Article

A Large Genetic Causal Analysis of the Gut Microbiota and Urological Cancers: A Bidirectional Mendelian Randomization Study

Zhaofa Yin †, Bohan Liu †, Shijian Feng †, Yushi He, Cai Tang, Pengan Chen, Xinyi Wang and Kunjie Wang *

Department of Urology, Institute of Urology (Laboratory of Reconstructive Urology), National Clinical Research Center for Geriatrics, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu 610041, China; ldszxyyyzf@163.com (Z.Y.); liubohan@stu.scu.edu.cn (B.L.); fengshijian@wchscu.cn (S.F.); 13348939543@163.com (Y.H.); tc1989@163.com (C.T.); cpa_scu@163.com (P.C.); wxy751431349@163.com (X.W.)
* Correspondence: wangkj@scu.edu.cn; Tel.: +86-028-8542-2444
† These authors contributed equally to this work.

Abstract: Background: Several observational studies and clinical trials have shown that the gut microbiota is associated with urological cancers. However, the causal relationship between gut microbiota and urological cancers remains to be elucidated due to many confounding factors. Methods: In this study, we used two thresholds to identify gut microbiota GWAS from the MiBioGen consortium and obtained data for five urological cancers from the UK biobank and Finngen consortium, respectively. We then performed a two-sample Mendelian randomization (MR) analysis with Wald ratio or inverse variance weighted as the main method. We also performed comprehensive sensitivity analyses to verify the robustness of the results. In addition, we performed a reverse MR analysis to examine the direction of causality. Results: Our study found that family *Rikenellaceae*, genus *Allisonella*, genus *Lachnospiraceae UCG001*, genus *Oscillibacter*, genus *Eubacterium coprostanoligenes group*, genus *Eubacterium ruminantium group*, genus *Ruminococcaceae UCG013*, and genus *Senegalimassilia* were related to bladder cancer; genus *Ruminococcus torques group*, genus *Oscillibacter*, genus *Barnesiella*, genus *Butyricicoccus*, and genus *Ruminococcaceae UCG005* were related to prostate cancer; class *Alphaproteobacteria*, class *Bacilli*, family *Family XI*, genus *Coprococcus2*, genus *Intestinimonas*, genus *Lachnoclostridium*, genus *Lactococcus*, genus *Ruminococcus torques group*, and genus *Eubacterium brachy group* were related to renal cell cancer; family *Clostridiaceae 1*, family *Christensenellaceae*, genus *Eubacterium coprostanoligenes group*, genus *Clostridium sensu stricto 1*, and genus *Eubacterium eligens group* were related to renal pelvis cancer; family *Peptostreptococcaceae*, genus *Romboutsia*, and genus *Subdoligranulum* were related to testicular cancer. Comprehensive sensitivity analyses proved that our results were reliable. Conclusions: Our study confirms the role of specific gut microbial taxa on urological cancers, explores the mechanism of gut microbiota on urological cancers from a macroscopic level, provides potential targets for the screening and treatment of urological cancers, and is dedicated to providing new ideas for clinical research.

Keywords: gut microbiota; urological cancer; mendelian randomization; genetics

1. Introduction

With the proliferation of the populace and the escalation of societal senescence, the incidence and prevalence of cancer have dramatically grown [1]. The data showed that urological cancers accounted for 13.1% of new cancer cases and 7.9% of total cancer mortality [2]. The prevalent urological cancers are bladder cancer, prostate cancer, renal cell cancer, renal pelvis cancer, and testicular cancer [3]. Compared with 1990, the number of patients with urological cancers has increased by 2.5 fold, and the number of deaths has increased by 1.6 fold [4]. According to statistics, in the United States, there were 17,100 additional deaths from bladder cancer, 34,500 from prostate cancer, and 13,920 from

renal cell and renal pelvis cancer in 2022 [2]. Testicular cancer remains relatively rare, accounting for 0.4% of new cancer cases [5]. Compared to other cancers, urological cancers develop slowly and can easily be cured by early detection and treatment [6]. However, the early symptoms of urological cancers are not obvious, and cystoscopy, tissue biopsy, and imaging examinations limit their use in large-scale screening due to invasiveness, cost, and ionizing radiation [7]. Therefore, it is very important to find new targets suitable for large-scale screening and prevention of urological cancers.

In recent years, with the discovery of the gut–kidney axis, gut–prostate axis, and gut–testis axis, the association between gut microbiota and urological cancers has gained significant attention [8–10]. The gut microbiota refers to the vast community of microorganisms residing in the gastrointestinal tract, primarily composed of bacteria, fungi, viruses, and other microbial species, 98% of which are bacteria [11]. The gut microbiota is a mutualistic symbiosis in humans that plays a crucial role in maintaining human health and has a profound impact on various disease processes, including urological cancers [12–14]. To date, several observational studies have shown differences between healthy individuals and patients with urological cancers in the composition and diversity of the gut microbiota. In a Chinese case–control study, He et al. observed a lower abundance of *Prevotella* in the intestines of bladder cancer patients [15]. In an observational study in the United States, Liss et al. found that *Bacteroides* and *Streptococci* were more abundant in the intestines of prostate cancer patients [16]. Several animal model experiments have also reported the involvement of the gut microbiota in the progression of urological cancers via intricate signaling pathways [13,17].

The association between gut microbiota and urological cancers is a rapidly evolving field of research. It holds great promise for the development of novel diagnostic, preventive, and therapeutic strategies. Understanding the intricate relationships between the gut microbiota and urological cancers could develop the microbiota-based therapies such as microbiota transplantation, probiotic therapy, and dietary therapy, as well as novel targets for urologic cancer screening [18]. Clinicians can help identify individuals at higher risk based on their gut microbiota profiles and guide personalized interventions to mitigate that risk, making personalized treatment for urologic cancers possible.

Unfortunately, the conclusions of most current observational studies predominantly rely on the analysis of the composition and changes in gut microbiota in patients' feces. Traditional observational studies are limited by inherent flaws, including environmental confounders, selection bias, and reverse causation [19]. The establishment of models in animal experiments focuses on transplanting gut microbiota into mice and deriving results, and the gut microbiota of these mice is susceptible to various influences, including factors such as dietary patterns and antibiotic usage [20]. In conclusion, the relationship between gut microbiota and urological cancers remains to be elucidated. Although randomized controlled trials (RCTs) are the gold standard for verifying causality, the extremely large number of gut microbial species and the long latency period from gut microbiota imbalance to cancer development make RCT difficult to apply in a real clinical setting [21]. Therefore, a new approach is needed to explore the causal relationship between gut microbiota and urological cancers.

We use Mendelian randomization (MR) to explore the causal relationship between the gut microbiota and urological cancers. In MR analysis, genetic variations are utilized as instrumental variables (IVs) to explore the causal association between exposure and outcome [22]. Since genetic variants originate from parents and are randomly assigned at conception, properly conducted MR analysis can prevent reverse causality and lessen bias caused by environmental variables [23].

In this study, we performed a bidirectional two-sample MR analysis to explore the causal relationship between gut microbiota and five urological cancers, including bladder cancer, prostate cancer, renal cell cancer, renal pelvis cancer, and testicular cancer. Based on the results of the MR analysis, we tried to elucidate the role of the gut microbiota in urological cancers, find new targets for cancer screening and prevention, and pave the way

for the development of microbiota-based interventions, such as microbiota transplantation, probiotic therapy, and dietary therapy [18].

2. Materials and Methods

2.1. Study Design

Gut microbiota was defined as exposure and five urological cancers as the outcome (reverse MR analysis: urological cancer as exposure and gut microbiota as outcome). The IVs were screened out with a series of quality control procedures and analyzed using MR (Figure 1). Our MR analysis was based on three assumptions: (1) IVs were significantly associated with exposure; (2) IVs were not associated with any confounding factors; (3) IVs did not affect outcomes in any way other than exposure [24].
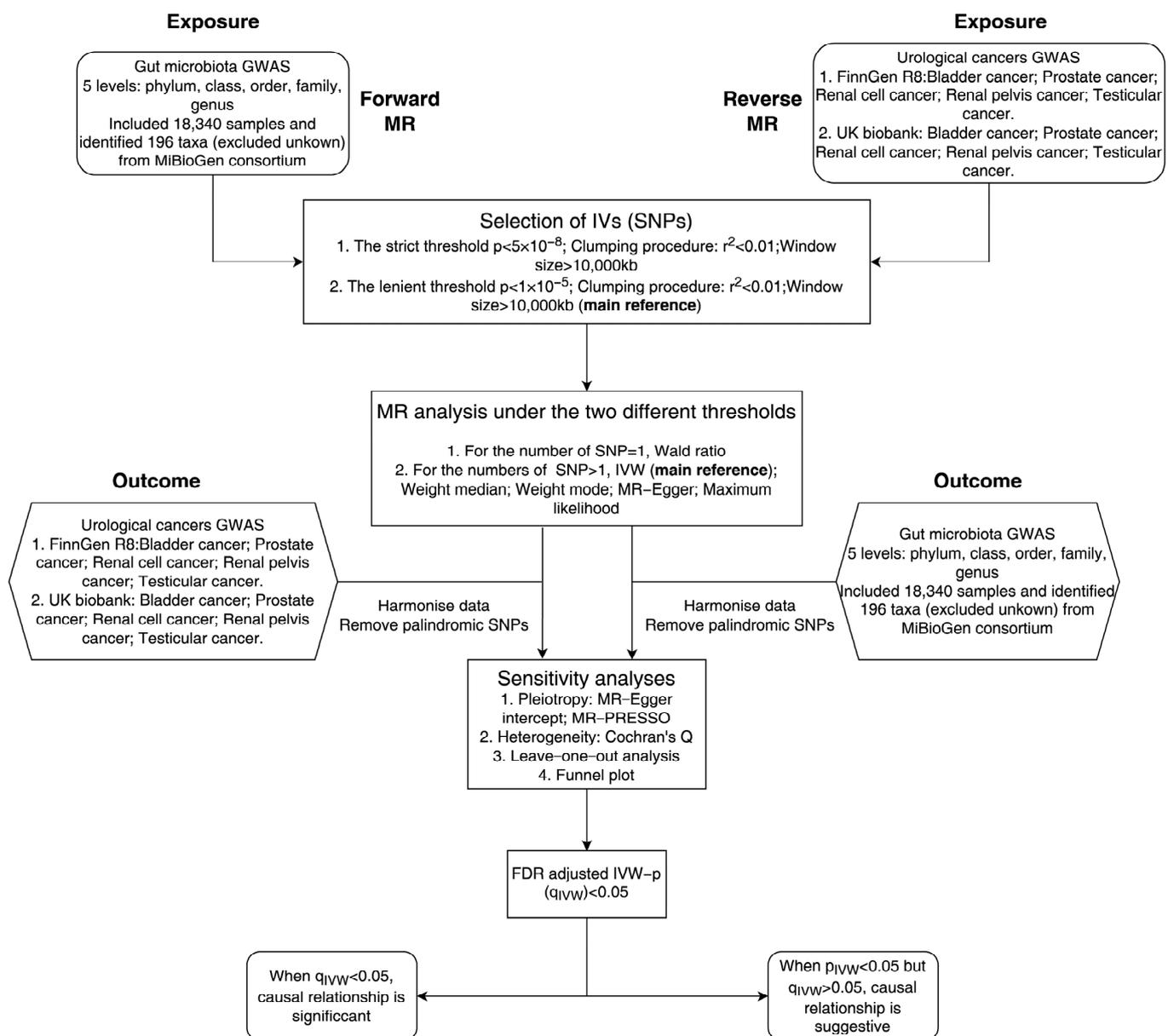

Figure 1. Study design and flowchart of MR analysis in this study.

2.2. Data Sources

Single-nucleotide polymorphisms (SNPs) associated with the gut microbiota were obtained from the largest genome-wide association study (GWAS) summary data to

date published by the MiBioGen consortium [25]. This is a large-scale multi-ethnic GWAS involving 18,340 participants in 24 cohorts, 72.3% of whom had European ancestry (n = 13,266). This study analyzed the microbial composition and classified microbiota using direct taxonomic binning by targeting three distinct variable regions (V4, V3–V4, V1–V2) of 16S ribosomal RNA in participants. Genetic variants in microbiota hosts were identified using microbiota quantitative trait loci mapping analysis. Kurilshikov A et al. also adjusted for sex, age, technical covariates, and genetic principal components. GWAS finally included 211 taxa (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla) [25].

We obtained GWAS summary data for five urological cancers from FinnGen Biobank R8 and UK biobank, respectively [26,27]. Five urological cancers were diagnosed according to ICD-O-3, controls excluding all cancers. More information on the above can be found in the Supplementary Tables S1–S6.

2.3. Instrument Variable Selection

To ensure that the results of MR analysis are stable and reliable, we used the following criteria to screen IVs: (1) SNPs that were statistically significantly associated with the gut microbiome were chosen as IVs ($p < 5 \times 10^{-8}$). However, only a small amount of SNPs were chosen as IVs. To explore more comprehensive results, we set a more lenient threshold and used the filtered SNPs as the second IV set ($p < 1 \times 10^{-5}$). (2) To avoid linkage disequilibrium (LD), we selected the independent SNPs ($r^2 < 0.01$ and distance > 10,000 kb) using the clumping procedure. (3) SNPs with minor allele frequencies (MAF) below 0.01 were excluded. (4) We removed duplicate SNPs and palindromic SNPs. (5) We searched Phenoscanner (http://www.phenoscanner.medschl.cam.ac.uk/, accessed on 28 April 2023), a database that can be used to find SNP-associated phenotypes, and removed SNPs directly related ($p = 5 \times 10^{-8}$) to five urological cancers [28]. (6) We calculated F-statistics (F), preventing the impact of weak IVs on the results, with the formula $F = \frac{R^2 \times (N - 1 - K)}{(1 - R^2) \times K}$, ($R^2 = 2 \times beta^2 \times MAF \times (1 - MAF)$, N = sample size, K = number of IVs). $F > 10$ was generally considered a threshold for strong IVs [29]. Therefore, we excluded IVs with $F < 10$ when performing MR analysis.

2.4. MR Analysis

We performed MR analysis to explore the causal relationship between the gut microbiota and five urological cancers. For taxa with only one IV, we performed the Wald ratio for MR analysis [30]. For taxa with more than one IV, inverse variance-weighted (IVW) was the main statistical method for MR analysis. Through meta-analysis, the IVW integrated the impacts of individual IVs into a total weighted effect. Therefore, this method was reliable when all IVs were valid [31]. In addition, we used weighted median, maximum likelihood, weighted mode, and MR–Egger methods for complementary and alternative analyses. When more than 50% of IVs were valid, the results of the weighted-median method were reliable [32]. The maximum likelihood ratio measured the probability of a distribution parameter [33]. Based on the similarity of causality, the weighted mode method could divide SNPs into different subsets and assess the causal connection between exposure and outcome using the subset with the highest number of SNPs [34]. Horizontal pleiotropy could be calculated with MR egger. However, affected by the SNPs, the result may not be accurate [32]. When the results of the five MR analysis methods were different, we gave priority to the results of IVW.

To avoid increased Type 1 errors from multiple hypothesis testing, we corrected the significance threshold using the false discovery rate (FDR) correction [35]. Considering that the q-value (q) corrected with microbiota counts were too stringent, we used the number of species of the MR analysis method for correction. Corrected threshold $q < 0.05$ was considered significant. When $p < 0.05$ but $q > 0.05$, gut microbiota were considered associated with urological cancers.

2.5. Sensitivity Analyses

For taxa with more than 2 SNPs, we performed a series of sensitivity analyses. In the MR–Egger regression, intercepts were used to test directional horizontal pleiotropic effects [36]. We also used MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) global test for pleiotropy analysis and corrected estimates by removing outliers (outlier test) if necessary [37]. In addition, we used Cochran's Q statistic and funnel plot to examine the heterogeneity of IVW and the MR–Egger regression methods. If there was heterogeneity in the results ($p < 0.05$), MR analysis was performed using the random effects model IVW. To determine if certain SNPs may affect on the results of the MR analysis, we used the leave-one-out test.

2.6. Reverse MR Analysis

To explore whether urological cancer has a causal effect on the important gut microbial taxa identified in the forward MR analysis, we performed a reverse MR analysis (five urological cancers as exposures and important gut microbial taxa as outcomes). The analysis process was consistent with the forward MR analysis. The MR Steiger directionality test was used to examine whether there was a directional causality between exposure and outcome [38].

The MR analyses were performed using "TwoSampleMR" (version 0.5.6), "Mendelian-Randomization" (version 0.6.0), "MRPRESSO" (version 1.0), and "qvalue" (version 1.0) packages in R (version 4.2.1). Statistical significance was assigned to a result with $p < 0.05$ (two-sided).

3. Results

3.1. SNPs Selection

After removing 15 unknown taxa, our study included 196 taxa (9 phyla, 16 classes, 20 orders, 33 families, and 119 genera) for MR analysis. According to the screening criteria of IVs, we selected 22 SNPs (one SNP for phylum, one SNP for class, three SNPs for order, five SNPs for family, and twelve SNPs for genus) at $p < 5 \times 10^{-8}$ level and 2238 SNPs (108 SNPs for phylum, 194 SNP for class, 237 SNPs for order, 414 SNPs for family, and 1306 SNPs for genus) at $p < 1 \times 10^{-5}$. The F-statistics for all SNPs were all > 10 (27.8–106.6 at $p < 5 \times 10^{-8}$, 10.2–106.6 at $p < 1 \times 10^{-5}$) (Supplementary Tables S1–S6). The results showed that all SNPs were effective strong IVs, and instrumental bias would not affect the results of MR analysis.

3.2. Forward MR Analysis

For the taxa with only one SNP, $q = p$. For the taxa with more than 1 SNP, $q = p \times \frac{m}{k}$. M represented the number of MR analysis methods. Sorting the p-values from smallest to largest, k represented the ranking of p-values.

3.3. Bladder Cancer

In MR analysis at the $p < 5 \times 10^{-8}$ level, we found that the genus *Allisonella* reduced the risk of bladder cancer (odds ratio (OR) = 0.55, 95% confidence interval (CI) = 0.37–0.82, $p = 3.37 \times 10^{-3}$, Wald ratio) and the genus *Eubacterium coprostanoligenes group* (OR = 4.27, 95% CI = 1.56–11.68, $p = 4.72 \times 10^{-3}$, Wald ratio) increased the risk of bladder cancer (Table 1).

In MR analysis at the $p < 1 \times 10^{-5}$ level, we found that family *Rikenellaceae* (OR = 0.69, 95% CI = 0.53–0.90, $q = 1.59 \times 10^{-2}$, IVW), genus *LachnospiraceaeUCG001* (OR = 0.74, 95% CI = 0.58–0.94, $q = 2.66 \times 10^{-2}$, IVW), and genus *Oscillibacter* (OR = 0.66, 95% CI = 0.52–0.83, $q = 1.21 \times 10^{-3}$, IVW) reduced the risk of bladder cancer; genus *Eubacterium ruminantium group* (OR = 1.31, 95% CI = 1.09–1.56, $q = 1.53 \times 10^{-2}$, IVW), genus *RuminococcaceaeUCG013* (OR = 1.67, 95% CI = 1.21–2.31, $q = 8.76 \times 10^{-3}$, IVW), and genus *Senegalimassilia* (OR = 1.61, 95% CI = 1.17–2.21, $q = 1.32 \times 10^{-2}$, IVW) increased the risk of bladder cancer (Table 2 and Figure 2).

Table 1. Mendelian randomization (MR) results of significant causal relationship between gut microbiome and urological cancers ($p < 5 \times 10^{-8}$).

Exposure	Outcome	No. SNP	Methods	β	SE	OR	95% CI	*p*-Value	Causal Direction	Steiger *p*
genus *Allisonella*	Bladder cancer	1	Wald ratio	−0.594	0.202	0.55	0.37–0.82	3.40×10^{-3}	TRUE	2.60×10^{-6}
genus *Ruminococcus torques group*	Bladder cancer	1	Wald ratio	1.451	0.513	4.27	1.56–11.68	4.70×10^{-3}	TRUE	1.54×10^{-6}
genus *Ruminococcus torques group*	Prostate cancer	1	Wald ratio	0.671	0.258	1.96	1.18–3.25	9.20×10^{-3}	TRUE	8.62×10^{-6}
genus *Ruminococcus torques group*	Renal cell cancer	1	Wald ratio	1.414	0.573	4.11	1.34–12.67	1.40×10^{-2}	TRUE	1.02×10^{-6}
genus *Eubacterium coprostanoligenes group*	Renal pelvis cancer	1	Wald ratio	4.989	2.204	146.89	1.95–11,058.66	2.40×10^{-2}	TRUE	4.26×10^{-7}
family *Peptostreptococcaceae*	Testicular cancer	1	Wald ratio	2.672	1.261	14.48	1.22–171.37	3.40×10^{-2}	TRUE	2.88×10^{-6}
genus *Romboutsia*	Testicular cancer	1	Wald ratio	2.646	1.248	14.10	1.22–162.84	3.40×10^{-2}	TRUE	8.59×10^{-6}

Table 2. Mendelian randomization (MR) results of significant causal relationship between gut microbiome and urological cancers ($p < 1 \times 10^{-5}$).

Exposure	Outcome	No. SNP	Methods	β	SE	OR	95% CI	*p*-Value	Causal Direction	Steiger *p*
family *Rikenellaceae*	Bladder cancer	18	IVW	−0.372	0.136	0.69	0.53–0.90	6.40×10^{-3}	TRUE	1.24×10^{-74}
genus *Eubacterium ruminantium group*	Bladder cancer	18	IVW	0.267	0.090	1.31	1.09–1.56	3.10×10^{-3}	TRUE	3.21×10^{-73}
genus *Lachnospiraceae UCG001*	Bladder cancer	11	IVW	−0.302	0.120	0.74	0.58–0.94	1.20×10^{-2}	TRUE	3.65×10^{-46}
genus *Oscillibacte*	Bladder cancer	10	IVW	−0.423	0.121	0.66	0.52–0.83	4.80×10^{-4}	TRUE	2.40×10^{-40}
genus *Ruminococcaceae UCG013*	Bladder cancer	11	IVW	0.514	0.164	1.67	1.21–2.31	1.80×10^{-3}	TRUE	3.55×10^{-46}
genus *Senegalimassilia*	Bladder cancer	4	IVW	0.473	0.162	1.61	1.17–2.21	3.50×10^{-3}	TRUE	2.83×10^{-16}
genus *Barnesiella*	Prostate cancer	12	IVW	−1.422	0.485	0.24	0.09–0.62	3.40×10^{-3}	TRUE	4.18×10^{-48}
genus *Butyricicoccus*	Prostate cancer	3	IVW	−1.725	0.649	0.18	0.05–0.64	7.90×10^{-3}	TRUE	1.59×10^{-20}
genus *Oscillibacter*	Prostate cancer	13	IVW	−0.154	0.057	0.86	0.77–0.96	7.20×10^{-3}	TRUE	6.46×10^{-50}
genus *Ruminococcaceae UCG005*	Prostate cancer	14	IVW	0.413	0.150	1.51	1.13–2.03	6.00×10^{-3}	TRUE	7.84×10^{-57}
class *Alphaproteobacteria*	Renal cell cancer	6	IVW	0.456	0.192	1.58	1.08–2.30	1.80×10^{-2}	TRUE	3.89×10^{-26}
class *Bacilli*	Renal cell cancer	18	IVW	0.342	0.151	1.41	1.05–1.90	2.40×10^{-2}	TRUE	1.42×10^{-74}
family *FamilyXI*	Renal cell cancer	8	IVW	−0.273	0.098	0.76	0.63–0.92	5.60×10^{-3}	TRUE	1.09×10^{-34}
genus *Coprococcus 2*	Renal cell cancer	9	IVW	−0.468	0.179	0.63	0.44–0.89	9.10×10^{-3}	TRUE	2.73×10^{-36}
genus *Eubacterium brachy group*	Renal cell cancer	4	IVW	0.761	0.280	2.14	1.24–3.71	6.60×10^{-3}	TRUE	6.49×10^{-18}
genus *Intestinimonas*	Renal cell cancer	16	IVW	−0.374	0.134	0.69	0.53–0.89	5.20×10^{-3}	TRUE	4.54×10^{-68}
genus *Lachnoclostridium*	Renal cell cancer	13	IVW	−0.635	0.188	0.53	0.37–0.77	7.70×10^{-4}	TRUE	5.58×10^{-52}
genus *Lactococcus*	Renal cell cancer	6	IVW	−0.601	0.244	0.55	0.34–0.88	1.40×10^{-2}	TRUE	7.62×10^{-27}
family *Christensenellaceae*	Renal pelvis cancer	9	IVW	0.748	0.218	2.11	1.38–3.25	6.30×10^{-4}	TRUE	9.96×10^{-49}
family *Clostridiaceae 1*	Renal pelvis cancer	8	IVW	−0.751	0.234	0.47	0.30–0.75	1.40×10^{-3}	TRUE	1.89×10^{-30}
genus *Clostridium-sensustricto 1*	Renal pelvis cancer	7	IVW	−0.601	0.218	0.55	0.36–0.84	6.00×10^{-3}	TRUE	2.18×10^{-33}
genus *Eubacterium eligens group*	Renal pelvis cancer	6	IVW	0.886	0.287	2.43	1.38–4.26	2.00×10^{-3}	TRUE	3.96×10^{-22}
genus *Subdoligranulum*	Testicular cancer	7	IVW	−2.029	0.781	0.13	0.03–0.61	9.40×10^{-3}	TRUE	1.42×10^{-24}

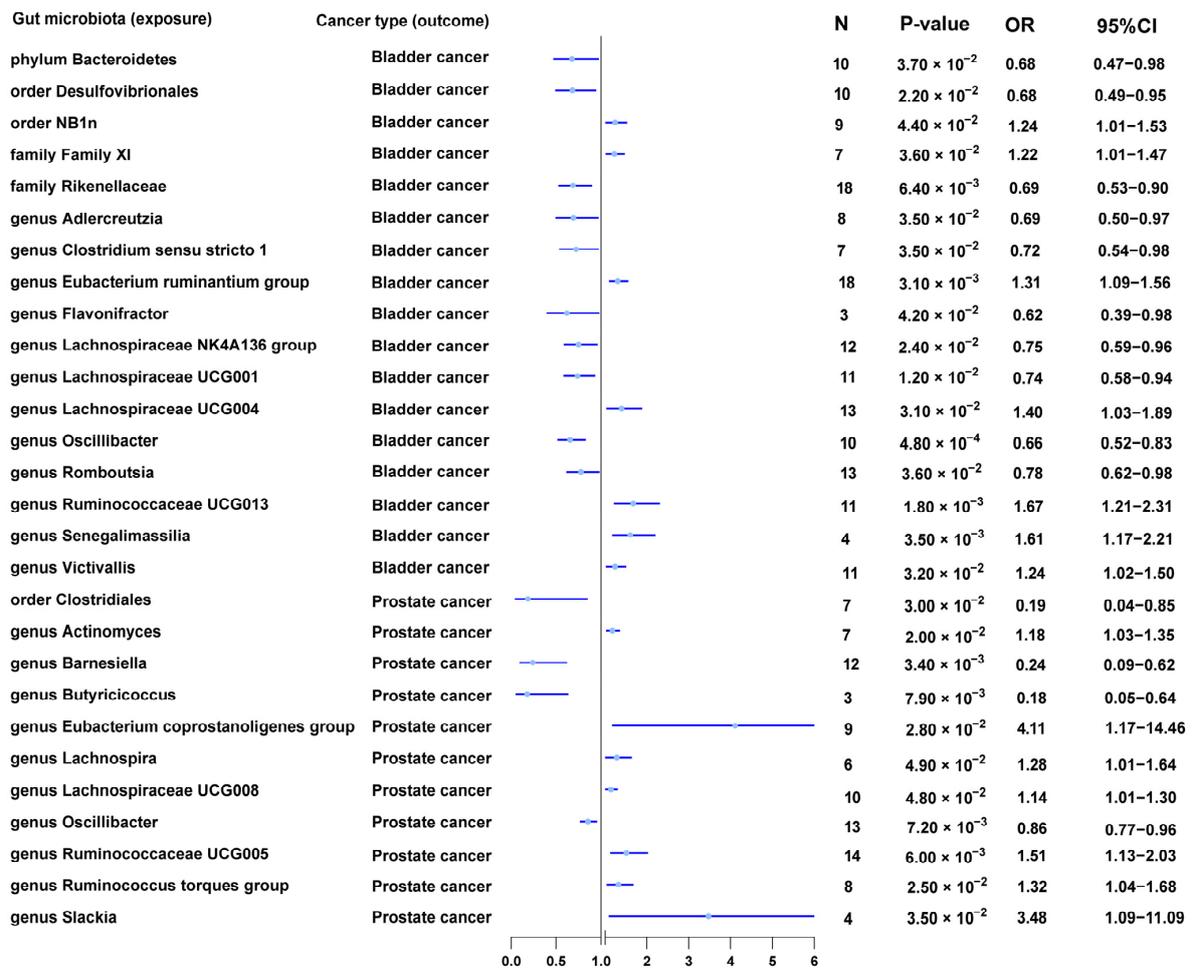| Gut microbiota (exposure)	Cancer type (outcome)		N	P-value	OR	95%CI
phylum Bacteroidetes	Bladder cancer		10	3.70×10^{-2}	0.68	0.47−0.98
order Desulfovibrionales	Bladder cancer		10	2.20×10^{-2}	0.68	0.49−0.95
order NB1n	Bladder cancer		9	4.40×10^{-2}	1.24	1.01−1.53
family Family XI	Bladder cancer		7	3.60×10^{-2}	1.22	1.01−1.47
family Rikenellaceae	Bladder cancer		18	6.40×10^{-3}	0.69	0.53−0.90
genus Adlercreutzia	Bladder cancer		8	3.50×10^{-2}	0.69	0.50−0.97
genus Clostridium sensu stricto 1	Bladder cancer		7	3.50×10^{-2}	0.72	0.54−0.98
genus Eubacterium ruminantium group	Bladder cancer		18	3.10×10^{-3}	1.31	1.09−1.56
genus Flavonifractor	Bladder cancer		3	4.20×10^{-2}	0.62	0.39−0.98
genus Lachnospiraceae NK4A136 group	Bladder cancer		12	2.40×10^{-2}	0.75	0.59−0.96
genus Lachnospiraceae UCG001	Bladder cancer		11	1.20×10^{-2}	0.74	0.58−0.94
genus Lachnospiraceae UCG004	Bladder cancer		13	3.10×10^{-2}	1.40	1.03−1.89
genus Oscillibacter	Bladder cancer		10	4.80×10^{-4}	0.66	0.52−0.83
genus Romboutsia	Bladder cancer		13	3.60×10^{-2}	0.78	0.62−0.98
genus Ruminococcaceae UCG013	Bladder cancer		11	1.80×10^{-3}	1.67	1.21−2.31
genus Senegalimassilia	Bladder cancer		4	3.50×10^{-3}	1.61	1.17−2.21
genus Victivallis	Bladder cancer		11	3.20×10^{-2}	1.24	1.02−1.50
order Clostridiales	Prostate cancer		7	3.00×10^{-2}	0.19	0.04−0.85
genus Actinomyces	Prostate cancer		7	2.00×10^{-2}	1.18	1.03−1.35
genus Barnesiella	Prostate cancer		12	3.40×10^{-3}	0.24	0.09−0.62
genus Butyricicoccus	Prostate cancer		3	7.90×10^{-3}	0.18	0.05−0.64
genus Eubacterium coprostanoligenes group	Prostate cancer		9	2.80×10^{-2}	4.11	1.17−14.46
genus Lachnospira	Prostate cancer		6	4.90×10^{-2}	1.28	1.01−1.64
genus Lachnospiraceae UCG008	Prostate cancer		10	4.80×10^{-2}	1.14	1.01−1.30
genus Oscillibacter	Prostate cancer		13	7.20×10^{-3}	0.86	0.77−0.96
genus Ruminococcaceae UCG005	Prostate cancer		14	6.00×10^{-3}	1.51	1.13−2.03
genus Ruminococcus torques group	Prostate cancer		8	2.50×10^{-2}	1.32	1.04−1.68
genus Slackia	Prostate cancer		4	3.50×10^{-2}	3.48	1.09−11.09

Figure 2. MR results of causal effects between gut microbiota and urological cancers (Bladder cancer and Prostate cancer) ($p < 1 \times 10^{-5}$).

3.4. Prostate Cancer

In MR analysis at the $p < 5 \times 10^{-8}$ level, we found that the genus *Ruminococcus torques group* (OR = 1.96, 95% CI = 1.18–3.25, $p = 9.24 \times 10^{-3}$, Wald ratio) increased the risk of prostate cancer (Table 1).

In MR analysis at the $p < 1 \times 10^{-5}$ level, we found that the genera *Oscillibacter* (OR = 0.86, 95% CI = 0.77–0.96, $q = 2.42 \times 10^{-2}$, IVW), *Barnesiella* (OR = 0.24, 95% CI = 0.09–0.62, $q = 1.23 \times 10^{-2}$, IVW), and *Butyricicoccus* (OR = 0.18, 95% CI = 0.05–0.64, $q = 2.94 \times 10^{-2}$, IVW) reduced the risk of prostate cancer; the genus *RuminococcaceaeUCG005* (OR = 1.51, 95% CI = 1.13–2.03, $q = 3.02 \times 10^{-2}$, IVW) increased the risk of prostate cancer (Table 2 and Figure 2).

3.5. Renal Cell Cancer

In MR analysis at the $p < 5 \times 10^{-8}$ level, we found that the genus *Ruminococcus torques group* (OR = 4.11, 95% CI = 1.34–12.67, $p = 1.37 \times 10^{-2}$, Wald ratio) increased the risk of renal cell cancer (Table 1).

In MR analysis at the $p < 1 \times 10^{-5}$ level, we found that the family *Family XI* (OR = 0.76, 95% CI = 0.63–0.92, $q = 1.80 \times 10^{-2}$, IVW), the genera *Coprococcus 2* (OR = 0.63, 95% CI = 0.44–0.89, $q = 2.44 \times 10^{-2}$, IVW), *Intestinimonas* (OR = 0.69, 95% CI = 0.53–0.89, $q = 1.89 \times 10^{-2}$, IVW), *Lachnoclostridium* (OR = 0.53, 95% CI = 0.37–0.77, $q = 1.92 \times 10^{-3}$, IVW), and *Lactococcus* (OR = 0.55, 95% CI = 0.34–0.88, $q = 3.64 \times 10^{-2}$, IVW) reduced the risk of renal cell cancer; the classes *Alphaproteobacteria* (OR = 1.58, 95% CI = 1.08–2.30,

$q = 4.76 \times 10^{-2}$, IVW) and *Bacilli* (OR = 1.41, 95% CI = 1.05–1.90, $q = 4.22 \times 10^{-2}$, IVW), and the genus *Eubacterium brachy group* (OR = 2.14, 95% CI = 1.24–3.71, $q = 2.27 \times 10^{-2}$, IVW) increased the risk of renal cell cancer (Table 2 and Figure 3).
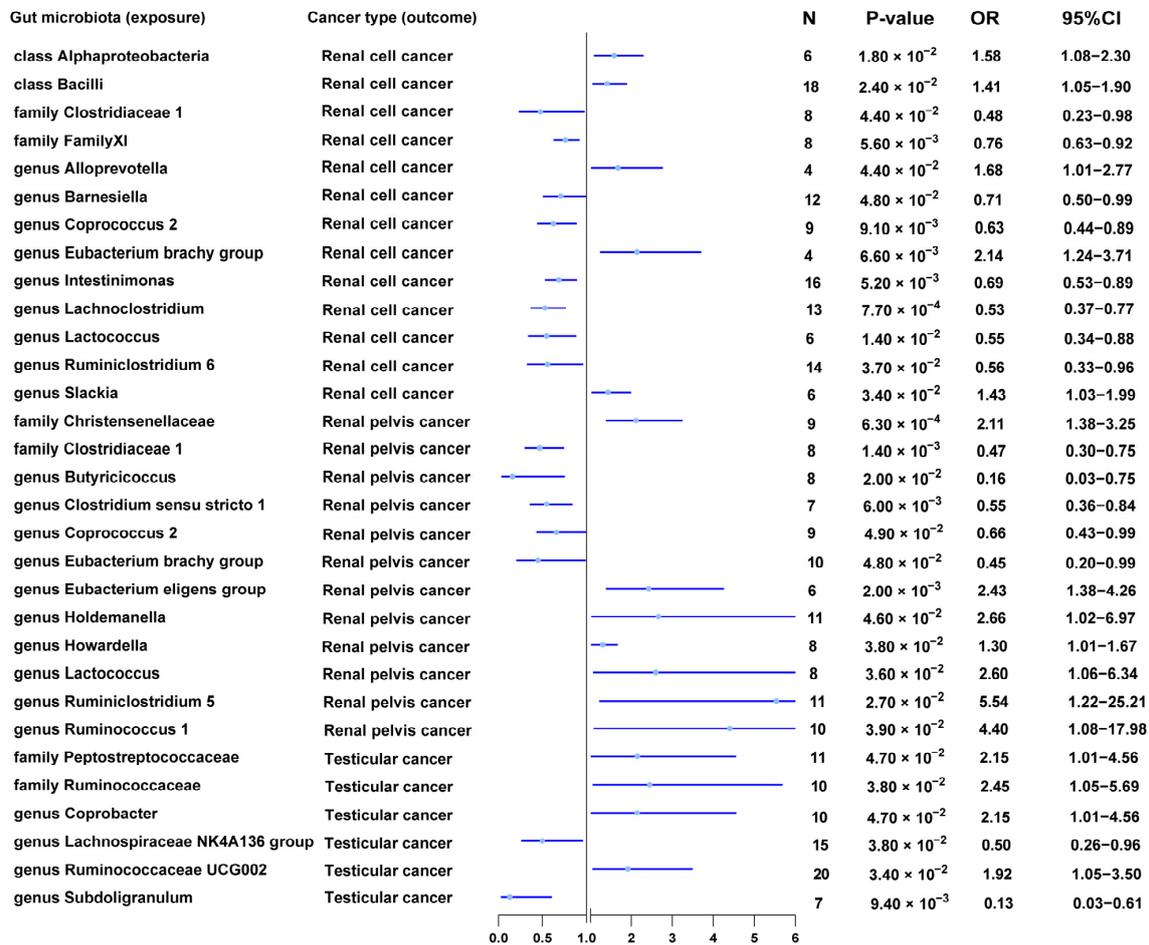
Gut microbiota (exposure)	Cancer type (outcome)		N	P-value	OR	95%CI
class Alphaproteobacteria	Renal cell cancer		6	1.80×10^{-2}	1.58	1.08–2.30
class Bacilli	Renal cell cancer		18	2.40×10^{-2}	1.41	1.05–1.90
family Clostridiaceae 1	Renal cell cancer		8	4.40×10^{-2}	0.48	0.23–0.98
family FamilyXI	Renal cell cancer		8	5.60×10^{-3}	0.76	0.63–0.92
genus Alloprevotella	Renal cell cancer		4	4.40×10^{-2}	1.68	1.01–2.77
genus Barnesiella	Renal cell cancer		12	4.80×10^{-2}	0.71	0.50–0.99
genus Coprococcus 2	Renal cell cancer		9	9.10×10^{-3}	0.63	0.44–0.89
genus Eubacterium brachy group	Renal cell cancer		4	6.60×10^{-3}	2.14	1.24–3.71
genus Intestinimonas	Renal cell cancer		16	5.20×10^{-3}	0.69	0.53–0.89
genus Lachnoclostridium	Renal cell cancer		13	7.70×10^{-4}	0.53	0.37–0.77
genus Lactococcus	Renal cell cancer		6	1.40×10^{-2}	0.55	0.34–0.88
genus Ruminiclostridium 6	Renal cell cancer		14	3.70×10^{-2}	0.56	0.33–0.96
genus Slackia	Renal cell cancer		6	3.40×10^{-2}	1.43	1.03–1.99
family Christensenellaceae	Renal pelvis cancer		9	6.30×10^{-4}	2.11	1.38–3.25
family Clostridiaceae 1	Renal pelvis cancer		8	1.40×10^{-3}	0.47	0.30–0.75
genus Butyricicoccus	Renal pelvis cancer		8	2.00×10^{-2}	0.16	0.03–0.75
genus Clostridium sensu stricto 1	Renal pelvis cancer		7	6.00×10^{-3}	0.55	0.36–0.84
genus Coprococcus 2	Renal pelvis cancer		9	4.90×10^{-2}	0.66	0.43–0.99
genus Eubacterium brachy group	Renal pelvis cancer		10	4.80×10^{-2}	0.45	0.20–0.99
genus Eubacterium eligens group	Renal pelvis cancer		6	2.00×10^{-3}	2.43	1.38–4.26
genus Holdemanella	Renal pelvis cancer		11	4.60×10^{-2}	2.66	1.02–6.97
genus Howardella	Renal pelvis cancer		8	3.80×10^{-2}	1.30	1.01–1.67
genus Lactococcus	Renal pelvis cancer		8	3.60×10^{-2}	2.60	1.06–6.34
genus Ruminiclostridium 5	Renal pelvis cancer		11	2.70×10^{-2}	5.54	1.22–25.21
genus Ruminococcus 1	Renal pelvis cancer		10	3.90×10^{-2}	4.40	1.08–17.98
family Peptostreptococcaceae	Testicular cancer		11	4.70×10^{-2}	2.15	1.01–4.56
family Ruminococcaceae	Testicular cancer		10	3.80×10^{-2}	2.45	1.05–5.69
genus Coprobacter	Testicular cancer		10	4.70×10^{-2}	2.15	1.01–4.56
genus Lachnospiraceae NK4A136 group	Testicular cancer		15	3.80×10^{-2}	0.50	0.26–0.96
genus Ruminococcaceae UCG002	Testicular cancer		20	3.40×10^{-2}	1.92	1.05–3.50
genus Subdoligranulum	Testicular cancer		7	9.40×10^{-3}	0.13	0.03–0.61

Figure 3. MR results of causal effects between gut microbiota and urological cancers (Renal cell cancer, Renal pelvis cancer, and Testicular cancer) ($p < 1 \times 10^{-5}$).

3.6. Renal Pelvis Cancer

In MR analysis at the $p < 5 \times 10^{-8}$ level, we found that the genus *Eubacterium co-prostanoligenes group* (OR = 146.89, 95% CI = 1.95–11,058.66, $p = 2.36 \times 10^{-2}$, Wald ratio) increased the risk of renal pelvis cancer (Table 1).

In MR analysis at the $p < 1 \times 10^{-5}$ level, we found that the family *Clostridiaceae 1* (OR = 0.47, 95% CI = 0.30–0.75, $q = 3.98 \times 10^{-3}$, IVW) and the genus *Clostridium sensu stricto 1* (OR = 0.55, 95% CI = 0.36–0.84, $q = 1.73 \times 10^{-2}$, IVW) reduced the risk of renal pelvis cancer; the family *Christensenellaceae* (OR = 2.11, 95% CI = 1.38–3.25, $q = 2.21 \times 10^{-3}$, IVW) and the genus *Eubacterium eligens group* (OR = 2.43, 95% CI = 1.38–4.26, $q = 6.33 \times 10^{-3}$, IVW) increased the risk of renal pelvis cancer (Table 2 and Figure 3).

3.7. Testicular Cancer

In MR analysis at the $p < 5 \times 10^{-8}$ level, we found that family *Peptostreptococcaceae* (OR = 14.48, 95% CI = 1.22–171.37, $p = 3.40 \times 10^{-2}$, Wald ratio) and the genus *Romboutsia* (OR = 14.10, 95% CI = 1.22–162.84, $p = 3.40 \times 10^{-2}$, Wald ratio) increased the risk of testicular cancer (Table 1).

In MR analysis at the $p < 1 \times 10^{-5}$ level, we found that the genus *Subdoligranulum* (OR = 0.13, 95% CI = 0.03–0.61, $q = 1.91 \times 10^{-2}$, IVW) reduced the risk of testicular cancer (Table 2 and Figure 3).

3.8. Sensitivity Analyses

Heterogeneity and pleiotropy analyses ensured the reliability and robustness of our MR analysis results. Cochran's Q statistic of IVW and MR–Egger showed no heterogeneity in five urological cancers (Table 3). The funnel plot suggested the same result as Cochran's Q statistic (Supplementary Figures S1–S91). The MR–Egger intercept and MR-PRESSO global test showed no potential horizontal pleiotropy (Table 3). We also performed the leave-one-out test and re-analyzed after removing one SNP each time, and our results were still stable (Supplementary Figures S1–S91). Forest plots are shown in Supplementary Figures S1–S91.

Table 3. The results of the sensitivity analyses of MR.

| Exposure | Outcome | Method | Heterogeneity | | MR-PRESSO | MR–Egger Pleiotropy Test | |
			Cochran's Q	*p*-Value	Global *p*-Value	MR–Egger Intercept	*p*-Value
family *Rikenellaceae*	Bladder cancer	IVW	14.77	0.61	0.62		
		MR–Egger	11.64	0.77		−0.050	0.10
genus *Eubacterium ruminantium group*	Bladder cancer	IVW	17.47	0.42	0.47		
		MR–Egger	17.43	0.36		0.005	0.86
genus *Lachnospiraceae UCG001*	Bladder cancer	IVW	5.31	0.87	0.882		
		MR–Egger	5.30	0.81		−0.005	0.92
genus *Oscillibacte*	Bladder cancer	IVW	9.54	0.39	0.45		
		MR–Egger	9.37	0.31		0.020	0.72
genus *Ruminococcaceae UCG013*	Bladder cancer	IVW	6.44	0.78	0.80		
		MR–Egger	3.48	0.94		−0.062	0.12
genus *Senegalimassilia*	Bladder cancer	IVW	1.79	0.62	0.69		
		MR–Egger	1.43	0.49		0.031	0.61
genus *Barnesiella*	Prostate cancer	IVW	3.70	0.98	0.99		
		MR–Egger	2.64	0.99		−0.138	0.33
genus *Butyricicoccus*	Prostate cancer	IVW	1.17	0.56			
		MR–Egger	1.15	0.28		0.031	0.93
genus *Oscillibacter*	Prostate cancer	IVW	10.60	0.56	0.59		
		MR–Egger	10.59	0.48		−0.002	0.94
genus *Ruminococcaceae UCG005*	Prostate cancer	IVW	16.32	0.23	0.17		
		MR–Egger	12.48	0.41		−0.060	0.08
class *Alphaproteobacteria*	Renal cell cancer	IVW	3.75	0.59	0.56		
		MR–Egger	3.22	0.52		0.046	0.51
class *Bacilli*	Renal cell cancer	IVW	17.61	0.41	0.46		
		MR–Egger	16.40	0.43		−0.033	0.29
family *FamilyXI*	Renal cell cancer	IVW	2.07	0.99	0.96		
		MR–Egger	0.85	0.96		0.091	0.31
genus *Coprococcus 2*	Renal cell cancer	IVW	4.77	0.78	0.80		
		MR–Egger	4.72	0.69		−0.015	0.84
genus *Eubacterium brachy group*	Renal cell cancer	IVW	0.37	0.95	0.95		
		MR–Egger	0.37	0.83		−0.012	0.97
genus *Intestinimonas*	Renal cell cancer	IVW	13.29	0.58	0.61		
		MR–Egger	13.24	0.51		−0.007	0.83
genus *Lachnoclostridium*	Renal cell cancer	IVW	12.97	0.37	0.43		
		MR–Egger	11.87	0.37		−0.044	0.33
genus *Lactococcus*	Renal cell cancer	IVW	4.66	0.46	0.47		
		MR–Egger	3.69	0.45		−0.138	0.38
family *Christensenellaceae*	Renal pelvis cancer	IVW	6.98	0.54	0.58		
		MR–Egger	6.12	0.53		0.036	0.38
family *Clostridiaceae 1*	Renal pelvis cancer	IVW	4.55	0.71	0.74		
		MR–Egger	3.24	0.78		0.065	0.30
genus *Eubacterium eligens group*	Renal pelvis cancer	IVW	3.93	0.56	0.55		
		MR–Egger	2.75	0.60		−0.111	0.34
genus *Clostridiumsensustricto 1*	Renal pelvis cancer	IVW	4.38	0.62	0.65		
		MR–Egger	4.34	0.50		−0.011	0.84
genus *Subdoligranulum*	Testicular cancer	IVW	9.99	0.13	0.18		
		MR–Egger	9.45	0.09		−0.083	0.62

3.9. Suggestive Associations between Gut Microbiota and Urological Cancers

In MR analysis at the $p < 1 \times 10^{-5}$ level, we found suggested relationships between some members of the gut microbiota and urological cancers ($p < 0.05$, $q > 0.05$, IVW). The phylum *Bacteroidetes*, order *Desulfovibrionales*, order *NB1n*, family *Family XI*, genus *Lachnospiraceae UCG004*, genus *Victivallis*, genus *Adlercreutzia*, genus *Clostridium sensu stricto 1*, genus *Flavonifractor*, genus *Lachnospiraceae NK4A136 group*, and genus *Romboutsia* were suggested to be related to bladder cancer. The order *Clostridiales*, genus *Eubacterium coprostanoligenes group*, genus *Slackia*, genus *Ruminococcus torques group*, genus *Actinomyces*, genus *Lachnospira*, and genus *Lachnospiraceae UCG008* were suggested to be related to prostate cancer. The *family Clostridiaceae1*, *family Family XI*, *genus Barnesiella*, *genus Slackia*, *genus Alloprevotella*, and *genus Ruminiclostridium 6* were suggested to be related to renal cell cancer. The genus *Coprococcus 2*, genus *Holdemanella*, genus *Howardella*, genus *Ruminiclostridium 5*, genus *Eubacterium brachy group*, genus *Eubacterium eligens group*, genus *Butyricicoccus*, genus *Lactococcus*, and genus *Ruminococcus 1* were suggested to be related to renal pelvis cancer. The family *Ruminococcaceae*, family *Peptostreptococcaceae*, genus *Lachnospiraceae NK4A136 group*, genus *Ruminococcaceae UCG002*, genus *Coprobacter*, and genus *Subdoligranulum* were suggested to be related to testicular cancer.

3.10. Reverse MR Analysis

Reverse MR analysis suggested that renal cell cancer may be related to class *Alphaproteobacteria* at $p < 1 \times 10^{-5}$ level ($p < 0.05$, $q > 0.05$, IVW) (Supplementary Tables S1–S6).

4. Discussion

To our knowledge, this study is the first MR analysis to genetically explore the causal relationship between gut microbiota and urologic cancers. Based on the largest GWAS of the gut microbiota, our MR study provides fairly strong genetic evidence that alterations in the abundance of specific gut microbiota play an important role in the occurrence and development of urologic cancers. Using genetic variables as tools, MR analysis largely avoids confounding factors and compensates for the lack of observational studies.

Our study supports previous observational evidence. A metagenomic analysis from China found dysregulated Eubacterium abundance in the gut of bladder cancer patients [39]. In another cohort study, upregulation of Eubacterium abundance in urine was associated with non-muscle invasive bladder cancer [40]. Our findings are identical and link this association specifically to the *Eubacterium coprostanoligenes group* and the *Eubacterium ruminantium group*. ECM1 is a glycoprotein that can induce tumor growth by promoting angiogenesis or enhancing epidermal growth factor signaling [41]. As a regulator of the tumor microenvironment, matrix metalloproteinases can degrade the extracellular matrix and infiltrate tumors into surrounding tissues [42]. ECM1-MMP9 plays an important role in the occurrence and development of tumors [43]. Zhang et al. found that Eubacterium can upregulate ECM1 in bladder tissue, increase the expression of MMP9 through the ERK1/2 phosphorylation pathway, and finally lead to the occurrence and development of bladder cancer [40]. Our study found that *Ruminococcus torques group* and *Ruminococcaceae UCG005* increase the risk of prostate cancer, which is consistent with previous observational studies. Liu et al. found that the abundance of Ruminococcus in the gut of castration-resistant prostate cancer (CRPC) patients was increased [44]. Pernigoni et al. also found that the guts of CRPC patients and mouse models were enriched for Ruminococcus [45]. Ruminococcus belongs to Firmicutes, and its main metabolites are short-chain fatty acids (SCFAs) [46]. SCFAs can promote the production of insulin growth factor 1 (IGF-1) in the whole body and the prostate, and IGF-1 can activate the proliferation of prostate cancer cells through the MAPK and PI3K pathways [47]. Ruminococcus has also been shown to positively correlate with serum testosterone levels, possibly through deglucuronidation of testosterone into a bioactive form and reabsorption [48,49]. Testosterone levels are closely related to the development of prostate cancer [50].

Our MR analysis identified more than 20 gut microbial taxa causally associated with urological cancers, most of which were absent or rarely reported in previous studies. To explore the mechanism of this causal relationship, we interpreted it in several ways. (1) As a compound in the bacterial cell wall, lipopolysaccharide is considered a "danger signal" recognized by the immune system [51]. Gut microbial imbalance can lead to increased LPS, which binds to TLR4 to activate NF-κB, mediates the transcription of stress-related compounds, and increases cancer risk [52]. In addition, higher levels of LPS can cause LPS-endotoxemia to promote carcinogenesis [53]. (2) Gut microbial imbalance can lead to abnormal differentiation of T cells [54]. Regulatory T cells (Tregs) can promote tumor development by silencing the immune clearance of tumor cells [51]. Treg levels may be upregulated during gut ecological dysbiosis, creating a friendly environment for cancer initiation, progression, and metastasis [55]. Gut microbial imbalance induces chronic inflammation in the urinary system through NF-B and mTOR pathways, which can trigger oxidative stress and lead to the accumulation of ROS and NOS [3,56]. (3) Gut microbial imbalance can lead to fluctuations in sex hormone levels, and elevated androgen levels increase the risk of prostate cancer [50]. Elevated estrogen levels may activate polycyclic hydrocarbons, leading to the production of carcinogenic metabolites such as free radical cations that induce DNA damage and increase the risk of cancer [57,58]. Most of the current mechanisms remain at the macroscopic whole gut microbiota level, and more mechanistic studies are needed in the future to explore the role of individual taxa on urologic cancers.

As risk factors for urologic cancers, we propose that obesity and smoking may affect urological cancers by targeting the gut microbiota. Nowadays, a high-fat diet based on carbohydrates and fats is becoming one of the major components of the population's diet, and the incidence of obesity is increasing significantly [59,60]. The positive relationship between obesity and urologic cancers has been confirmed by several studies, and the mechanisms may be related to insulin resistance, abnormal IGF system, ectopic fat deposition, and microbiome alterations [3]. However, microbiome alterations have only been mentioned in observational studies [61–63]. Our study has made a certain contribution to filling the gap in this field. Compared with a healthy population, there are certain alterations in the abundance of specific gut microbes in obesity, which we have shown to be strongly associated with urological cancers. For example, the abundance of *Ruminococcus torques* is increased in the gut of obese people, and *Ruminococcus torques* is associated with an increased risk of prostate cancer and renal cell cancer [61]. *Oscillibacter* is associated with normal body weight and has a protective effect on bladder and prostate cancer [64]. BMI and *Rikenellaceae* show a negative linear relationship, and *Rikenellaceae* may reduce the risk of bladder cancer [65]. The discovery of key gut microbial taxa may open up new avenues for the dietary treatment of cancer. On the other hand, the effect of smoking on urological cancer is surprising. A high-quality MR study demonstrated that smoking leads to a decrease in the abundance of *Ruminococcaceae UCG005*, which we believe increases the risk of prostate cancer [66]. This seems to be contrary to the conventional view that "smoking is a risk factor for cancer". However, a large prospective study from Sweden also came to the same conclusion that smokers have a lower risk of prostate cancer [67]. In conclusion, the relationship between high-fat diet, smoking, gut microbiota, and urologic cancer is complex, and more prospective studies and mediation MR analyses are needed to explore the associations and mechanisms in the future.

Understanding the bidirectional relationship between gut microbiota and urological cancers may contribute to personalized medicine approaches. Our study provides an emerging approach to large-scale screening for urological cancers. Currently, cystoscopy and bladder tissue biopsy are considered to be the gold standard for bladder cancer diagnosis [68]. However, both examinations are invasive, limiting their use in large-scale screening [7]. In recent years, serum prostate-specific antigen (PSA) has been widely used for screening and early detection of prostate cancer due to its non-invasive advantages [69]. Typically, a prostate biopsy is performed when a patient's PSA level is greater than 4 ng/mL [70]. However, PSA is organ-specific rather than tumor-specific, leading to

increased PSA levels in non-neoplastic lesions of the prostate such as benign prostatic hyperplasia [71]. The low specificity of PSA may lead to overdiagnosis and treatment of some nonneoplastic lesions of the prostate [72]. Diagnosis of renal cell cancer is challenging because renal cell cancer is often clinically silent [73]. The clinical manifestations of early renal cell cancer are variable and nonspecific, and when the renal cancer triad (hematuria, pain, mass) appears, renal cell cancer is usually in an advanced state [74]. In fact, renal cell cancer is usually not diagnosed until distant metastases have developed [75]. As mentioned in the introduction, the value of early screening for urological cancers is tremendous, which will greatly improve the five-year survival rate and prognosis of patients. We believe that it is necessary and feasible to construct a risk assessment model for urological cancers based on changes in the abundance of specific taxa of gut microbiota. The model should take into account the changes in abundance of all taxa that are genetically predicted to be related to urologic cancers, not just a specific taxon. In fact, it has been demonstrated that a risk score based on changes in gut microbiota abundance can diagnose prostate cancer more accurately than PSA examination (area under the curve = 0.81 vs. 0.67) [76].

Our study may pave the way for the development of targeted interventions. When microbes are responsible for the development and progression of urologic cancers, modulating the microbiota may yield potential benefits in the urinary tract [77]. A study including 67 patients with renal cell cancer found that antibiotic use before or after anti-PD-2/PD-L1 immunotherapy resulted in significantly shorter progression-free survival and overall survival [78]. Antibiotic use leads to disturbances in gut microbiota abundance, with changes in Ruminococcus abundance correlating with clinical response to checkpoint inhibition [79]. Another study on differences in gut microbiota composition in prostate cancer patients showed a significant increase in Lachnospiraceae abundance in the gut of patients with oral androgen receptor axis-targeted therapy [80]. Karen et al. suggested that the presence of Lachnospiraceae may be associated with a positive response to anti-PD-1 immunotherapy [80]. Our study suggests that Lactococcus, a significant bacterial model for Lactobacillus, reduces the risk of renal cell cancer. Lactococcus is a potent binder of fibronectin, contributing to the maintenance of barrier homeostasis and moderating inflammatory processes triggered by potentially opportunistic microorganisms and other injuries [81]. The increased strength of fibronectin can induce specific immunity to bladder cancer [82]. We suggest that performing fecal Lactococcus transplantation or direct bladder instillation may induce an immune response to treat bladder cancer. Gut microbiota directly or indirectly involved in the treatment of urological cancers, which may be related to hormones, inflammatory responses, and immunity [77]. Our study identified some gut microbiota associated with reduced risk of urologic cancers, laying the groundwork for translating microbiome research into clinical action and opening avenues for microbial-based therapies. Based on our study, clinicians can target individuals at higher risk of urological cancers based on their gut microbial profile and guide personalized interventions to mitigate risk, such as fecal transplantation, probiotic therapy, and dietary therapy.

Additionally, we provide insights for the optimization of the RCT [83]. (1) RCT is the gold standard for exploring causality [20]. Since gut microbiota plays an irreplaceable role in body homeostasis, it is worthwhile to conduct RCT to explore the role of gut microbiota on urological cancer. Our findings provide a solid theoretical basis for conducting RCTs. (2) Due to the extremely complex composition and a large amount of gut microbiota, it is unrealistic to conduct RCT studies on every taxon. A bidirectional MR study could help identify new avenues of research by revealing a hitherto unknown causal relationship between the gut microbiota and urologic cancers. We screened out a series of specific taxa through gene prediction and determined the cancers that have causal relationships with them. Furthermore, we quantified the risk factors utilizing the OR value and provided references for variables and outcomes within RCTs.

Our study has several limitations. Firstly, the participants in the GWAS for urological cancers were all of the European ancestry, so extrapolation of our results to other ethnic groups requires caution. Secondly, GWAS of the gut microbiota included many ethnicities,

which may still result in some bias even though the ancestry of most participants was European (greater than 72.3%). Thirdly, we used the largest GWAS of the gut microbiota to date, but its sample size is still very limited (n = 14,306). In the future, the GWAS of gut microbiota needs to expand the sample size to the traditional GWAS level (n > 100,000) to increase power and reduce errors. Fourthly, urologic cancers are more prevalent in the male population, and the composition of the gut microbiota displays divergence based on gender [2]. Due to the use of summary-level GWAS, our work could not be analyzed separately for the two genders. Gender-specific MR analysis should be carried out in future research. Fifthly, in the process of using drugs to treat diseases, the gut microbiota may develop drug resistance, thus affecting the therapeutic effect. Exploring the drug resistance of taxa that are causally related to urological cancer is meaningful to improve the therapeutic effect of urological cancer. Due to the limitations of the GWAS database, our study cannot explore drug resistance in specific taxa. Therefore, future studies should conduct in-depth studies on drug resistance in the gut microbiota.

5. Conclusions

In conclusion, using publicly accessible gene databases, our study comprehensively explored the causal relationship between gut microbiota and five urological cancers. We verified the existing observational evidence and explored the mechanism of gut microbiota on urological cancer from a macroscopic level, providing potential targets for the screening and treatment of urological cancer and new ideas for future clinical research.

Abbreviations

CI	confidence interval
RCTs	randomized controlled trials
MR	Mendelian randomization
IVs	instrumental variables
SNPs	single-nucleotide polymorphisms
GWAS	genome-wide association study
LD	linkage disequilibrium
MAF	minor allele frequencies
F	F-statistics
IVW	inverse variance weighted
FDR	false discovery rate
q	q-value
MR-PRESSO	MR Pleiotropy RESidual Sum and Outlier
OR	odds ratio
CRPC	castration-resistant prostate cancer
SCFAs	short-chain fatty acids
IGF-1	insulin growth factor 1
Tregs	regulatory T cells
PSA	prostate-specific antigen

References

1. Fitzmaurice, C.; Dicker, D.; Pain, A.; Hamavid, H.; Moradi-Lakeh, M.; MacIntyre, M.F.; Allen, C.; Hansen, G.; Woodbrook, R.; Wolfe, C.; et al. The Global Burden of Cancer 2013. *JAMA Oncol.* **2015**, *1*, 505–527. [CrossRef] [PubMed]
2. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA A Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
3. Papavasileiou, G.; Tsilingiris, D.; Spyrou, N.; Vallianou, N.G.; Karampela, I.; Magkos, F.; Dalamaga, M. Obesity and main urologic cancers: Current systematic evidence, novel biological mechanisms, perspectives and challenges. *Semin. Cancer Biol.* **2023**, *91*, 70–98. [CrossRef] [PubMed]
4. Dy, G.W.; Gore, J.L.; Forouzanfar, M.H.; Naghavi, M.; Fitzmaurice, C. Global Burden of Urologic Cancers, 1990–2013. *Eur. Urol.* **2017**, *71*, 437–446. [CrossRef] [PubMed]
5. Mitchell, K.A.; Williams, H. Emerging genomic biomarkers for improving kidney, prostate, and bladder cancer health disparities outcomes. *Urol. Oncol.* **2022**, *40*, 126–132. [CrossRef]
6. Su, P.; Zhang, M.; Kang, X. Targeting c-Met in the treatment of urologic neoplasms: Current status and challenges. *Front. Oncol.* **2023**, *13*, 1071030. [CrossRef]
7. Li, S.; Xin, K.; Pan, S.; Wang, Y.; Zheng, J.; Li, Z.; Liu, X.; Liu, B.; Xu, Z.; Chen, X. Blood-based liquid biopsy: Insights into early detection, prediction, and treatment monitoring of bladder cancer. *Cell Mol. Biol. Lett.* **2023**, *28*, 28. [CrossRef]
8. Stavropoulou, E.; Kantartzi, K.; Tsigalou, C.; Konstantinidis, T.; Romanidou, G.; Voidarou, C.; Bezirtzoglou, E. Focus on the Gut-Kidney Axis in Health and Disease. *Front. Med.* **2021**, *7*, 620102. [CrossRef]
9. Matsushita, M.; Fujita, K.; Hatano, K.; De Velasco, M.A.; Tsujimura, A.; Uemura, H.; Nonomura, N. Emerging Relationship between the Gut Microbiome and Prostate Cancer. *World J. Men's Health* **2023**, *41*, 759–768. [CrossRef]
10. Yan, X.; Feng, Y.; Hao, Y.; Zhong, R.; Jiang, Y.; Tang, X.; Lu, D.; Fang, H.; Agarwal, M.; Chen, L.; et al. Gut-Testis Axis: Microbiota Prime Metabolome to Increase Sperm Quality in Young Type 2 Diabetes. *Microbiol. Spectr.* **2022**, *10*, e0142322. [CrossRef]
11. Kc, D.; Sumner, R.; Lippmann, S. Gut microbiota and health. *Postgrad. Med.* **2020**, *132*, 274. [CrossRef] [PubMed]
12. Moszak, M.; Szulińska, M.; Bogdański, P. You Are What You Eat-The Relationship between Diet, Microbiota, and Metabolic Disorders-A Review. *Nutrients* **2020**, *12*, 1096. [CrossRef] [PubMed]
13. Patterson, E.; Ryan, P.M.; Cryan, J.F.; Dinan, T.G.; Ross, R.P.; Fitzgerald, G.F.; Stanton, C. Gut microbiota, obesity and diabetes. *Postgrad. Med. J.* **2016**, *92*, 286–300. [CrossRef] [PubMed]
14. Long, Y.; Tang, L.; Zhou, Y.; Zhao, S.; Zhu, H. Causal relationship between gut microbiota and cancers: A two-sample Mendelian randomisation study. *BMC Med.* **2023**, *21*, 66. [CrossRef]
15. He, C.; Li, B.; Huang, L.; Teng, C.; Bao, Y.; Ren, M.; Shan, Y. Gut microbial composition changes in bladder cancer patients: A case-control study in Harbin, China. *Asia Pac. J. Clin. Nutr.* **2020**, *29*, 395–403. [CrossRef]
16. Liss, M.A.; White, J.R.; Goros, M.; Gelfond, J.; Leach, R.; Johnson-Pais, T.; Lai, Z.; Rourke, E.; Basler, J.; Ankerst, D.; et al. Metabolic Biosynthesis Pathways Identified from Fecal Microbiome Associated with Prostate Cancer. *Eur. Urol.* **2018**, *74*, 575–582. [CrossRef]
17. Vivarelli, S.; Falzone, L.; Leonardi, G.C.; Salmeri, M.; Libra, M. Novel insights on gut microbiota manipulation and immune checkpoint inhibition in cancer (Review). *Int. J. Oncol.* **2021**, *59*, 75. [CrossRef]

18. Ting, N.L.-N.; Lau, H.C.-H.; Yu, J. Cancer pharmacomicrobiomics: Targeting microbiota to optimise cancer therapy outcomes. *Gut* **2022**, *71*, 1412–1425. [CrossRef]

19. Boyko, E.J. Observational research--opportunities and limitations. *J. Diabetes Its Complicat.* **2013**, *27*, 642–648. [CrossRef]

20. Wan, Y.; Wang, F.; Yuan, J.; Li, J.; Jiang, D.; Zhang, J.; Li, H.; Wang, R.; Tang, J.; Huang, T.; et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: A 6-month randomised controlled-feeding trial. *Gut* **2019**, *68*, 1417–1429. [CrossRef]

21. Steeger, C.M.; Buckley, P.R.; Pampel, F.C.; Gust, C.J.; Hill, K.G. Common Methodological Problems in Randomized Controlled Trials of Preventive Interventions. *Prev. Sci.* **2021**, *22*, 1159–1172. [CrossRef] [PubMed]

22. Smith, G.D.; Ebrahim, S. 'Mendelian randomization': Can genetic epidemiology contribute to understanding environmental determinants of disease? *Int. J. Epidemiol.* **2003**, *32*, 1–22. [CrossRef] [PubMed]

23. Emdin, C.A.; Khera, A.V.; Kathiresan, S. Mendelian Randomization. *JAMA* **2017**, *318*, 1925–1926. [CrossRef]

24. Bowden, J.; Holmes, M.V. Meta-analysis and Mendelian randomization: A review. *Res. Synth. Methods* **2019**, *10*, 486–496. [CrossRef] [PubMed]

25. Kurilshikov, A.; Medina-Gomez, C.; Bacigalupe, R.; Radjabzadeh, D.; Wang, J.; Demirkan, A.; Le Roy, C.I.; Garay, J.A.R.; Finnicum, C.T.; Liu, X.; et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. *Nat. Genet.* **2021**, *53*, 156–165. [CrossRef]

26. Kurki, M.I.; Karjalainen, J.; Palta, P.; Sipilä, T.P.; Kristiansson, K.; Donner, K.M.; Reeve, M.P.; Laivuori, H.; Aavikko, M.; Kaunisto, M.A.; et al. FinnGen provides genetic insights from a well-phenotyped isolated population. *Nature* **2023**, *613*, 508–518. [CrossRef]

27. Sudlow, C.; Gallacher, J.; Allen, N.; Beral, V.; Burton, P.; Danesh, J.; Downey, P.; Elliott, P.; Green, J.; Landray, M.; et al. UK biobank: An open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* **2015**, *12*, e1001779. [CrossRef]

28. Staley, J.R.; Blackshaw, J.; Kamat, M.A.; Ellis, S.; Surendran, P.; Sun, B.B.; Paul, D.S.; Freitag, D.; Burgess, S.; Butterworth, A.S.; et al. PhenoScanner: A database of human genotype-phenotype associations. *Bioinformatics* **2016**, *32*, 3207–3209. [CrossRef]

29. Burgess, S.; Thompson, S.G.; CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. *Int. J. Epidemiol.* **2011**, *40*, 755–764. [CrossRef]

30. Burgess, S.; Small, D.S.; Thompson, S.G. A review of instrumental variable estimators for Mendelian randomization. *Stat. Methods Med. Res.* **2017**, *26*, 2333–2355. [CrossRef]

31. Burgess, S.; Butterworth, A.; Thompson, S.G. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet. Epidemiol.* **2013**, *37*, 658–665. [CrossRef]

32. Bowden, J.; Davey Smith, G.; Haycock, P.C.; Burgess, S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted-median Estimator. *Genet. Epidemiol.* **2016**, *40*, 304–314. [CrossRef]

33. Milligan, B.G. Maximum-likelihood estimation of relatedness. *Genetics* **2003**, *163*, 1153–1167. [CrossRef]

34. Hartwig, F.P.; Davey Smith, G.; Bowden, J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. *Int. J. Epidemiol.* **2017**, *46*, 1985–1998. [CrossRef] [PubMed]

35. Storey, J.D.; Tibshirani, R. Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9440–9445. [CrossRef]

36. Bowden, J.; Davey Smith, G.; Burgess, S. Mendelian randomization with invalid instruments: Effect estimation and bias detection through Egger regression. *Int. J. Epidemiol.* **2015**, *44*, 512–525. [CrossRef] [PubMed]

37. Verbanck, M.; Chen, C.Y.; Neale, B.; Do, R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat. Genet.* **2018**, *50*, 693–698. [CrossRef] [PubMed]

38. Hemani, G.; Tilling, K.; Davey Smith, G. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet.* **2017**, *13*, e1007081. [CrossRef]

39. Qin, C.; Chen, Z.; Cao, R.; Shi, M.; Tian, Y. Integrated Analysis of the Fecal Metagenome and Metabolome in Bladder Cancer in a Chinese Population. *Genes* **2022**, *13*, 1967. [CrossRef]

40. Zhang, Y.; Wang, W.; Zhou, H.; Cui, Y. Urinary *Eubacterium* sp. CAG:581 Promotes Non-Muscle Invasive Bladder Cancer (NMIBC) Development through the ECM1/MMP9 Pathway. *Cancers* **2023**, *15*, 809. [CrossRef]

41. Lee, K.-M.; Nam, K.; Oh, S.; Lim, J.; Kim, R.K.; Shim, D.; Choi, J.-H.; Lee, S.-J.; Yu, J.-H.; Lee, J.W.; et al. ECM1 regulates tumor metastasis and CSC-like property through stabilization of β-catenin. *Oncogene* **2015**, *34*, 6055–6065. [CrossRef]

42. Lu, P.; Takai, K.; Weaver, V.M.; Werb, Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a005058. [CrossRef] [PubMed]

43. Lee, K.-M.; Nam, K.; Oh, S.; Lim, J.; Kim, Y.-P.; Lee, J.W.; Yu, J.-H.; Ahn, S.-H.; Kim, S.-B.; Noh, D.-Y.; et al. Extracellular matrix protein 1 regulates cell proliferation and trastuzumab resistance through activation of epidermal growth factor signaling. Breast cancer research. *Breast Cancer Res.* **2014**, *16*, 479. [CrossRef] [PubMed]

44. Liu, Y.; Yang, C.; Zhang, Z.; Jiang, H. Gut Microbiota Dysbiosis Accelerates Prostate Cancer Progression Through Increased LPCAT1 Expression and Enhanced DNA Repair Pathways. *Front. Oncol.* **2021**, *11*, 679712. [CrossRef] [PubMed]

45. Pernigoni, N.; Zagato, E.; Calcinotto, A.; Troiani, M.; Mestre, R.P.; Calì, B.; Attanasio, G.; Troisi, J.; Minini, M.; Mosole, S.; et al. Commensal bacteria promote endocrine resistance in prostate cancer through androgen biosynthesis. *Science* **2021**, *374*, 216–224. [CrossRef] [PubMed]

46. Yuan, X.; Chen, R.; Zhang, Y.; Lin, X.; Yang, X. Gut microbiota: Effect of pubertal status. *BMC Microbiol.* **2020**, *20*, 334. [CrossRef] [PubMed]
47. Matsushita, M.; Fujita, K.; Hayashi, T.; Kayama, H.; Motooka, D.; Hase, H.; Jingushi, K.; Yamamichi, G.; Yumiba, S.; Tomiyama, E.; et al. Gut Microbiota-Derived Short-Chain Fatty Acids Promote Prostate Cancer Growth via IGF1 Signaling. *Cancer Res.* **2021**, *81*, 4014–4026. [CrossRef]
48. Shin, J.-H.; Park, Y.-H.; Sim, M.; Kim, S.-A.; Joung, H.; Shin, D.-M. Serum level of sex steroid hormone is associated with diversity and profiles of human gut microbiome. *Res. Microbiol.* **2019**, *170*, 192–201. [CrossRef]
49. Cross, T.-W.L.; Kasahara, K.; Rey, F.E. Sexual dimorphism of cardiometabolic dysfunction: Gut microbiome in the play? *Mol. Metab.* **2018**, *15*, 70–81. [CrossRef]
50. Sandhu, S.; Moore, C.M.; Chiong, E.; Beltran, H.; Bristow, R.G.; Williams, S.G. Prostate cancer. *Lancet* **2021**, *398*, 1075–1090. [CrossRef]
51. Wheeler, K.M.; Liss, M.A. The Microbiome and Prostate Cancer Risk. *Curr. Urol. Rep.* **2019**, *20*, 66. [CrossRef] [PubMed]
52. Vykhovanets, E.V.; Shankar, E.; Vykhovanets, O.V.; Shukla, S.; Gupta, S. High-fat diet increases NF-κB signaling in the prostate of reporter mice. *Prostate* **2011**, *71*, 147–156. [CrossRef]
53. Avgerinos, K.I.; Spyrou, N.; Mantzoros, C.S.; Dalamaga, M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. *Metab. Clin. Exp.* **2019**, *92*, 121–135. [CrossRef] [PubMed]
54. Lee, Y.K.; Mazmanian, S.K. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science* **2010**, *330*, 1768–1773. [CrossRef] [PubMed]
55. Pandiyan, P.; Bhaskaran, N.; Zou, M.; Schneider, E.; Jayaraman, S.; Huehn, J. Microbiome Dependent Regulation of Tregs and Th17 Cells in Mucosa. *Front. Immunol.* **2019**, *10*, 426. [CrossRef]
56. Che, B.; Zhang, W.; Xu, S.; Yin, J.; He, J.; Huang, T.; Li, W.; Yu, Y.; Tang, K. Prostate Microbiota and Prostate Cancer: A New Trend in Treatment. *Front. Oncol.* **2021**, *11*, 805459. [CrossRef]
57. Katongole, P.; Sande, O.J.; Joloba, M.; Reynolds, S.J.; Niyonzima, N. The human microbiome and its link in prostate cancer risk and pathogenesis. *Infect. Agents Cancer* **2020**, *15*, 53. [CrossRef]
58. Plottel, C.S.; Blaser, M.J. Microbiome and malignancy. *Cell Host Microbe* **2011**, *10*, 324–335. [CrossRef]
59. Yang, J.; Yu, J. The association of diet, gut microbiota and colorectal cancer: What we eat may imply what we get. *Protein Cell* **2018**, *9*, 474–487. [CrossRef]
60. McGill, C.R.; Fulgoni, V.L., III; Devareddy, L. Ten-year trends in fiber and whole grain intakes and food sources for the United States population: National Health and Nutrition Examination Survey 2001–2010. *Nutrients* **2015**, *7*, 1119–1130. [CrossRef]
61. Spyrou, N.; Vallianou, N.; Kadillari, J.; Dalamaga, M. The interplay of obesity, gut microbiome and diet in the immune check point inhibitors therapy era. *Semin. Cancer Biol.* **2021**, *73*, 356–376. [CrossRef] [PubMed]
62. Gupta, S.; Kanwar, S.S. The influence of dysbiosis on kidney stones that risk up renal cell carcinoma (RCC). *Semin. Cancer Biol.* **2021**, *70*, 134–138. [CrossRef] [PubMed]
63. Crocetto, F.; Boccellino, M.; Barone, B.; Di Zazzo, E.; Sciarra, A.; Galasso, G.; Settembre, G.; Quagliuolo, L.; Imbimbo, C.; Boffo, S.; et al. The Crosstalk between Prostate Cancer and Microbiota Inflammation: Nutraceutical Products Are Useful to Balance This Interplay? *Nutrients* **2020**, *12*, 2648. [CrossRef]
64. Gophna, U.; Konikoff, T.; Nielsen, H.B. Oscillospira and related bacteria—From metagenomic species to metabolic features. *Environ. Microbiol.* **2017**, *19*, 835–841. [CrossRef] [PubMed]
65. Lv, Y.; Qin, X.; Jia, H.; Chen, S.; Sun, W.; Wang, X. The association between gut microbiota composition and BMI in Chinese male college students, as analysed by next-generation sequencing. *Br. J. Nutr.* **2019**, *122*, 986–995. [CrossRef] [PubMed]
66. Fan, J.; Zhou, Y.; Meng, R.; Tang, J.; Zhu, J.; Aldrich, M.C.; Cox, N.J.; Zhu, Y.; Li, Y.; Zhou, D. Cross-talks between gut microbiota and tobacco smoking: A two-sample Mendelian randomization study. *BMC Med.* **2023**, *21*, 163. [CrossRef]
67. Jochems, S.H.; Fritz, J.; Häggström, C.; Järvholm, B.; Stattin, P.; Stocks, T. Smoking and Risk of Prostate Cancer and Prostate Cancer Death: A Pooled Study. *Eur. Urol.* **2023**, *83*, 422–431. [CrossRef]
68. Kong, C.; Zhang, S.; Lei, Q.; Wu, S. State-of-the-Art Advances of Nanomedicine for Diagnosis and Treatment of Bladder Cancer. *Biosensors* **2022**, *12*, 796. [CrossRef]
69. Li, X.; Li, C.; Chen, M. Patients With "Gray Zone" PSA Levels: Application of Prostate MRI and MRS in the Diagnosis of Prostate Cancer. *J. Magn. Reson. Imaging JMRI* **2023**, *57*, 992–1010. [CrossRef]
70. Welch, H.G.; Schwartz, L.M.; Woloshin, S. Prostate-specific antigen levels in the United States: Implications of various definitions for abnormal. *J. Natl. Cancer Inst.* **2005**, *97*, 1132–1137. [CrossRef]
71. Litwin, M.S.; Tan, H.-J. The Diagnosis and Treatment of Prostate Cancer: A Review. *JAMA* **2017**, *317*, 2532–2542. [CrossRef] [PubMed]
72. Ilic, D.; Djulbegovic, M.; Jung, J.H.; Hwang, E.C.; Zhou, Q.; Cleves, A.; Agoritsas, T.; Dahm, P. Prostate cancer screening with prostate-specific antigen (PSA) test: A systematic review and meta-analysis. *BMJ (Clin. Res. Ed.)* **2018**, *362*, k3519. [CrossRef] [PubMed]
73. Osca-Verdegal, R.; Beltrán-García, J.; Górriz, J.L.; Jabaloyas, J.M.M.; Pallardó, F.V.; García-Giménez, J.L. Use of Circular RNAs in Diagnosis, Prognosis and Therapeutics of Renal Cell Carcinoma. *Front. Cell Dev. Biol.* **2022**, *10*, 879814. [CrossRef]
74. Gibbons, R.P.; Montie, J.E.; Correa, R.J.; Mason, J.T. Manifestations of renal cell carcinoma. *Urology* **1976**, *8*, 201–206. [CrossRef]

75. Brufau, B.P.; Cerqueda, C.S.; Villalba, L.B.; Izquierdo, R.S.; González, B.M.; Molina, C.N. Metastatic renal cell carcinoma: Radiologic findings and assessment of response to targeted antiangiogenic therapy by using multidetector CT. *Radiographics* **2013**, *33*, 1691–1716. [CrossRef]

76. Matsushita, M.; Fujita, K.; Motooka, D.; Hatano, K.; Fukae, S.; Kawamura, N.; Tomiyama, E.; Hayashi, Y.; Banno, E.; Takao, T.; et al. The gut microbiota associated with high-Gleason prostate cancer. *Cancer Sci.* **2021**, *112*, 3125–3135. [CrossRef] [PubMed]

77. Markowski, M.C.; Boorjian, S.A.; Burton, J.P.; Hahn, N.M.; Ingersoll, M.A.; Vareki, S.M.; Pal, S.K.; Sfanos, K.S. The Microbiome and Genitourinary Cancer: A Collaborative Review. *Eur. Urol.* **2019**, *75*, 637–646. [CrossRef]

78. Routy, B.; le Chatelier, E.; DeRosa, L.; Duong, C.P.M.; Alou, M.T.; Daillère, R.; Fluckiger, A.; Messaoudene, M.; Rauber, C.; Roberti, M.P.; et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **2018**, *359*, 91–97. [CrossRef]

79. Gopalakrishnan, V.; Spencer, C.N.; Nezi, L.; Reuben, A.; Andrews, M.C.; Karpinets, T.V.; Prieto, P.A.; Vicente, D.; Hoffman, K.; Wei, S.C.; et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* **2018**, *359*, 97–103. [CrossRef]

80. Sfanos, K.S.; Markowski, M.C.; Peiffer, L.B.; Ernst, S.E.; White, J.R.; Pienta, K.J.; Antonarakis, E.S.; Ross, A.E. Compositional differences in gastrointestinal microbiota in prostate cancer patients treated with androgen axis-targeted therapies. *Prostate Cancer Prostatic Dis.* **2018**, *21*, 539–548. [CrossRef]

81. Lindgren, S.E.; Swaisgood, H.E.; Janolino, V.G.; Axelsson, L.T.; Richter, C.S.; Mackenzie, J.M.; Dobrogosz, W.J. Binding of Lactobacillus reuteri to fibronectin immobilized on glass beads. *Zent. Fur Bakteriol./Int. J. Med. Microbiol.* **1992**, *277*, 519–528. [CrossRef] [PubMed]

82. Pettenati, C.; Ingersoll, M.A. Mechanisms of BCG immunotherapy and its outlook for bladder cancer. *Nat. Rev. Urol.* **2018**, *15*, 615–625. [CrossRef] [PubMed]

83. Ference, B.A.; Holmes, M.V.; Smith, G.D. Using Mendelian Randomization to Improve the Design of Randomized Trials. *Cold Spring Harb. Perspect. Med.* **2021**, *11*, a040980. [CrossRef] [PubMed]