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Analysis

Circulating metabolic biomarkers mediated causal relationship between gut microbiota and bladder cancer: a two-step mendelian randomization study

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Abstract

Background Dysbiosis of the gut microbiota (GM) has been reported to be associated with cancers, including bladder cancer (BLCA). However, the specific causal relationship between GM and BLCA, as well as the mediating role of circulating metabolic biomarkers (CMBs), has remained unclear. Therefore, we aimed to elucidate the causal relationship among GM, CMBs, and BLCA, through a mendelian randomization (MR) approach.

Method The summary statistics of 473 GM (n = 5959) and 233 CMBs (n = 136,016) from the NHGRI-EBI GWAS Catalog, and BLCA (cases n = 2053 and controls n = 287,137) from the FinnGen study were leveraged for our research. Bidirectional MR analysis was conducted to investigate the causal link between GM and BLCA, and two-step MR (TSMR) was employed to identified potential mediating CMBs. The inverse-variance weighted (IVW) was primarily utilized for effect estimation. Additionally, the Cochrane's Q test was used to evaluate heterogeneity, and the MR-Egger method was employed to evaluate pleiotropy.

Result The study revealed that 15 GM and 12 CMBs were causally associated with BLCA ($p < 0.05$). Specially, dorea was found to significantly increase the risk of developing BLCA (OR = 2.20, 95% CI: 1.29–3.75). Furthermore, TSMR analysis indicated that total cholesterol levels in small HDL and cholesterol esters in small HDL mediate the causal relationship between dorea and BLCA, with mediated proportions of 2.46% and 2.14%, respectively.

Conclusion The findings of this study provide compelling evidence supporting the mediating role of CMBs in the causal relationship on GM and BLCA.

Keywords Gut microbiota · Circulating metabolic biomarkers · Bladder cancer · Mendelian randomization · TSMR

Abbreviations

BLCA	Bladder cancer
CMBs	Circulating metabolic biomarkers
CI	Confidence interval

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GM Gut microbiota
IVW Inverse-variance weighted
IVs Instrumental variables
MR Mendelian randomization
OR Odds ratio
SNPs Single nucleotide polymorphisms
TSMR Two-step MR

1 Introduction

Bladder cancer (BLCA) is one of the most prevalent genitourinary cancers. According to the latest GLOBOCAN estimates, the incidence of BLCA ranks the ninth most common malignant neoplasm, with new cases continuing to rise [1]. The Global Burden of Disease analysis revealed that the age-standardized prevalence rate of BLCA has demonstrated a significant increasing trend, imposing a substantial detrimental impact on patients'quality of life [2]. Currently, recurrence and progression remain significant challenges in the treatment of BLCA [3, 4], necessitating the early identification of risk factors to aid in prevention.

Previous studies have suggested that gut microbiota (GM) may play a pathogenic role in the recurrence and progression of BLCA [5, 6]. Studies in mouse models have indicated that the depletion of GM affects the toxicokinetics of nitrosamines, thereby significantly reducing the development and severity of BLCA [7]. Given the advancements in detection technologies, an increasing number of GM have been identified, warranting further investigation into their causal relationship with BLCA. Genome-wide association studies (GWAS) conducted in large cohorts have identified 473 GM associated with at least one genetic variant [8]. However, no study focuses on the causal relationship between above GM and BLCA.

Recently, the application of biomarkers, such as circulating tumor cells, in the diagnosis and treatment of BLCA has been extensively reported [9]. Thanks to large GWAS combined with metabolic profiling platforms, loci associated with circulating metabolic traits have been successfully identified [10]. Recently, Karjalainen et.al conducted a GWAS on 233 circulating metabolic biomarkers (CMBs) from 136,016 participants across 33 cohorts, providing insights into metabolic pathways and disease etiology [11]. However, the mediating role of CMBs in the causal relationship between GM and BLCA remains unknown.

In previous studies, the association between GM and BLCA has attracted considerable attention. Unfortunately, most conclusions are primarily based on observational studies of GM abundance in stool or in urine [12, 13]. Observational studies can be influenced by environmental factors and reverse causality [14]. In contrast to observational studies, mendelian randomization (MR) study utilizes genetic variations to assess the causal link between exposure and outcome, thereby mitigating the impact of certain environmental factors and reverse causality [15]. The mediation MR provides an opportunity to identify mediating factors of the causal relationship [16]. Therefore, we aim to utilize MR to infer the causal relationship among GM and BLCA, especially the mediating role of CMBs, thereby offering new insights into the causal relationships among them.

2 Method

2.1 MR study design

Currently, to minimize the influence of confounding factors, mainstream MR analysis must satisfy the following three assumptions [17]: (1) The instrumental variables (IVs) are strongly related to the exposure; (2) The IVs are independent of confounding factors; (3) The IVs are related to the outcome solely through the exposure. Specially, two-step MR (TSMR) analysis emphasizes the moderating role of mediators between exposure and outcome [18]. This study examined the GM as the exposure, CMBs as the mediator, and BLCA as the outcome (Fig. 1). Moreover, TSMR approach was completed in this study primarily consists of three components: (1) the effect estimates of GM on BLCA (β); (2) the effect estimates of GM on CMBs (β1); (3) the effect estimates of CMBs on BLCA (β2) (Fig. 1).

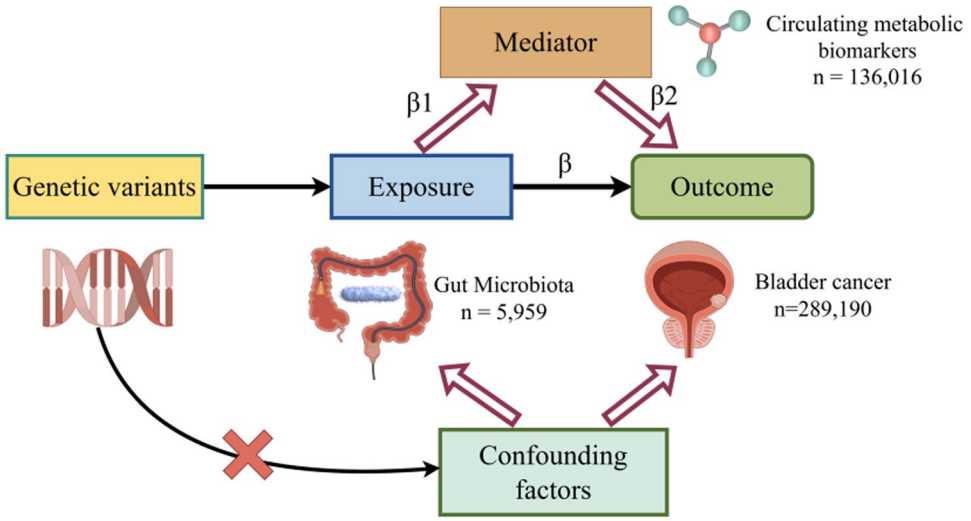

Fig. 1 Design of our study. In our MR hypothesis, genetic variants as the instrumental variables are strongly associated with the exposure (GM) and related to the outcome (BLCA) solely through GM, without confounding factors. For two-step MR study, the causal relationship between GM and the mediator (CMBs) was constructed. Finally, the mediating role of CMBs in the connection of GM and BLCA was demonstrated. β: the causal effect estimates of GM on BLCA; β1: the causal effect estimates of GM on CMBs; β2: the causal effect of CMBs on BLCA. MR, mendelian randomization; GM, gut microbiota; BLCA, bladder cancer; CMBs, circulating metabolic biomarkers. The figure was drawn by Figdraw

2.2 Data source

In their study, Qin et al. conducted a GWAS on 2801 microbial taxa from a cohort of 5959 individuals enrolled in the FR02 study, generating data for 473 GM [8]. The FR02 study focused on common and relatively abundant microbial taxa, defined as those with prevalence in over 25% of the study cohort. Using a genome-wide significance threshold ($p < 5 \times 10^{-8}$), they identified 473 GM taxa. Furthermore, adjustments for antibiotic prescriptions did not alter any genome-wide significant associations. The relevant information can be accessed in the NHGRI-EBI GWAS Catalog under the accession numbers GCST90032172 to GCST90032644. Similarly, Karjalainen et al. performed a GWAS on 233 CMBs using data from 136,016 participants across 33 cohorts [11]. The data for this study can be found in the NHGRI-EBI GWAS Catalog with accession numbers GCST90301941 to GCST90302173. These data represent an unprecedented sample size, enhancing the credibility of the MR study findings. Additionally, the summarized statistics for BLCA (cases n = 2053 and controls n = 287,137) were collected by screening the FinnGen R9 database. Although the statistics excluded all types of BLCA, their exclusion did not adversely impact the study due to the larger sample size.

2.3 Extraction of IVs

For GWAS, single nucleotide polymorphisms (SNPs) are considered as the IVs. SNPs strongly associated with the exposure are crucial for ensuring the reliability of MR. Initially, SNPs linked to GM or CMBs were selected at a significance level of $p < 1 \times 10^{-5}$ [19]. Furthermore, to account for linkage disequilibrium, the chosen SNPs must satisfy the criteria of $r^2 < 0.001$ and a distance > 10,000 kb [20]. Subsequently, to mitigate potential weak instrument bias, instruments with F-statistic parameters < 10 will be excluded [21]. The specific calculation formula was derived from previous literature [22].

2.4 MR analysis

2.4.1 MR for GM or CMBs on BLCA

To evaluate the causal impact of GM or CMBs on BLCA, a MR analysis was carried out. The inverse variance weighted (IVW) method was the primary analytical approach [23]. The IVW estimate is obtained through a meta-analysis of Wald ratios for all genetic variants, assuming no horizontal pleiotropy among SNPs. Under this assumption, IVW offers the most robust and precise evaluation of causal effects. Pleiotropy analyses confirmed the stability of IVW results, with no significant horizontal pleiotropy detected. The outcomes were presented through odds ratio (OR) with 95% confidence interval (CI). Results were considered statistically significant if the p value < 0.05.

2.4.2 Mediation analysis

To assess the mediating effect of CMBs, the GM with the highest OR was regarded as the exposure, and CMBs identified as causally related to BLCA were considered as the mediators. In the TSMR analysis, the mediating effect was calculated using basic effect estimates from the MR analysis. The proportion of mediation can be calculated as the"indirect effect/total effect"([β1 × β2]/β)[24].

2.4.3 Heterogeneity and pleiotropy

Eliminating heterogeneity and pleiotropy are crucial steps to enhance the credibility of the analysis results. The Cochran Q test was used to evaluate the heterogeneity of each SNP [25]. Additionally, MR-Egger regression was employed to assess pleiotropy [26]. The final outcomes demonstrated no heterogeneity or pleiotropy.

2.4.4 Bidirectional MR analysis

To ensure the validity of the MR analysis, it is crucial to exclude reverse causation. Therefore, we designated BLCA as the exposure, and the associated GM or CMBs related to BLCA as the outcome. To prevent bidirectional causal relationship, we implemented a stringent threshold of $p < 5 \times 10^{-6}$ to identify relevant SNPs for BLCA, thereby preparing for the bidirectional MR analysis.

2.4.5 Statistical analysis

All analyses were conducted using the R software (v4.3.3). The R packages TwoSampleMR and forestploter were employed for the MR analysis and the generation of forest plot, respectively [27].

3 Result

3.1 Causal effect of GM and BLCA

The results revealed the causal relationship between 15 GM and BLCA. Specifically, an increased in dorea abundance in stool significantly elevate the risk of BLCA (OR = 2.20, 95% CI: 1.29–3.75) (Fig. 2 and Table 1). Through bidirectional MR analysis, BLCA was identified to lead to change in 42 GM (Fig S1). The supplementary figure displayed only the top ten items.

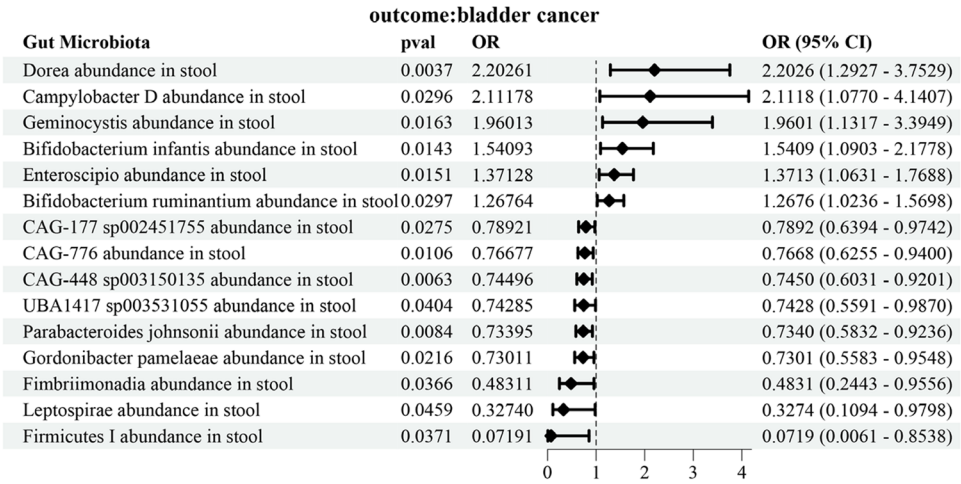

Fig. 2 Forest plot to identified the GM related to BLCA. By MR analysis, 15 GM were revealed to be related to BLCA ($p < 0.05$). Specially, dorea abundance in stool was found to significantly elevate the risk of BLCA with a larger OR (2.20, 95% CI: 1.29–3.75). MR, mendelian randomization; GM, gut microbiota; BLCA, bladder cancer; OR, odds ratio

3.2 Causal effect of CMBs and BLCA

Furthermore, causal relationship between 12 CMBs and BLCA was observed. Notably, it mainly included the lactate levels and metabolites associated with high-density lipoprotein (HDL) (Fig. 3). In the bidirectional MR analysis, BLCA was showed to influence 22 CMBs (Fig S2).

3.3 Mediating role of CMBs in the Dorea-BLCA axis

In the mediation analysis, we aimed to pinpoint CMBs with mediating effects on dorea and BLCA. Leveraging TSMR, we identified the mediated role of total cholesterol levels in small HDL and cholesterol esters in small HDL on the causal impact of dorea on BLCA (Table 1), with mediation proportions of 2.46% and 2.14% respectively (Table 2).

4 Discussion

Current reports on the relationship between GM and BLCA primarily rely on observational studies. For instance, He et al. observed a decrease in the abundances of Clostridium cluster XI and Prevotella in BLCA patients [28]. However, observational studied cannot definitively establish a causal relationship between GM and BLCA. Nevertheless, MR analysis can solve this problem, to a certain extent. Utilizing large population cohorts of GWAS, data concerning 473 GM and 233 CMBs have been reported, but the causal impact of above GM and CMBs on BLCA remains ambiguous. For the first time, we explored the causal influence of 473 GM on BLCA through MR analysis, and demonstrated the mediating CMBs. Our study uncovered dorea-total cholesterol levels in small HDL-bladder axis and dorea-cholesterol esters in small HDL-bladder axis, providing new insights into exploring the mechanism linking GM and BLCA.

Recent studies have established a connection between GM and the occurrence of BLCA. Specifically, alterations in GM may lead to systemic metabolic changes that contribute to immune dysregulation, which could have tumorigenic effects [29]. This dysregulation may partially explain the heightened mortality rates associated with BLCA in the elderly population. Moreover, GM also play a role in the treatment outcomes of BLCA. For instance, Wu et al. demonstrated that Parabacteroides distasonis enhances the efficacy of immunotherapy for BLCA by activating anti-tumor immune responses [30]. In addition, Miyake's research reported that a probiotic mixture of Lactobacillus and Bifidobacterium enhances anti-tumor immune responses in urothelial carcinoma through a gut-tumor immune response axis [31]. Our study identified a causal association between 15 GM and BLCA through mendelian randomization analysis, particularly highlighting dorea. This is the first time we proposed that dorea may increase the risk of BLCA. As a putative pro-inflammatory microorganism, dorea demonstrates significantly elevated abundance in subjects with bladder Clonorchis sinensis infection compared to healthy controls [32]. However, current evidence regarding dorea-cancer associations remains sparse, particularly in bladder carcinogenesis. To date, its oncogenic mechanisms have been only partially elucidated in gastrointestinal

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Table 1 The Results of important MR outcomes

Outcome	Exposure	Odds ratio (95% CI)	*P*	Q-statistics	P_h	Egger intercept	P_p
Total cholesterol levels in small HDL	Dorea abundance in stool	1.08 (1.01, 1.15)	0.024	5.482	0.856	0.0067	0.126
Cholesterol esters in small HDL	Dorea abundance in stool	1.07 (1.00, 1.14)	0.042	4.122	0.941	0.0054	0.209
Bladder cancer	Dorea abundance in stool	2.20 (1.29, 3.75)	0.003	8.067	0.622	–0.0338	0.300
Bladder cancer	Total cholesterol levels in small HDL	1.27 (1.03, 1.58)	0.024	89.96	0.511	–0.006	0.401
Bladder cancer	Cholesterol esters in small HDL	1.26 (1.04, 1.53)	0.015	85.12	0.871	–0.006	0.333

CMBs, circulating metabolic biomarkers; HDL, high-density lipoprotein. The precise values cannot be presented due to formatting constraints

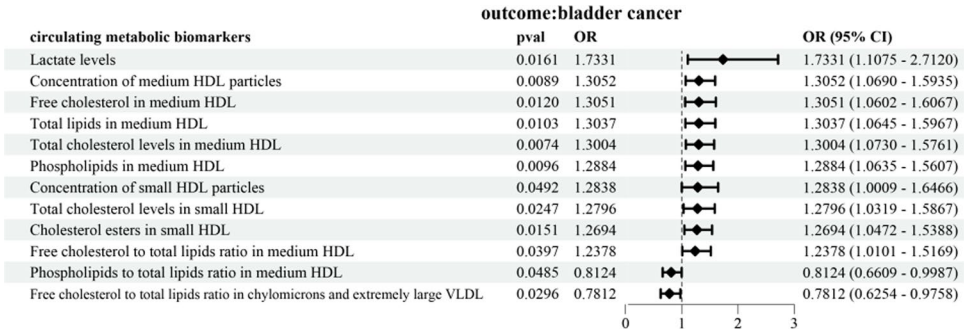

Fig. 3 Forest plot to infer the causal relationship among CMBs and BLCA. 12 CMBs were identified to be associated with BLCA ($p < 0.05$). Among them, the lactate levels and metabolites associated with HDL were major kind of CMBs. CMBs, circulating metabolic biomarkers; BLCA, bladder cancer; HDL, high-density lipoprotein

Table 2 Mediated proportions of CMBs in causal relationship of dorea and BLCA

Exposure	Mediator	Outcome	Direct effect	Indirect effect (β1 × β2)	Total effect (β)	Mediated proportion (%)
Dorea abundance in stool	Total cholesterol levels in small HDL	Bladder cancer	0.770	0.0193	0.789	2.46
Dorea abundance in stool	Cholesterol esters in small HDL	Bladder cancer	0.773	0.0168	0.789	2.14

β: the causal effect estimates of GM on BLCA; β1: the causal effect estimates of GM on CMBs; β2: the causal effect of CMBs on BLCA. CMBs, circulating metabolic biomarkers; HDL, high-density lipoprotein; BLCA, bladder cancer. The precise values cannot be presented due to formatting constraints

malignancies. Prior research has indicated that dorea as a significant bacterium linked to colorectal cancer and noted its pro-inflammatory properties [33]. Given the distinct immunological landscape of BLCA, the pathobiological role of dorea in urothelial malignancy warrants systematic investigation.

Previous studies have revealed that GM contributes to the onset and progression of diseases through metabolites. Research has indicated that tryptophan metabolites derived from GM regulate various components of the tumor microenvironment [34]. Several compounds produced from tryptophan metabolism are critical in inflammation and immune responses [35]. Additionally, studies have indicated significantly reduced plasma tryptophan levels in BLCA [36]. Consequently, the role of metabolites in the causal relationship between GM and BLCA merits further investigation. Notably, metabolites related to HDL have been implicated in cancer risk in numerous studies [37]. Dysfunctional HDL may contribute to the progression of BLCA by exacerbating cellular oxidative stress and potentially disrupting cholesterol homeostasis [38]. Similarly, smoking is a well-established risk factor for BLCA. MR analysis has showed that smoking indirectly impacts BLCA through specific blood lipids [39]. Hence, we hypothesize that particular GM influence the occurrence of BLCA via specific CMBs, necessitating further in vivo and in vitro validation.

Accumulating evidence has demonstrated the critical involvement of lipid metabolism in BLCA pathogenesis, tumor recurrence, and malignant progression [40]. Notably, Cheng et al. revealed that inhibition of adipocyte-mediated lipid trafficking substantially impairs tumor growth through (PPAR) γ-dependent mechanisms, highlighting the metabolic vulnerability of BLCA cells [41]. Furthermore, metabolic reprogramming of lipid biosynthesis has been identified as a molecular determinant of therapeutic resistance, particularly in cisplatin-refractory BLCA models [42]. In this study, the mediated effect of CMBs appears relatively modest. This phenomenon may stem from the multifaceted roles of GM beyond CMBs in BLCA pathogenesis. Specifically, GM may modulate immune responses, tissue repair, and prognostic outcomes in BLCA, with CMBs likely representing only a partial component of these underlying mechanisms [43]. Undiscovered CMBs might still play pivotal roles in the initiation and progression of BLCA, warranting further exploration.

We firstly examine the mediating role of CMBs in the causal relationship between CM and BLCA. Nonetheless, there are still some limitations in our research. Firstly, most of the included data originates from European populations, potentially limiting the generalizability of our findings to broader populations. This limitation primarily stems from the scarcity of GWAS data across diverse demographic groups. Secondly, external validation of our study was not conducted. Differences in the frequencies and effect sizes of genetic variants across populations may limit the generalizability of our findings

to broader populations. We anticipate that increased sharing of GWAS data from diverse ethnic groups will enable more precise translation of research outcomes to benefit global health initiatives. Thirdly, the GM-CMBs-bladder axis discovered in this study is solely based on MR analysis, so in vivo and in vitro experiments for validation to enhance the credibility of our findings is very essential.

5 Conclusion

Our study indicated that GM is associated with risk of BLCA. Furthermore, it is noteworthy that dorea may influence the occurrence of BLCA through total cholesterol levels in small HDL and cholesterol esters in small HDL.

Authors' contributions Fa-Ye Wei performed most of the data analysis and visualization. Qi-Huan He and Bin-Tong Yin contributed to screen and collect datasets from public databases. Jia-Wen Zhao and Yu-Tong Zhao wrote and checked the manuscript. Zheng-Shu Wei, Yu-Jian Li, and Hai-Qi Liang reviewed the data and revised the manuscript. Ji-Wen Cheng and Min Qin conceived and finished the study design. All authors read and approved the manuscript.

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Data availability All R scripts in our study are available from the corresponding author upon reasonable request. Publicly available datasets were analyzed in this study, from NHGRI-EBI GWAS Catalog and FinnGen R9 database.

Declarations

Competing interests We declare that there are no conflicts of interests.

Informed consent Ethical approval does not apply to this article.

Statement for institutional email Because some authors are not formal hospital employees, so we cannot provide institutional email addresses for all authors.

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