Egyptian Journal of Medical Human Genetics

RESEARCH

Open Access

Causal associations between gut microbiota and chronic prostatitis/chronic pelvic pain syndrome: a two-sample Mendelian randomization study

Hao Xu[1,2†], Yu Zhang[1,3†], Yinglang Zhang[1,4], Chong Shen[1], Zhe Zhang[1], Jian Wang[1], Diansheng Zhou[1], Zhouliang Wu[1], Yunkai Qie[1], Shenglai Liu[1], Dawei Tian[1], Hailong Hu[1] and Changli Wu[1*]

Abstract

Background Recent researches have increasingly indicated a strong correlation between the gut microbiota and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Nevertheless, the impact of gut microbiota on CP/CPPS still requires further elucidation.

Methods Employing the summary statistics provided by the MiBioGen consortium, we executed a two-sample Mendelian randomization (MR) analysis. The study involved 18,340 participants and considered gut microbiota as the instrumental variable. Chronic prostatitis summary statistics, representing 500 cases and 208,308 controls, were extracted from the GWAS Catalog release data as the disease outcome. Various methods, including weighted inverse variance, MR-Egger and weighted median, were employed to assess how gut microbiota interact and correlate with CP/CPPS. Sensitivity analysis was used to eliminate heterogeneity and horizontal pleiotropy.

Results Our findings, primarily derived from the IVW approach, provided evidence for a causal link between five categories of gut microbiota and CP/CPPS. Resultantly, the genus *Christensenellaceae* (OR = 0.39, 95% CI 0.17–0.87, *P* = 0.02), genus *Eisenbergiella* (OR = 0.62, 95% CI 0.40–0.97, *P* = 0.04), genus *Hungatella* (OR = 0.49, 95% CI 0.28–0.85, *P* = 0.01) and genus *Terrisporobacter* (OR = 0.39, 95% CI 0.20–0.75, *P* = 0.00) exhibited a protective impact on CP/CPPS, while family *Prevotellaceae* (OR = 1.78, 95% CI 1.01–3.15, *P* = 0.05) had the opposite effect. No notable heterogeneity of instrumental variables or horizontal pleiotropy was detected.

Conclusions The findings of this study, which used a two-sample Mendelian randomization approach, indicate a causal link between gut microbiota and CP/CPPS. This could be valuable in offering fresh perspectives for additional mechanistic and clinical investigations of microbiota-related CP/CPPS. Nevertheless, additional randomized controlled trials are necessary for validation.

Keywords Gut microbiota, Chronic prostatitis, Mendelian randomization, Causal associations

† Hao Xu and Yu Zhang have contributed equally to this work.

*Correspondence:
Changli Wu
zkxh_1989@163.com
Full list of author information is available at the end of the article

Introduction

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a common condition that affects a significant number of young males, with reported prevalence rates varying from 1.8 to 8.2% [1]. The diagnosis and treatment of this condition have presented a considerable clinical difficulty due to the uncertain cause and the varied and diverse symptoms. Manifesting frequently as persistent and recurring pelvic or systemic pain episodes, lower urinary tract dysfunction, and diminished quality of life stemming from depression and anxiety are common occurrences in patients. Despite being nonlethal, it affects millions of people globally, while the potential mechanism of CP/CPPS is unknown.

The largest microecosystem within the human body is the gut microbiota, and it is intricately linked to metabolic processes, immunity regulation, and the maintenance of stability in the intestinal mucosal barrier [2]. Furthermore, the gut microbiota assume a pivotal role as an integral component of the broader intestinal microbial system [3]. A wealth of empirical evidence supports the notion that individuals encountering simultaneous challenges affecting both the bladder and the intestines, notably conditions like functional bowel disorders and inflammatory intestinal conditions, are subject to notable health implications [4]. Moreover, the gut-bladder axis, a widely recognized interaction between the gut and bladder, may play a crucial role in altering bladder activity and vice versa. Hence, investigating the link between gut microbiome and CP/CPPS could offer novel avenues and concepts for preventing and treating of CP/CPPS.

The cause-and-effect link between exposure and result can be deduced employing a single nucleotide polymorphism (SNP) as an instrumental variable (IV) through the statistical approach called Mendelian randomization (MR) [5]. In contrast to observational investigations, MR studies possess the capacity to eliminate confounding factors and counteract reverse causation through the haphazard distribution of genetic variations. This allows for the simulation of randomized controlled trials (RCTS) and prevents the interference of reverse causality and potential confounders commonly faced in traditional RCTS [6]. In the current landscape of scientific research, the widespread adoption of MR analysis stands out as a prominent methodology extensively used to evaluate the conceivable cause-and-effect association between the composition and dynamics of the gut microbiota and illness [7–10]. The objective of this investigation was to scrutinize how gut microbiota influence CP/CPPS, through an exhaustive two-sample MR analysis. This analysis made use of the summary statistics from genome-wide association studies (GWAS) provided by the MiBioGen and GWAS Catalog consortiums, presenting innovative biomarkers for the clinical management of CP/CPPS.

Materials and methods

Study design

In this study, gut microbiota were used as exposure factors, SNPs that were significantly related to gut microbiota were selected as the IVs. The outcome variable utilized was chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). Two-sample MR method was employed for the causality analysis. Cochran's Q test was performed to test the heterogeneity of the results, and sensitivity analysis was conducted to verify the reliability. Figure 1a illustrates that for IVs to be considered valid, they must satisfy three key assumptions: (1) Correlation hypothesis: IVs are significantly associated with gut microbiota; (2) Independence hypothesis: IVs are not associated with confounding factors other than gut microbiota; (3) Exclusivity hypothesis: IVs can only affect the CP/CPPS through gut microbiota [11]. The workflow, illustrating the sequential steps and key components of our process, is displayed in Fig. 1b. Given that the information used as study material herein comprises public GWAS data, no further ethical consent was deemed necessary.

Data source

Initially, data concerning gut microbial taxa were gathered through MiBioGen (https://mibiogen.gcc.rug.nl), an extensive GWAS meta-analysis encompassing 18,340 participants across 24 groups, predominantly of European ancestry (n = 13,266) [12]. Examining the microbial composition by focusing on three distinct variable regions of the 16S rRNA gene revealed insightful data. This comprehensive analysis resulted in the categorization of a total of 211 bacterial taxa, encompassing a diverse spectrum of biological classifications, including 9 phyla, 16 classes, 20 orders, 35 families, and 131 genera. This extensive taxonomic profiling provides a nuanced elaboration of the complex diversity within the gut microbiota, capturing a holistic view of its structural and compositional aspects. Following this, we excluded three unknown families and 12 unknown genera. In subsequent steps, our attention narrowed down to the genus level, the lowest classification in bacterial taxonomy. This refined dataset comprised 9 phyla, 16 classes, 20 orders, 32 families, and 119 genera, laying the foundation for the forthcoming MR analysis. This meticulous categorization at the genus level ensures a granular exploration of the associations between specific bacterial groups and the targeted outcomes in our research. In this current investigation, GWAS summary statistics was utilized for CP/CPPS extracted from the GWAS Catalog release

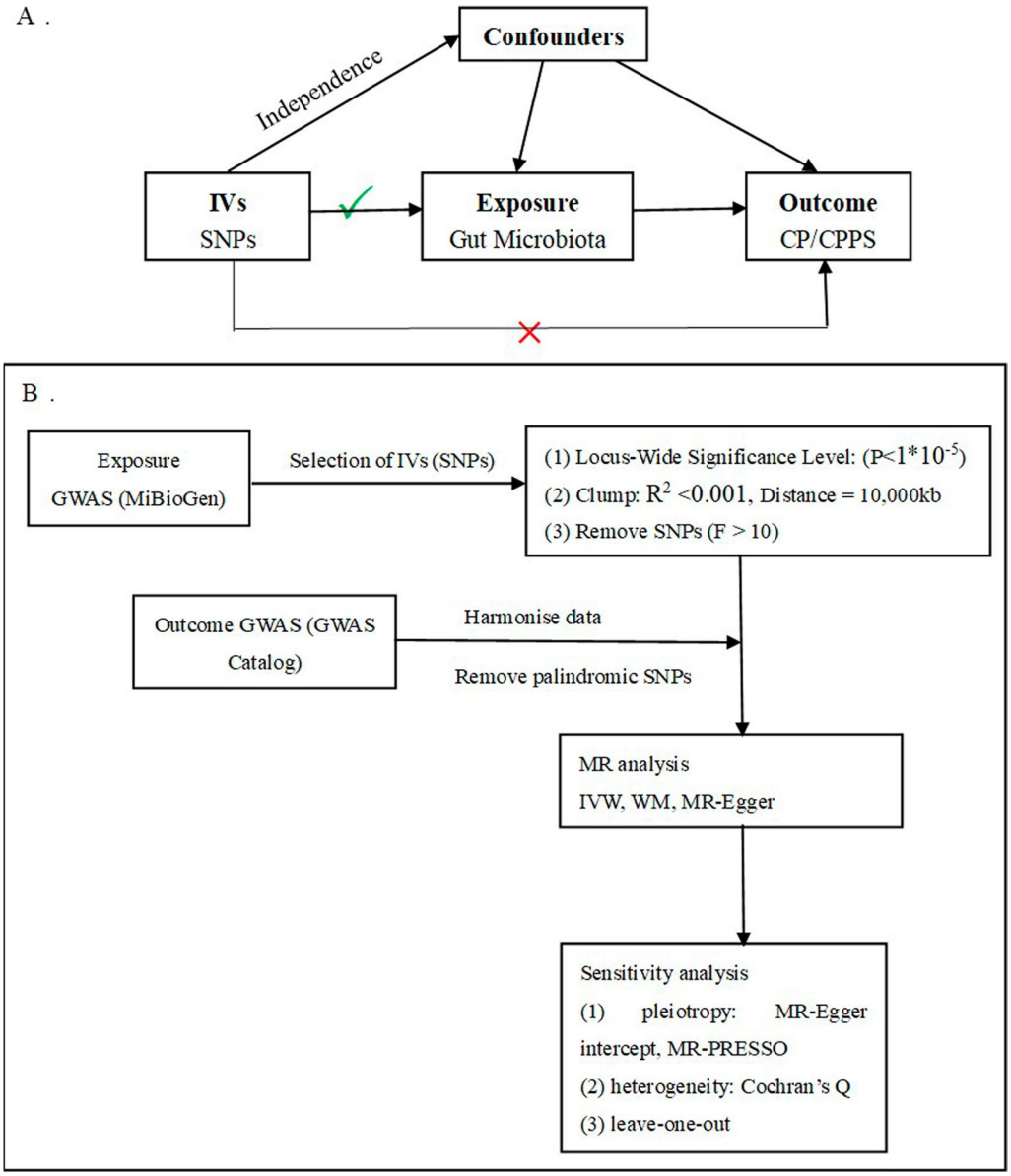

Fig. 1 **a** Three assumptions of Mendelian randomization. **b** Flowchart of this Mendelian randomization study

data, which encompassed 500 cases and 208,308 controls. The term "chronic prostatitis" was specifically utilized in our investigation. In the course of the analysis, thorough adjustments were systematically integrated to account for gender, age, the first 10 genetic principal components, and the genotyping batch. This meticulous approach aimed to facilitate a thorough and well-controlled scrutiny of the dataset.

Instrumental variable (IV)

To ensure the accuracy of the results, we screened the data extracted from MiBioGen. As the number of eligible IVs ($P<1*10^{-8}$) was extremely small, a significance threshold of less than $1*10^{-5}$ was selected as the *p*-value [13]. Furthermore, in order to prevent any linkage disequilibrium (LD) between gene tools, we established the chain imbalance threshold $r^2<0.001$ and clumping distance = 10,000 kb. The *F* statistics for individual bacterial taxa were calculated by applying the given formula: $F=R^2\times(N-K-1)/(1-R^2)\times K$, to assess the efficacy of the selected SNPs. Here, R^2 denotes the proportion of exposure variance explained by the IVs, *N* refers to the sample size, and *K* represents the number of SNPs [14]. A *F*-statistic value equal to or greater than 10 indicates

the lack of substantial instrumental bias, signifying that the instrumental variables employed in the analysis exhibit a robust and reliable influence on the outcomes under examination. Hence, SNPs having < 10 *F* value were removed as they lacked adequate validity [15]. Additionally, the presence of palindromic variation entails that the plus and minus chains will exhibit identical alleles, specifically A and T (or C and G). As a result, the exclusion of palindromic SNPs was implemented to mitigate the risk of potential disruptions in strand orientation or allele coding, especially in instances involving A/T or G/C alleles [16]. In the end, PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) was screened to further assess whether the IVs were potentially associated with confounders or risk factors for CP/CPPS in order to prevent potential pleiotropy. The final MR estimations were obtained by rerunning the MR analysis after excluding the IVs that did not meet the described criteria above.

Statistical analysis

Following the alignment of the SNPs in the data source with matching alleles, we conducted a two-sample MR analysis. The MR analysis utilized the Wald ratio method for bacterial genera that had a single IV. To assess the connections between CP/CPPS and the human gut microbiota, we employed the inverse-variance weighted (IVW) approach as the primary method for MR analysis. In addition, MR-Egger regression and weighted median analysis (WME) were also considered as secondary references. For a comprehensive assessment of the influence of gut microbiota on CP/CPPS, the IVW technique adopted a meta-analysis approach, utilizing Wald estimates for each SNP. In the absence of horizontal pleiotropy, the IVW findings would remain impartial. MR-Egger regression method was following in this study to determine the horizontal pleiotropy, wherein the *p*-value greater than 0.05 confirms that each SNP adheres to the Mendelian hypothesis, reinforcing the reliability of outcomes derived via IVW approach. Conversely, the significance level < 0.05 for the MR-Egger intercept suggests the potential presence of directional pleiotropy, prompting a cautious interpretation of the IVW results. The accurate estimation of a causal connection can be achieved by the weighted median approach when valid instrumental variables contribute not less than 50% of the weights. Additionally, we employed MR-PRESSO analysis, a method that detects and adjusts for the impacts of diverse outliers within the instrument. Heterogeneity of IVs was estimated using Cochran's IVW Q statistics [17]. Existence of atypical IVs was confirmed through the analysis known as "leave-one-out," excluding individual instrumental SNP one by one [18].

To examine the potential reverse causal relationship between CP/CPPS and gut microbiota, we embarked on a reverse MR analysis specifically targeting bacteria that had initially demonstrated a causal association with CP/CPPS in the primary MR analysis. Notably, this method helped us to scrutinize the bidirectional influences between CP/CPPS and gut microbiota, along with a detailed overview of their interplay. The methodologies and configurations utilized remained consistent with those of progressive MR. Regrettably, due to the insufficient number of IVs, the reverse MR analysis was failed to conduct.

Conducting all statistical analyses within the R software (version 4.1.2), we employed the TwoSampleMR (version 0.5.6) and MR-PRESSO packages (version 1.0) for the execution of MR analyses. Additionally, the R package "forestploter" was utilized to create specific graphical representations, ensuring a comprehensive and visually accessible presentation of the results.

Results

We screened 2197 SNPs as instrumental variables (IVs) for 196 bacterial taxa based on the IV selection criteria. The breakdown of these taxa, as presented in Table 1, revealed the distribution across various taxonomic levels, encompassing 9 phyla (105 SNPs), 16 classes (191 SNPs), 20 orders (235 SNPs), 32 families (385 SNPs), and 119 genera (1,281 SNPs). All the F statistics of the IVs were over 10. The results of the MR analysis for IVs are visually illustrated in the circus plot (Fig. 2) and further elaboration is provided in Additional file 1: Excel 1. According to primary IVW analysis, one family, four genera showed significant association with CP/CPPS. Specifically, genus *Christensenellaceae* (OR = 0.39, 95% CI 0.17–0.87, *P* = 0.02), genus *Eisenbergiella* (OR = 0.62, 95%CI 0.40–0.97, *P* = 0.04), genus *Hungatella* (OR = 0.49, 95%CI 0.28–0.85, *P* = 0.01) and genus *Terrisporobacter* (OR = 0.39, 95%CI 0.20–0.75, *P* = 0.00) exhibited a protective impact on CP/CPPS, while family *Prevotellaceae* (OR = 1.78, 95%CI 1.01–3.15, *P* = 0.05) is tentatively linked to a higher likelihood of developing CP/CPPS (Fig. 3). The results confirmed the relationship between

Table 1 Selection of IVs after quality control

Taxonomies	Taxa	NSNP	Palindromic	IVs
Phylum	9	126	21	105
Class	16	233	42	191
Order	20	293	58	235
Family	32	468	83	385
Genus	119	1579	298	1281
Total	196	2699	502	2197

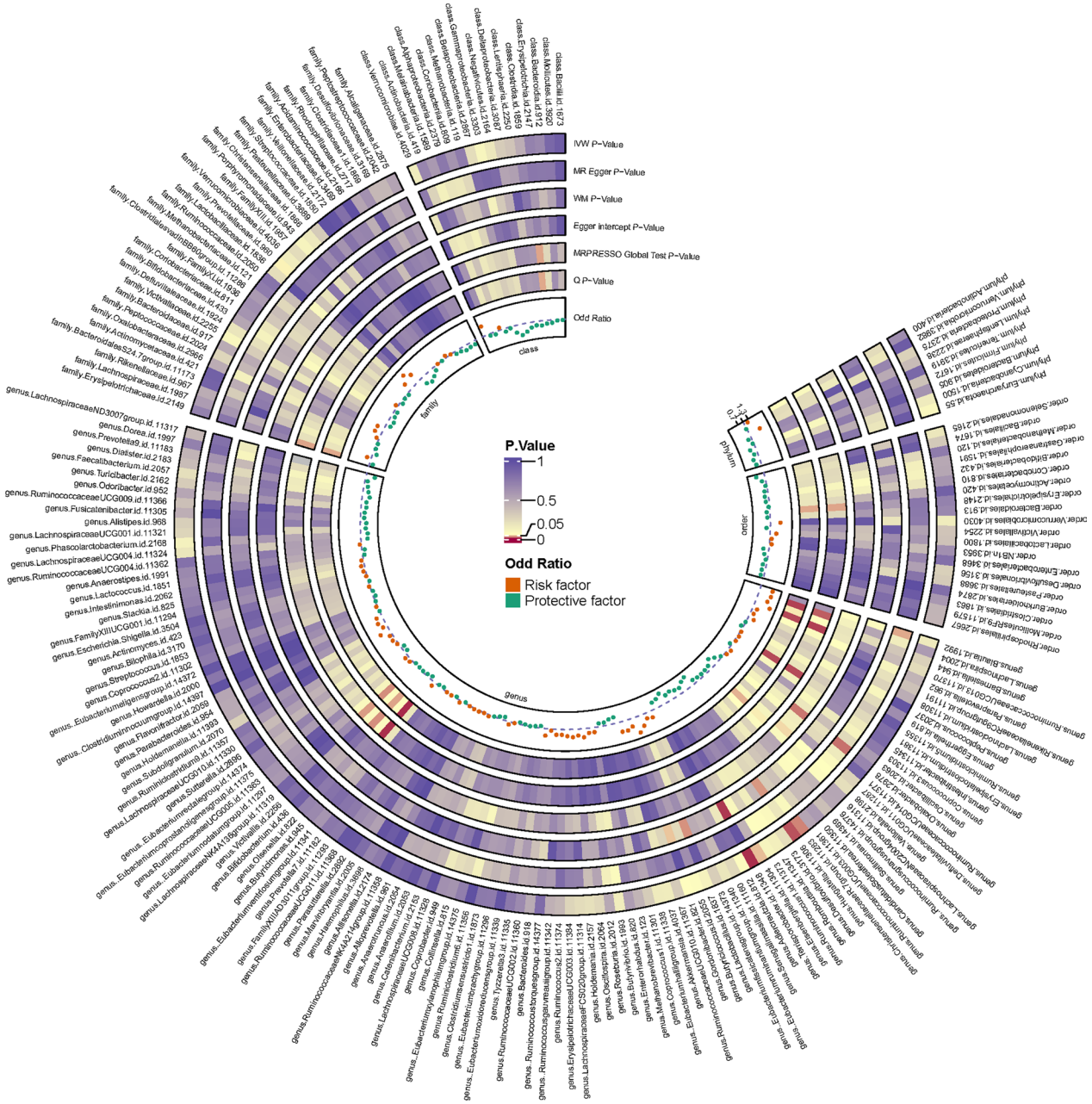

Fig. 2 The circus plot showing all results of MR analysis and sensitivity analysis between GM and CP/CPPS

specific microbial taxa and the susceptibility to or protection against CP/CPPS, shedding light on potential microbial contributors to the etiology of this condition.

The outcomes of sensitivity analyses listed in Table 2 provide a comprehensive overview. Cochran's *Q*-test results, as presented, revealed the absence of significant values for any of the gut microbiota, signifying that there was no heterogeneity among the IVs. The examination of MR-Egger's intercept yielded results that were statistically insignificant, indicating the absence of horizontal pleiotropy in the analyzed data. Furthermore, leave-one-out analysis affirms the obtained results, demonstrating that individual SNPs did not unduly influence the overall results, as depicted in Fig. 4. In essence, these collective findings consistently point toward a strong connection between specific gut microbiota and CP/CPPS, underpinned by genetic factors. The meticulous analyses conducted, coupled with the nonexistence

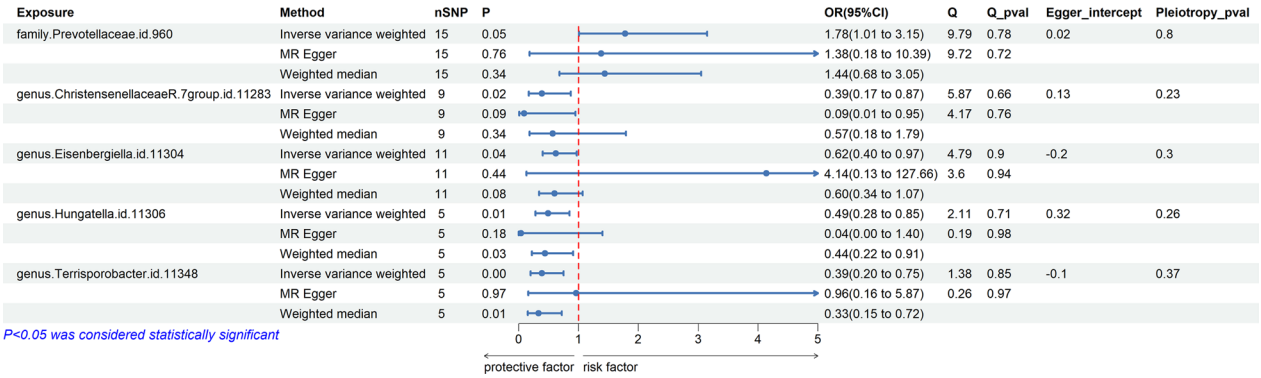

Fig. 3 Forest plot of the associations between genetically determined 5 gut microbial genera with the risks of CP/CPPS

Table 2 MR estimates for the association between gut microbiota and CP/CPPS

Classification	Nsnp	SE	*P*-val	OR (95%CI)	Heterogeneity		Pleiotropy		MR-PRESSO
					Q	*Q*-pval	Egger-intercept	*P*-val	
family.Prevotellaceae.id.960	15	0.291	0.047	1.782 (1.007–3.152)	9.790	0.777	0.018	0.799	0.799
genus.ChristensenellaceaeR.7group.id.11283	9	0.416	0.023	0.387 (0.171–0.875)	5.875	0.661	0.126	0.233	0.694
genus.Eisenbergiella.id.11304	11	0.223	0.035	0.625 (0.403–0.969)	4.789	0.905	-0.197	0.304	0.906
genus.Hungatella.id.11306	5	0.280	0.012	0.493 (0.285–0.854)	2.113	0.715	0.318	0.260	0.767
genus.Terrisporobacter.id.11348	5	0.337	0.005	0.387 (0.200–0.749)	1.376	0.848	-0.103	0.368	0.839

of heterogeneity and horizontal pleiotropy, enhance the confidence in the reliability and robustness of the established causal connection between specific gut microbiota and CP/CPPS.

Discussion

CP/CPPS is a complicated and multifaceted disorder with an uncertain origin. An increasing body of research has discovered a potential connection between the gastrointestinal microbiome and various illnesses in humans. Since the brain-gut-bladder axis theory emerged, numerous clinical and animal model studies have validated the correlation between the gut microbiome and bladder symptoms. In recent years, there has been a growing acknowledgement of the connection between gut microbiota and CP/CPPS [19, 20]. But the exact mechanism is not clear. Intestinal flora may influence CP/CPPS via mediating immune inflammatory response, neurotransmitters, androkinin, and direct infection.

Urinary disorders, such as urinary urgency, increased urination frequency, and difficulty initiating or completing voiding, were commonly observed in patients with gastrointestinal disorders [21]. Leue et al. have reported that there is a connection between functional bladder disorders and bowel disease [22]. Considering the frequent occurrence of urinary and colonic dysfunctions together, as well as the possibility of one organ affecting the functioning of the other, it can be concluded that there is an interaction between these two organs. Gut-bladder axis is the most famous hypothesis regarding bladder-gut cross-talk. The gut microbiota play a vital role as an essential mediator in the bidirectional communication between the gastrointestinal tract and the urinary bladder. In these exchanges, inflammasomes, complexes of multiple proteins that can trigger inflammatory reactions, and metabolites produced by the microbiome like short-chain fatty acids (SCFAs), have a significant impact.

SCFAs have become significant microbial byproducts involved in the regulation of immune inflammation and metabolism through interactions with the gut microbiome and host receptors [23]. Within the microenvironment of the proximal colon, three noteworthy SCFAs—acetate, propionate, and butyrate—hold prominence, with concentrations ranging from 50 to 120 mM (mM), showcasing considerable variability within this specified concentration range [24]. They are generated by two primary categories of bacteria, specifically *Bacteroidetes* and *Firmicutes* [25]. SCFAs display a versatile capability to influence various aspects of immune cell function, as they possess the ability to modulate gene expression, alter cellular differentiation processes, impact chemotaxis, regulate cellular proliferation, and even induce apoptosis within immune cells, actively participating in the comprehensive process of immune response

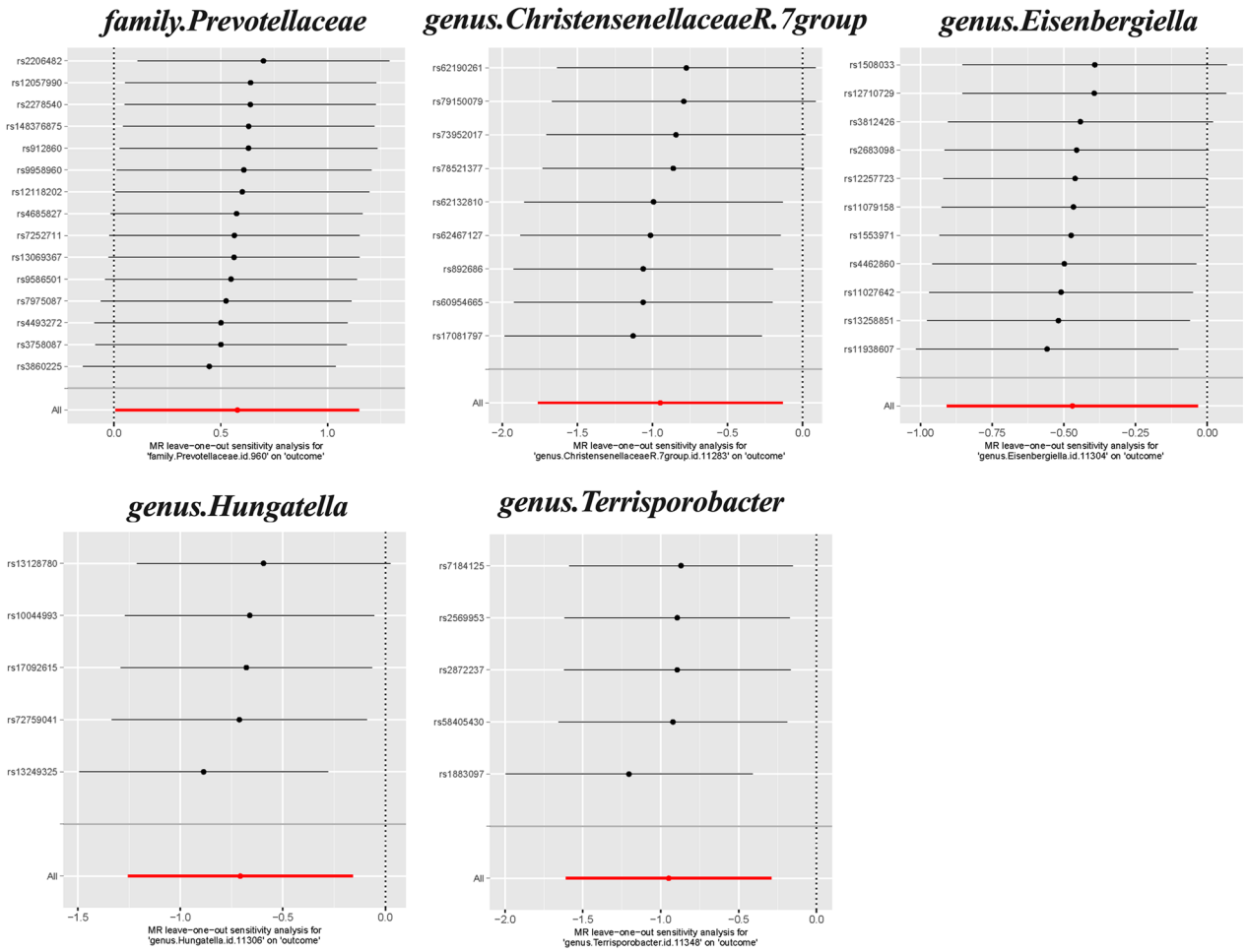

Fig. 4 Leave-one-out plots for the causal association between gut microbiota and CP/CPPS

[26]. According to the findings presented by Du HX et al., the occurrence of gut dysbiosis plays a contributory role in generating an imbalance in the differentiation of Th17 and Treg cells within the context of experimental autoimmune prostatitis (EAP). This imbalance, observed in the differentiation patterns of these immune cells, is intricately linked to the lower levels of propionic acid—a significant SCFA originated from gut microbiota [27]. Chen et al. observed that the oral consumption of glycated whey proteins may induce a prebiotic effect, leading to heightened levels of *Allobaculum, Anaerostipes, Bacteroides, Parabacteroides*, and *Prevotella*, while concurrently lowering the levels of *Adlercreutzia* and *Roseburia* at the genus level. Consequently, this led to a decrease in the immune inflammatory response of mice in the EAP model [28].

Interestingly, 4 GM taxa, including family *Christensenellaceae*, genus *Eisenbergiella*, genus *Terrisporobacter*, and genus *hungatella* of our results, all belong to the phylum *Firmicutes*. In the healthy population, there was a greater presence of *Christensenellaceae*, which showed an inverse correlation with inflammation [29]. Kropp C et al. reported that *Christensenellaceae minuta* has the ability to inhibit intestinal harm, decrease inflammation in the colon, and facilitate the healing of the mucosal layer in both in vitro and in vivo experiments [30]. Similarly, Relizani K et al. presented a comprehensive screening procedure that integrates in vitro and in vivo tests to systematically choose a viable strain of *Christensenellaceae minuta* with potent immunomodulatory characteristics. This indicates that *Christensenella minuta* has the potential to be utilized as a future biotherapy for Crohn's disease [31]. The relationship between *Genus Eisenbergiella* and eubiosis is likely due to its ability to generate significant metabolic products such as butyrate, acetate, lactate, and succinate, which have a nourishing impact on the mucosa[32]. Moreover, the genus *Eisenbergiella* might be strongly associated with the observed reduction in inflammation of intestine among mice with ulcerative colitis [33]. In accordance

with *Eisenbergiella*, the genus *Terrisporobacter* was found to have significantly linked to SCFAs and oxidative stress in a study involving animals[34]. Qiao J et al. reported that rapeseed bee pollen can inhibit pathogenic bacteria and enhance probiotics, particularly in the Firmicutes-to-Bacteroidetes (F/B) ratio to alleviate chronic non-bacteria prostatitis [19], which is basically consistent with our findings. However, to the best of our knowledge, there have been no prior examinations exploring the potential link between the genera *Eisenbergiella/Terrisporobacter* and CP/CPPS.

CP/CPPS may be also related to abnormal release of neurotransmitters. The main neurotransmitters involved in the occurrence and development of CP/CPPS are "5-hydroxytryptamine(5-HT), noradrenaline (NE), and dopamine (DA)." They have a significant impact on this condition. Statistics show that about 40% of CP/CPPS men suffer from premature ejaculation [35]. Moreover, 78% of CP/CPPS patients exhibit psychological distress, including anxiety and depression [36]. The concentrations of 5-HT and various other neurotransmitters in both peripheral blood and the brain may be subject to influence by the gut microbiota. Yano et al. showed that the 5-HT level in germ-free mice was lower than that in healthy mice. When normal intestinal flora was transplanted into germ-free mice, it was possible to restore it to normal levels [37]. Du et al. reported that the EAP mice showed obvious depression-like behavior and the composition of intestinal bacteria such as *Dantamoesophagota*, *Ruminococcus*, and *Bacteroidetes* was significantly different from that in normal mice. Fecal bacteria from EAP mice transplanted into pseudogerm-free mice treated with antibiotics could aggravate the depression-like behavior of the host [38]. Disrupted neurotransmitter release can impact the functioning of the nervous system, leading to symptoms of depression and sexual dysfunction in individuals with CP/CPPS. 5-HT reuptake inhibitors can be used for targeted treatment [39].

Besides, gut microbiota correlate with androgen levels, which regulates prostate. Sufficient androgens can promote prostate growth and development, maintain secretion and differentiation function of prostate. Androgen imbalance can cause CP, the mechanism may be caused by the decrease of androgen content level, which has the effect of inhibiting humoral immunity, and when its content is reduced, it can cause autoimmune reaction. In addition, androgen can promote prostate autophagy, while high levels of estrogen inhibiting autophagy [40, 41]. Once androgen level decreased and the balance of male and female hormones disturbed, prostate cells may be damaged due to insufficient autophagy, inducing prostate inflammation. Poutahidis et al. found that the serum testosterone level was significantly increased after feeding *Lactobacillus reuteri* to mice [42]. Konkol Y et al. induced nonbacterial chronic prostate inflammation (CPI) in the Wistar rat strain for 18 weeks with subcutaneous testosterone and 17β-estradiol (E2) hormone pellets. A decrease in *Bacteroides* uniformis, *Lactobacillus*, and *Lachnospiraceae* levels was observed in rats suffering from CPI. In the fecal samples from those rats, SCFA butyric-, valeric-, and caproic-acid concentrations were also decreased [20]. These results and theories are similar to our findings. Liu et al. demonstrated that poria cocos polysaccharides and finasteride had the ability to alter the composition of intestinal flora in a rat model of CP/CPPS. This led to a decrease in the levels of pro-inflammatory cytokines (TNF-α, IL-2, and IL-8). Simultaneously, there was a reduction in androgens (dihydrotestosterone and testosterone), resulting in an improvement in both prostatic inflammation and histological damage, which is suggested that CP/CPPS can be treated by regulating the gut microbiota and androgen level [43].

Finally, gut microbiota may affect CP/CPPS through direct infection. Although it has not been confirmed that there is a direct pathway between the rectum and the prostate to explain how gut microbiota infuse the urogenital tract. The two organs are anatomically adjacent, and there is the possibility of "direct infiltration" [44].

In the current study, we utilized the summary data of gut microbiota from the MiBioGen Consortium's extensive GWAS meta-analysis and the GWAS Catalog's summary data of CP/CPPS to investigate the causal link. Mendelian randomization and sensitivity analysis were performed to assess the causal relationship between gut microbiota and CP/CPPS. Protective effects against CP/CPPS were observed in various genera of gut microbiota, including *ChristensenellaceaeR.7group*, *Eisenbergiella*, *Hungatella*, *Terrisporobacter* (OR < 1), whereas the *family Prevotellaceae* (OR > 1) had the opposite effect.

According to recent researches, rheumatoid arthritis [45], periodontitis [46], and intestinal and vaginal dysbiosis [47–49] are linked to the higher presence of *Prevotellaceae family* members in various microbial ecosystems. *Prevotella* colonization may lead to metabolic alterations in the microbiome, resulting in decreased IL-18 synthesis. This, in turn, exacerbates intestinal inflammation and contributes to the development of systemic autoimmunity. Regrettably, given the intricacy of gut microbiota, there is indeed a lack of agreement between our findings and the current supporting data. For example, Shoskes DA et al. used 16S rRNA sequencing, discovering reduced gut microbiota richness and diversity in CPPS patients compared to controls, along with markedly diminished levels of *Prevotella* [50]. Hence, additional research is required to authenticate these connections.

Our research brings distinct strengths. Firstly, through a two-sample MR analysis, we amplified the influence of gut microbiota on CP/CPPS causality. Secondly, the strict quality control procedures and robust MR methods applied ensure the reliability and stability of the causal estimates. Thirdly, the identification of potential causal links through the IVW method serves as a guiding beacon for further exploration into specific gut bacteria. This enlightens the investigation of their roles in shaping the intricate landscape of CP/CPPS development.

However, this task also possesses compelling constraints. While meeting the MR assumptions by ensuring the strong correlation of instrumental variables with gut microbiota taxa, our study remains vulnerable to the absence of a guarantee against weak instrumental bias. Firstly, the relatively modest sample size of CP/CPPS may cause some bias in the selection process of IVs, and there may be weak instrumental variables, which may reduce the reliability of the results, impeding the full scope of our research insights. Secondly, the reverse MR analysis was impeded by a limited number of IVs, preventing the establishment of a potential reciprocal causal connection between CP/CPPS and gut microbiota. Thirdly, although MR techniques can offer fresh perspectives on the causal relationships between exposure characteristics and outcome characteristics, the accuracy of estimating the strength of these associations may be limited. Therefore, further investigation is necessary to validate the results. Fourthly, it is crucial to recognize that the predominant participants in this GWAS hail from European ancestry. This demographic specificity raises a noteworthy consideration—the outcomes of this study may not be universally applicable to ethnically diverse populations. Finally, due to the lack of specific country, region, age, and other information, stratification analysis cannot be carried out, which may cause bias to research results. These limitations currently present hurdles in definitively grounding the cause–effect correlation between gut microbiota and CP/CPPS, urging further research endeavors that encompass a broader spectrum of ethnic backgrounds for comprehensive insights. In the future, if a GWAS dataset of CP/CPPS is publicly released with a larger number of participants and SNPs, or from other ethnic groups, it is hoped that additional researches can confirm these connections.

Conclusions

Our study assessed the potential causal role of gut microbiota on the risk of CP/CPPS. Various gut microbiota, such as *genus ChristensenellaceaeR.7group*, *Eisenbergiella*, *Hungatella*, *Terrisporobacter*, and *family Prevotellaceae*, emerged as potential associates with the occurrence of CP/CPPS. These findings hold the promise of introducing fresh perspectives for understanding the genesis and exploring innovative approaches for the management of CP/CPPS.

Abbreviations

CP/CPPS	Chronic prostatitis/chronic pelvic pain syndrome
SNP	Single nucleotide polymorphism
IVs	Instrumental variables
MR	Mendelian randomization
RCTS	Randomized controlled trials
GWAS	Genome-wide association study
16S rRNA	16S ribosomal RNA
LD	Linkage disequilibrium
IVW	Inverse-variance weighted
SCFA	Short-chain fatty acids
5-HT	5-Hydroxytryptamine
NE	Noradrenaline
DA	Dopamine
TNF	Tumor necrosis factor
IL-2	Interleukin-2
IL-8	Interleukin-8

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43042-024-00540-3.

Supplementary Materials 1 All results of MR analysis and sensitivity analysis between GM and CP/CPPS.

Acknowledgements

The MiBioGen consortium and GWAS Catalog consortia are acknowledged for sharing genetic data.

Author contributions

CW conceptualized the study; DT and HH designed this study; HX, YZ analyzed the data and wrote the manuscript; CS, YZ, ZZ, and JW acquired the data; DZ and ZW involved in the quality control of the data and algorithms; YQ interpreted the data; SL revised the manuscript.

Funding

The present study was supported by Tianjin Municipal Health Industry Key Project (Grant No. TJWJ2022XK014), Scientific Research Project of Tianjin Municipal Education Commission (Grant No. 2022ZD069), Technology Project of Tianjin Binhai New Area Health Commission (Grant No. 2019BWKY026), the Youth Fund of Tianjin Medical University Second Hospital (Grant No. 2022ydey15), Tianjin Health Science and Technology Project (Grant No. ZC20119), and The Talents Cultivated Project of Department of Urology, the Second Hospital of Tianjin Medical University (Grant No. MNRC202313).

Availability of data and materials

The datasets analyzed during the current study are available in the MiBioGen (https://mibiogen.gcc.rug.nl), GWAS Catalog (https://ftp.ebi.ac.uk/pub/databases/gwas/summary_statistics/GCST90044001-GCST90045000/GCST90044259/). The overall results of this study can be found in the article/Supplementary Material.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

Author details
[1]Department of Urology, Tianjin Institute of Urology, The Second Hospital of Tianjin Medical University, No. 23, Pingjiang Road, Tianjin 300211, China. [2]Department of Urology, First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, National Clinical Research Center for Chinese Medicine Acupuncture and Moxibustion, Tianjin 300193, China. [3]Department of Urology, The Eco-City Hospital of Tianjin Fifth Central Hospital, Tianjin 300451, China. [4]Department of Urology, Affiliated Hospital of Chifeng University, Chifeng 024000, Inner Mongolia, China.

Received: 13 January 2024 Accepted: 14 June 2024
Published online: 21 June 2024

References

1. Collins MM, Stafford RS, O'Leary MP, Barry MJ (1998) How common is prostatitis? A national survey of physician visits. J Urol 159(4):1224–1228
2. Hitch TCA, Hall LJ, Walsh SK et al (2022) Microbiome-based interventions to modulate gut ecology and the immune system. Mucosal Immunol 15(6):1095–1113. https://doi.org/10.1038/s41385-022-00564-1
3. Jandhyala SM, Talukdar R, Subramanyam C et al (2015) Role of the normal gut microbiota. World J Gastroenterol 21(29):8787–8803. https://doi.org/10.3748/wjg.v21.i29.8787
4. Alagiri M, Chottiner S, Ratner V, Slade D, Hanno PM (1997) Interstitial cystitis: unexplained associations with other chronic disease and pain syndromes. Urology 49(5A Suppl):52–57. https://doi.org/10.1016/s0090-4295(99)80332-x
5. Emdin CA, Khera AV, Kathiresan S (2017) Mendelian randomization. JAMA 318(19):1925–1926. https://doi.org/10.1001/jama.2017.17219
6. Bowden J, Holmes MV (2019) Meta-analysis and Mendelian randomization: a review. Res Synth Methods 10(4):486–496. https://doi.org/10.1002/jrsm.1346
7. Li P, Wang H, Guo L et al (2022) Association between gut microbiota and preeclampsia-eclampsia: a two-sample Mendelian randomization study. BMC Med 20(1):443. https://doi.org/10.1186/s12916-022-02657-x
8. Long Y, Tang L, Zhou Y, Zhao S, Zhu H (2023) Causal relationship between gut microbiota and cancers: a two-sample Mendelian randomisation study. BMC Med 21(1):66. https://doi.org/10.1186/s12916-023-02761-6
9. Li W, Ren A, Qin Q et al (2023) Causal associations between human gut microbiota and cholelithiasis: a mendelian randomization study. Front Cell Infect Microbiol 13:1169119. https://doi.org/10.3389/fcimb.2023.1169119
10. Xu Q, Ni JJ, Han BX et al (2021) Causal relationship between gut microbiota and autoimmune diseases: a two-sample mendelian randomization study. Front Immunol 12:746998. https://doi.org/10.3389/fimmu.2021.746998
11. Slob EAW, Burgess S (2020) A comparison of robust Mendelian randomization methods using summary data. Genet Epidemiol 44(4):313–329. https://doi.org/10.1002/gepi.22295
12. Kurilshikov A, Medina-Gomez C, Bacigalupe R et al (2021) Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet 53(2):156–165. https://doi.org/10.1038/s41588-020-00763-1
13. Sanna S, van Zuydam NR, Mahajan A et al (2019) Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet 51(4):600–605. https://doi.org/10.1038/s41588-019-0350-x
14. Pierce BL, Ahsan H, Vanderweele TJ (2011) Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. Int J Epidemiol 40(3):740–752. https://doi.org/10.1093/ije/dyq151
15. Burgess S, Thompson SG, Collaboration CCG (2011) Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol 40(3):755–764. https://doi.org/10.1093/ije/dyr036
16. Girault JM, Menigot S (2022) Palindromic vectors, symmetropy and symmentropy as symmetry descriptors of binary data. Entropy (Basel). https://doi.org/10.3390/e24010082
17. Bowden J, Del Greco MF, Minelli C et al (2017) A framework for the investigation of pleiotropy in two-sample summary data Mendelian randomization. Stat Med 36(11):1783–1802. https://doi.org/10.1002/sim.7221
18. Hemani G, Tilling K, Davey SG (2017) Orienting the causal relationship between imprecisely measured traits using GWAS summary data. PLoS Genet 13(11):e1007081. https://doi.org/10.1371/journal.pgen.1007081
19. Qiao J, Xiao X, Wang K et al (2023) Rapeseed bee pollen alleviates chronic non-bacterial prostatitis via regulating gut microbiota. J Sci Food Agric 103(15):7896–7904. https://doi.org/10.1002/jsfa.12878
20. Konkol Y, Keskitalo A, Vuorikoski H et al (2019) Chronic nonbacterial prostate inflammation in a rat model is associated with changes of gut microbiota that can be modified with a galactoglucomannan-rich hemicellulose extract in the diet. BJU Int 123(5):899–908. https://doi.org/10.1111/bju.14553
21. Klingele CJ, Lightner DJ, Fletcher JG, Gebhart JB, Bharucha AE (2010) Dysfunctional urinary voiding in women with functional defecatory disorders. Neurogastroenterol Motil 22(10):1094-e1284. https://doi.org/10.1111/j.1365-2982.2010.01539.x
22. Leue C, Kruimel J, Vrijens D et al (2017) Functional urological disorders: a sensitized defence response in the bladder-gut-brain axis. Nat Rev Urol 14(3):153–163. https://doi.org/10.1038/nrurol.2016.227
23. Rosser EC, Piper CJM, Matei DE et al (2020) Microbiota-derived metabolites suppress arthritis by amplifying aryl-hydrocarbon receptor activation in regulatory B cells. Cell Metab 31(4):837–851. https://doi.org/10.1016/j.cmet.2020.03.003
24. Needell JC, Ir D, Robertson CE et al (2017) Maternal treatment with short-chain fatty acids modulates the intestinal microbiota and immunity and ameliorates type 1 diabetes in the offspring. PLoS ONE 12(9):e0183786. https://doi.org/10.1371/journal.pone.0183786
25. Levy M, Thaiss CA, Elinav E (2016) Metabolites: messengers between the microbiota and the immune system. Genes Dev 30(14):1589–1597. https://doi.org/10.1101/gad.284091.116
26. Kim CH (2018) Immune regulation by microbiome metabolites. Immunology 154(2):220–229. https://doi.org/10.1111/imm.12930
27. Du HX, Yue SY, Niu D et al (2022) Gut microflora modulates Th17/Treg cell differentiation in experimental autoimmune prostatitis via the short-chain fatty acid propionate. Front Immunol 13:915218. https://doi.org/10.3389/fimmu.2022.915218
28. Chen Y, Guo KM, Nagy T, Guo TL (2020) Chronic oral exposure to glycated whey proteins increases survival of aged male NOD mice with autoimmune prostatitis by regulating the gut microbiome and anti-inflammatory responses. Food Funct 11(1):153–162. https://doi.org/10.1039/c9fo01740b
29. Waters JL, Ley RE (2019) The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. BMC Biol 17(1):83. https://doi.org/10.1186/s12915-019-0699-4
30. Kropp C, Le Corf K, Relizani K et al (2021) The Keystone commensal bacterium Christensenella minuta DSM 22607 displays anti-inflammatory properties both in vitro and in vivo. Sci Rep 11(1):11494. https://doi.org/10.1038/s41598-021-90885-1
31. Relizani K, Le Corf K, Kropp C et al (2022) Selection of a novel strain of Christensenella minuta as a future biotherapy for Crohn'ss disease. Sci Rep 12(1):6017. https://doi.org/10.1038/s41598-022-10015-3
32. Andriulli A, Bevilacqua A, Palmieri O et al (2022) Healthy and pro-inflammatory gut ecology plays a crucial role in the digestion and tolerance of a novel Gluten Friendly bread in celiac subjects: a randomized, double blind, placebo control in vivo study. Food Funct 13(3):1299–1315. https://doi.org/10.1039/d1fo00490e
33. Wu X, Xu N, Ye Z et al (2022) Polysaccharide from *Scutellaria barbata* D. Don attenuates inflammatory response and microbial dysbiosis in ulcerative colitis mice. Int J Biol Macromol 206:1–9. https://doi.org/10.1016/j.ijbiomac.2022.02.119
34. Li H, Shang Z, Liu X et al (2021) Clostridium butyricum alleviates enterotoxigenic *Escherichia coli* K88-induced oxidative damage through regulating the p62-Keap1-Nrf2 signaling pathway and remodeling the cecal microbial community. Front Immunol 12:771826. https://doi.org/10.3389/fimmu.2021.771826
35. Magistro G, Wagenlehner FM, Grabe M et al (2016) Contemporary management of chronic prostatitis/chronic pelvic pain syndrome. Eur Urol 69(2):286–297. https://doi.org/10.1016/j.eururo.2015.08.061

36. Li HJ, Kang DY (2016) Prevalence of sexual dysfunction in men with chronic prostatitis/chronic pelvic pain syndrome: a meta-analysis. World J Urol 34(7):1009–1017. https://doi.org/10.1007/s00345-015-1720-3
37. Yano JM, Yu K, Donaldson GP et al (2015) Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 161(2):264–276. https://doi.org/10.1016/j.cell.2015.02.047
38. Du HX, Liu Y, Zhang LG et al (2020) Abnormal gut microbiota composition is associated with experimental autoimmune prostatitis-induced depressive-like behaviors in mice. Prostate 80(9):663–673. https://doi.org/10.1002/pros.23978
39. Lao Y, He L, Zhang P, Dong Z (2022) Efficacy and safety of selective serotonin re-uptake inhibitors for chronic prostatitis/chronic pelvic pain syndrome: a systematic review and network meta-analysis. Asian J Surg 45(12):2810–2812. https://doi.org/10.1016/j.asjsur.2022.06.047
40. Kapahi P, Chen D, Rogers AN et al (2010) With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. Cell Metab 11(6):453–465. https://doi.org/10.1016/j.cmet.2010.05.001
41. Ma Y, Yang HZ, Xu LM et al (2015) Testosterone regulates the autophagic clearance of androgen binding protein in rat Sertoli cells. Sci Rep 5:8894. https://doi.org/10.1038/srep08894
42. Poutahidis T, Springer A, Levkovich T et al (2014) Probiotic microbes sustain youthful serum testosterone levels and testicular size in aging mice. PLoS ONE 9(1):e84877. https://doi.org/10.1371/journal.pone.0084877
43. Liu J, Liu L, Zhang G, Peng X (2021) Poria cocos polysaccharides attenuate chronic nonbacterial prostatitis by targeting the gut microbiota: comparative study of Poria cocos polysaccharides and finasteride in treating chronic prostatitis. Int J Biol Macromol 189:346–355. https://doi.org/10.1016/j.ijbiomac.2021.08.139
44. Motrich RD, Breser ML, Sanchez LR et al (2016) IL-17 is not essential for inflammation and chronic pelvic pain development in an experimental model of chronic prostatitis/chronic pelvic pain syndrome. Pain 157(3):585–597. https://doi.org/10.1097/j.pain.0000000000000405
45. de Aquino SG, Abdollahi-Roodsaz S, Koenders MI et al (2014) Periodontal pathogens directly promote autoimmune experimental arthritis by inducing a TLR2- and IL-1-driven Th17 response. J Immunol 192(9):4103–4111. https://doi.org/10.4049/jimmunol.1301970
46. Ji S, Kim Y, Min BM, Han SH, Choi Y (2007) Innate immune responses of gingival epithelial cells to nonperiodontopathic and periodontopathic bacteria. J Periodontal Res 42(6):503–510. https://doi.org/10.1111/j.1600-0765.2007.00974.x
47. Onderdonk AB, Delaney ML, Fichorova RN (2016) The human microbiome during bacterial vaginosis. Clin Microbiol Rev 29(2):223–238. https://doi.org/10.1128/CMR.00075-15
48. Gosmann C, Anahtar MN, Handley SA et al (2017) Lactobacillus-deficient cervicovaginal bacterial communities are associated with increased HIV acquisition in young South African women. Immunity 46(1):29–37. https://doi.org/10.1016/j.immuni.2016.12.013
49. Elinav E, Strowig T, Kau AL et al (2011) NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 145(5):745–757. https://doi.org/10.1016/j.cell.2011.04.022
50. Shoskes DA, Wang H, Polackwich AS et al (2016) Analysis of gut microbiome reveals significant differences between men with chronic prostatitis/chronic pelvic pain syndrome and controls. J Urol 196(2):435–441. https://doi.org/10.1016/j.juro.2016.02.2959

Publisher's Note