DNA quality was assessed by agarose (0.8%) gel electrophoresis. The V3–V4 hypervariable region of the 16S rDNA gene was amplified with barcode fusion primers (338F: 5-ACTCCTACGGGAGGCAGCAG-3, 806R: 5-GGACTACHVGGGTWTCTAAT-3) with a 56°C annealing temperature. After purification, the PCR products were used to construct libraries and sequenced on the Illumina MiSeq platform (Illumina) at Major Bio. The data were analyzed on the online platform of the Majorbio Cloud Platform (www.majorbio.com).

Fecal microbiota transplantation. 16-week-old male *Cfd*$^{-/-}$ mice underwent a 5-day gut microbiota clearance regimen: Once daily via

intragastric administration of a combination antibiotic regimen (vancomycin 100 mg/kg, neomycin sulfate 200 mg/kg, metronidazole 200 mg/kg, and ampicillin 200 mg/kg), each dose administered as a 200μL bolus[57]. Feces from the same age of WT mice were collected and resuspended in PBS at 0.125 g/mL, then 200μL of this suspension was administered to *Cfd*$^{-/-}$ mice by oral gavage once a day for 5 days. Last, collect mice feces and colon tissue 6 hours after the last gavage of fecal bacteria.

Statistical analysis. Student's t test was performed to analyze the significance of differences between two groups, and a value of $p < 0.05$ was considered statistically significant. All the data were analyzed using GraphPad Prism 6.0 software (GraphPad Software, Inc., San Diego, CA) and are expressed as the means ± SEMs.

Date availability. The datasets analyzed during the current study are available in the SRA repository, https://www.ncbi.nlm.nih.gov/sra/PRJNA1425553.

Conflicts of interest. The authors have declared no conflict of interests.

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Author contributions. Conceptualization: Qing Wei; methodology: Qing Wei and Dengfeng Huang; validation: Dengfeng Huang and Shouxin Hu; formal analysis: Shouxin Hu and Hu Liu; investigation: Shouxin Hu and Hu Liu; data curation: Dengfeng Huang; writing—original draft preparation: Shouxin Hu; writing—review and editing: Qing Wei and Dengfeng Huang; supervision: Qing Wei; project administration: Feifei Song; funding acquisition: Feifei Song.

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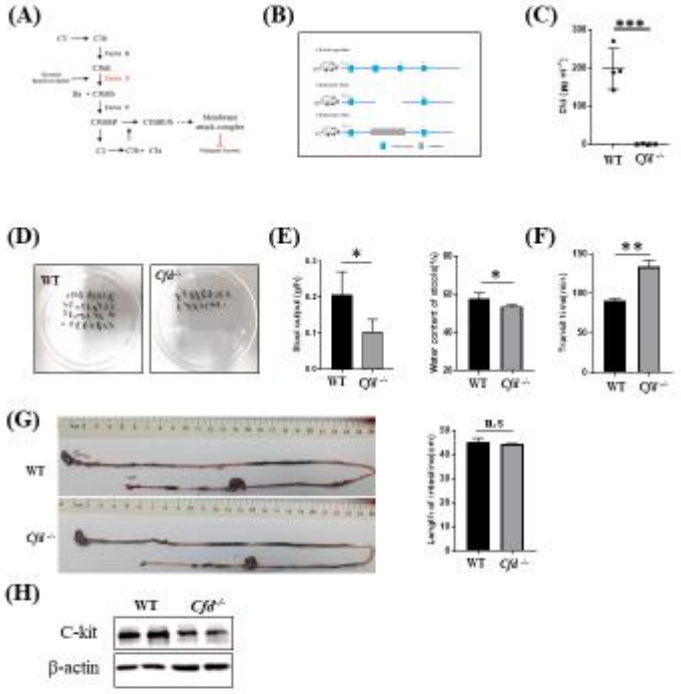

Figure1. Construction of *Cfd*$^{-/-}$ mice and their fecal parameters .
A, Alternative pathway. B, Construction of *Cfd*$^{-/-}$ mice. C, Quantification of CFD protein expression in serum of WT and *Cfd*$^{-/-}$ mice. D, Number of feces per hour in WT mice and *Cfd*$^{-/-}$ mice, 4-5 mice per group. E, Stool output and water content of stools in WT mice and *Cfd*$^{-/-}$ mice , 4 mice per group. F and G, Intestinal transit time and length in WT mice and *Cfd*$^{-/-}$ mice, 4 mice per group. H, The protein expression was examined by Western blot with the indicated antibodies after lysing colon tissues from mice, and β-actin was used as loading control. *, $P< 0.05$; **, $P< 0.01$; ***, $P< 0.001$. n.s.: not significant.

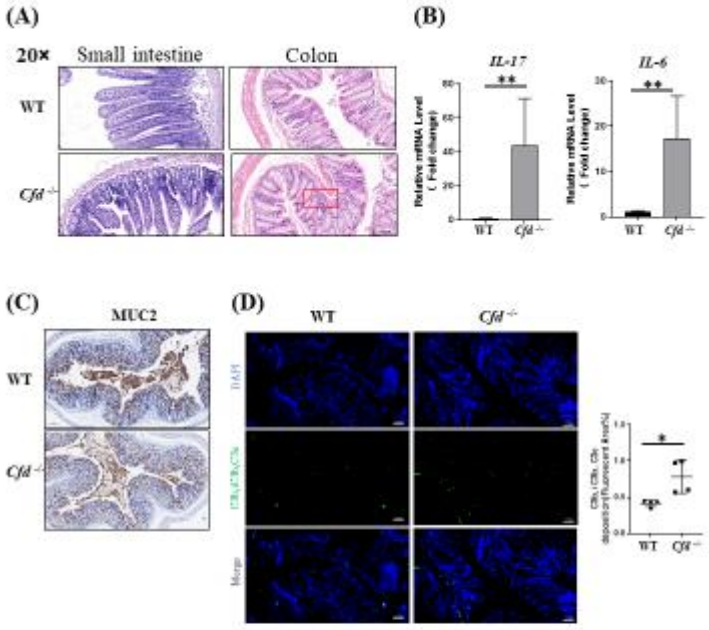

Figure2. The colon of *Cfd*$^{-/-}$ mice had inflammatory cell infiltration and high inflammatory factor expression.

A, Histopathological structure of small intestine, and colon of WT and *Cfd*$^{-/-}$ mice. B, IL-17, and IL-6 mRNA levels in the colon were measured by real-time PCR, n = 6. C, Representative IHC staining of MUC2 in colon of WT and *Cfd*$^{-/-}$ mice. D, Representative images of immunofluorescence analysis and quantitation of C3 cleavage product (C3b, iC3b, C3c) deposition in colon of WT and *Cfd*$^{-/-}$ mice. *, $P< 0.05$; **, $P< 0.01$.

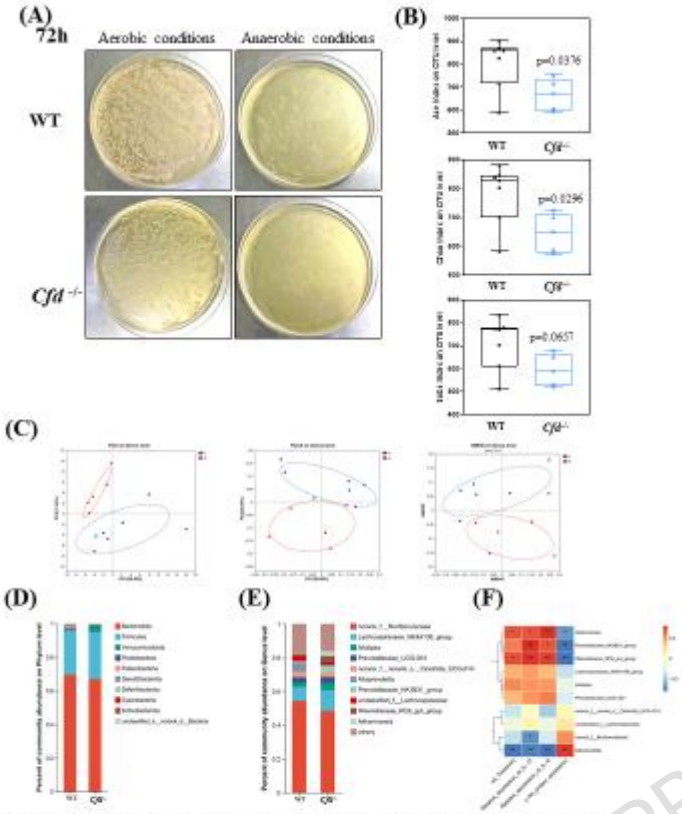

Figure3. Characterization of WT and *Cfd*$^{-/-}$ mice fecal microbiota. A,WT and *Cfd*$^{-/-}$ mice fecal microbiota were incubated for 72 hours in aerobic and anaerobic conditions. B, α-diversity analysis of fecal microbiota composition in WT and *Cfd*$^{-/-}$ mice. C, β-diversity analysis of fecal microbiota composition in WT and *Cfd*$^{-/-}$ mice. D and E, Microbiota composition of fecal microbiota and relative abundance (>0.01) at phylum and genus levels. F, Correlation analysis of transit rate, IL-17 and IL6mRNA, and C-kit protein expression with gut microbiota using Spearman analysis. *, P< 0.05; **, P< 0.01.

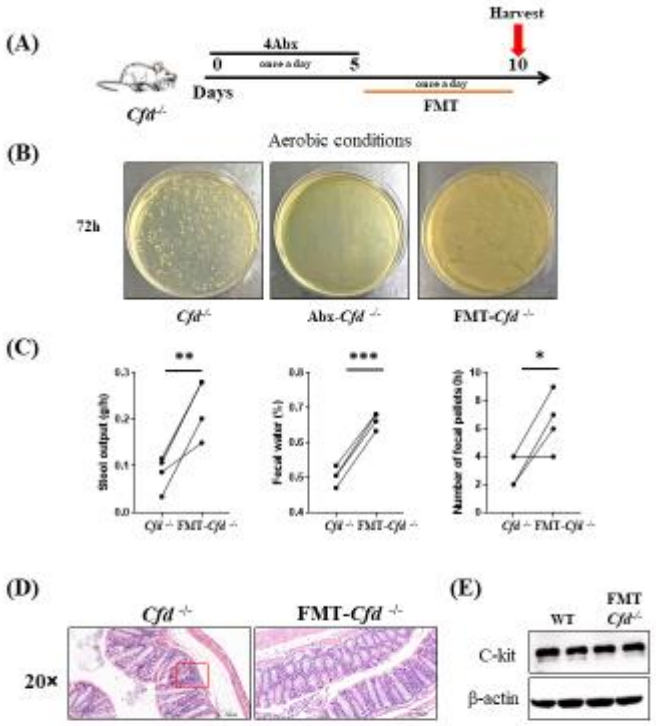

Figure4. Fecal parameters and C-kit expression in FMT mice.
A, FMT-*Cfd*$^{-/-}$ mice treatment schedule. B, *Cfd*$^{-/-}$, Abx-*Cfd*$^{-/-}$ and FMT-*Cfd*$^{-/-}$ mice fecal microbiota were incubated for 72 hours in aerobic conditions. C, Stool output, water content and fecal counts of *Cfd*$^{-/-}$ mice before and after treatment. D, Histopathological structure of colon of *Cfd*$^{-/-}$ and FMT-*Cfd*$^{-/-}$ mice. E, The protein expression was examined by Western blot with the indicated antibodies after lysing colon tissues from mice, and β-actin was used as loading control. *, $P< 0.05$; **, $P< 0.01$; ***, $P< 0.005$.