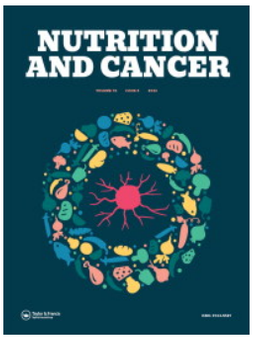

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An Atlas of Dietary Intakes and Medication Uses on Risk of Bladder Cancer: A Wide-Angle Mendelian Randomization Analysis

Ya-Ting Chen[a,b], Qiu-Yi Tang[c], Yan-Xi Zhang[a,b], Shi-Zhi Wang[b], Anke Wesselius[d,e], Wen-Chao Li[f], Maurice P. Zeegers[d,e] and Evan Yi-Wen Yu[a,b,d]

[a]Department of Epidemiology & Biostatistics, School of Public Health, Southeast University, Nanjing, China; [b]Key Laboratory of Environmental Medicine and Engineering of Ministry of Education, School of Public Health, Southeast University, Nanjing, China; [c]Medical School of Southeast University, Nanjing, China; [d]Department of Epidemiology, CAPHRI Care and Public Health Research Institute, Maastricht University, Maastricht, The Netherlands; [e]School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands; [f]Department of Urology, Affiliated Zhongda Hospital of Southeast University, Nanjing, China

ABSTRACT

Background: Observational studies suggests that diets and medications affect bladder cancer (BC) development, which are subject to confounding and difficult to make causal inference. Here we aimed to investigate whether those observational associations are causal and determining the potential directions and pathways.

Methods: We used 2-sample Mendelian randomization (MR) analysis to assess associations of dietary intakes, medication uses and molecules with BC risk. Genetic summary data were derived from participants of predominantly European ancestry with rigorous instruments selection, where univariable MR, mediation MR and multivariable MR were performed.

Results: The results of univariable MR showed 4 dietary intakes and 4 medication uses having a protective effect on BC, while 4 circulating metabolites, 440 circulating proteins and 2 gut microbes were observed to be causally associated with BC risk. Through mediation MR, we found 572 analytes showing consistent mediating effects between dietary intakes or medication uses and BC risk. Furthermore, 9 out of 16 diet-medication pairs showed significant interactions and alterations on BC when consumed jointly.

Conclusion: In summary, the findings obtained from the current study have important implications for informing prevention strategies that point to potential lifestyle interventions or medication prescriptions to reduce the risk of developing BC.

ARTICLE HISTORY

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HIGHLIGHTS

- The current study extends observational literature in showing the importance of diets and medications on bladder cancer prevention.
- The associations of diets and medications on bladder cancer prevention might be through circulating metabolites, circulating proteins and gut microbiota
- Our results provide a new understanding of interactions in certain diet-medication pairs which should be taken into account by both physicians and patients during the development of a treatment strategy.

Abbreviations: MR: Mendelian randomization; GWASs: genome-wide association studies; FFQs: food frequency questionnaires; SNVs: single nucleotide variants; OR: odd ratio; IVW: inverse-variance weighted; CI: confidence interval; *P*: *P* value; FDR: false discovery rate; MR-PRESSO: MR pleiotropy residual sum and outlier; SNP: single nucleotide polymorphism; IVs: instrumental variables; TwinsUK: The UK Adult Twin Registry; KORA: Cooperative Health Research in the Region of Augsburg; AGES-Reykjavik: The Age, Gene/Environment Susceptibility-Reykjavik Study; FINRISK: The North Karelia Project; LMM: linear mixed model; ICD-O-3: International Classification of Diseases for Oncology, 3rd Edition; LD: Linkage disequilibrium; MAF: minor allele frequency

CONTACT Evan Yi-Wen Yu evan.yu@maastrichtuniversity.nl Department of Epidemiology & Biostatistics, School of Public Health, Southeast University, Nanjing, China.

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Introduction

Bladder cancer (BC) is the most common malignancy of the urinary tract, which accounts for 0.6 million new diagnoses and 0.2 million deaths worldwide yearly (1). Despite advancements in treatment, the prognosis for BC remains poor in certain cases, with the recurrence rate ranging from 33.4% to 47.3%, regardless of the presence of comorbidities or infections (2, 3). Due to its high recurrence rate, BC has been reported to be among the most expensive lifetime treatment of all cancers, resulting in a heavy burden on the healthcare system and poor quality of life among BC patients (4). As suggested for many solid tumors, the development of BC is likely to be affected by lifestyle and environmental factors (5), particularly the role of dietary intakes and medication uses could be pronounced since the bladder is an excretory organ that is constantly exposed to both harmful and favorable components excreted through the urinary tract and come in direct contact with the bladder lining (6).

With the development of advanced techniques in high-throughput profiling, some complementary strategies to evaluate the effect of dietary intakes and medication uses on disease risk and assess their clinical relevance became popular and have already been widely applied (7). Previous literature showed that the use of intermediate molecules, i.e., circulating metabolites (8), circulating proteins (9), and gut microbiota (10) are successful to reveal the molecular responses and interplay between dietary intakes/medication uses and disease outcomes. However, so far, no study has systematically assessed the causal effects of dietary intakes or medication uses through the potential mediators (i.e., circulating metabolites, circulating proteins and gut microbiota) on BC risk.

In recent years, Mendelian randomization (MR) analysis has become an increasingly powerful tool for causal inference, which uses single nucleotide variants (SNVs) as unconfounded proxies for exposures to estimate their effects on outcomes of interest, thereby minimizing the bias affecting observational epidemiological studies. In addition, as an extension to simple MR analysis, mediation MR analysis (11) and multivariable MR analysis (12) emerged to decompose the effects of a single or multiple exposure(s), which act direct or via a mediating variable, on an outcome.

In the present study, leveraging GWAS summary statistics from various resources containing dietary intakes, medication uses, molecules and BC data, we examined the potential causal relationships between dietary intakes/medication uses and BC risk, then assessed the mediating roles of downstream molecules involved into the mechanistic pathways. We further used multivariable MR method (12) to assess the interaction of dietary intakes and medication uses owing to the fact that both of them may affect each other and BC risk.

Materials and Methods

Data Disclosure Statement and Study Design

This study is reported as per the STROBE-MR guideline (Supplementary STROBE-MR Checklist). All studies have existing ethical permissions from their respective institutional review boards, with participant written informed consent and rigorous quality control. Because all analyses herein are based on publicly available summary data, no ethical approval from an institutional review board was required for the current study. Although there is no formal or documented protocol for this study, the main analyses for the current study were prespecified.

The study design of the present work is shown in Figure 1; first, we reviewed and obtained publicly available summary statistics from six GWAS sources of predominantly European ancestry (Table S1). Second, we selected instrumental variables (IVs) for each exposure (i.e., dietary intakes or mediation uses), mediator (i.e., circulating metabolites, circulating proteins, and gut microbiota) according to the criteria explained in details below. Third, we performed a univariable MR analysis to estimate population-specific causal effects of each exposure (i.e., dietary intakes or mediation uses), mediator (i.e., circulating metabolites, circulating proteins, and gut microbiota) on BC risk using four established methods (refer to **Methods** further below). Fourthly, we investigated the mediation effects of each identified circulating metabolite, circulating protein or gut microbe on the effect of the identified dietary intakes/medication uses factors and BC risk. Finally, we assessed the combined effects of diet-medication pairs on BC risk using multivariable MR.

Genetic Instrumental Variables Selection

The details of data resources are presented in Supplementary Methods. Despite different genome-wide significance levels were set up across GWASs, genetic instruments for each exposure were selected at the threshold of $P < 5 \times 10^{-8}$ from corresponding GWASs, which is the most commonly accepted threshold based on performing a Bonferroni correction for all

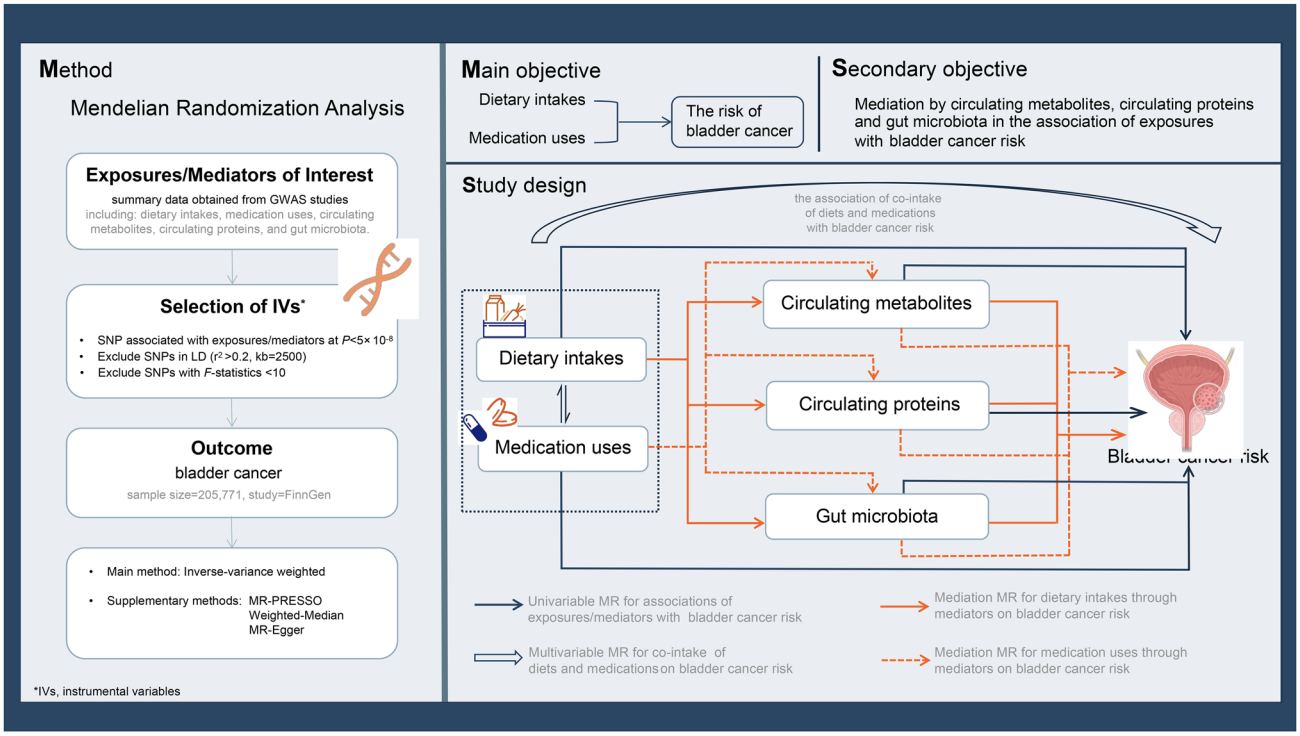

Figure 1. Overview of the study design.
First, we reviewed and obtained publicly available summary statistics from six GWAS sources of predominantly European ancestry. Second, we selected instrumental variables (IVs) for each exposure (i.e., dietary intakes or medication uses) and mediator (i.e., circulating metabolites, circulating proteins, and gut microbiota). Third, we performed a univariable MR analysis to estimate population-specific causal effects of each exposure (i.e., dietary intakes or medication uses) and mediator (i.e., circulating metabolites, circulating proteins, and gut microbiota) on bladder cancer risk using four established methods. Fourthly, we investigated the mediation effects of each identified circulating metabolite, circulating protein or gut microbe involved in the relation between identified dietary intakes/medication uses and bladder cancer risk. Finally, we assessed the combined effects of diet-medication pairs on bladder cancer risk by using multivariable MR.
Abbreviations: GWAS: genome-wide association study; SNP: single-nucleotide polymorphism; IV: instrumental variable; LD: linkage disequilibrium; MR: Mendelian randomization; MR-PRESSO: MR pleiotropy residual sum and outlier.

independent common SNPs across the human genome. Linkage disequilibrium (LD) among SNPs for each exposure was calculated based on 1000 genomes LD reference panel (European population) using the PLINK clumping approach (13). Independent SNPs were defined by r^2 <0.2 and clump window >2,500 kb and correlated SNPs (e.g., linkage disequilibrium) with the lowest *P*-value were retained. Furthermore, an in-silico approach through SNPnexus (https://www.snp-nexus.org/v4/) (14) was employed to annotate the selected genetic instrumental variables for indication of their biological functions.

Statistical Power for Genetic Instruments

The variance of each selected SNPs explained to the corresponding exposures or mediators by using "$2\beta^2MAF(1-MAF)$", where β was the effect size of the genetic variation and MAF represented the minor allele frequency (15). In addition, the *F* statistics for each instrument was estimated using the association of corresponding instrument with exposure and its standard error. SNPs which *F* statistics <10 were considered as weak and were therefore excluded. Detailed information on statistical power for genetic instruments of each exposure or mediator are shown in Tables S2–S6.

Statistical Analysis

A two-sample MR approach using summary data and the inverse-variance weighted (IVW) MR approach (single variable weighted linear regression) was used as the main methods (16) to assess the causal effect between two traits. Heterogeneity was measured by Cochrane's Q statistic based on the inverse-variance weighted model (17). To evaluate potential violations of the instrumental variable assumptions, i.e., the absence of horizontal pleiotropy, the MR pleiotropy residual sum and outlier (MR-PRESSO) test (18) was applied.

A two-step MR approach (19) based on the significant associations identified by IVW in univariable MR was used to determine whether the causal relationship between dietary intakes/medication uses and BC risk is mediated by molecules. In the first step of two-step MR, a genetic variant is used as an instrumental variable for the exposure of interest to estimate the causal impact

of the exposure on a hypothesized mediator premised on the significant association between the exposure and outcome of interest (i.e., BC risk). In the second step, an independent genetic variant was used as an instrument for the mediation effect, to establish the causal impact of the mediator on BC risk. The indirect effect of the exposure through the mediator on BC risk were then assessed by using the Sobel test (20).

A multivariable MR approach was used to estimate the effects of multiple exposures (i.e., co-intake of dietary intakes and medication uses) using multiple sets of overlapping SNPs identified by GWAS. For this stage, the extension of the MR method developed by Burgess et al. (12), performing multivariable weighted linear regression (variants uncorrelated, random effect model) with the intercept term set to zero, was applied.

In addition, several sensitivity analyses were used to evaluate the robustness of causal inferences. At first, the potential presence of horizontal pleiotropy was assessed using MR-Egger regression (21) based on its intercept term, where deviation from zero denotes the presence of directional pleiotropy. In addition, the complementary weighted-median method was applied. This method can guarantee MR estimates at least 50% of the included instruments are valid (22).

For detailed supplementary materials, including data and additional information, please refer to https://doi.org/10.6084/m9.figshare.24745152.v1. All the analyses were conducted in R (version 4.1.1, free software, see https://www.r-project.org/foundation/). All estimates were reported with two-tailed *P* values. We calculated the false discovery rate (FDR) to correct for multiple testing at $\alpha < 0.05$ significance level.

Results

Descriptive Statistics and Statistical Power

Characteristics of the 6 included cohorts/consortia are shown in Table S1. The median age of the cohort used for BC as the outcome was 63 years, with 1,701 BC cases out of 205,771 participants. After exclusion

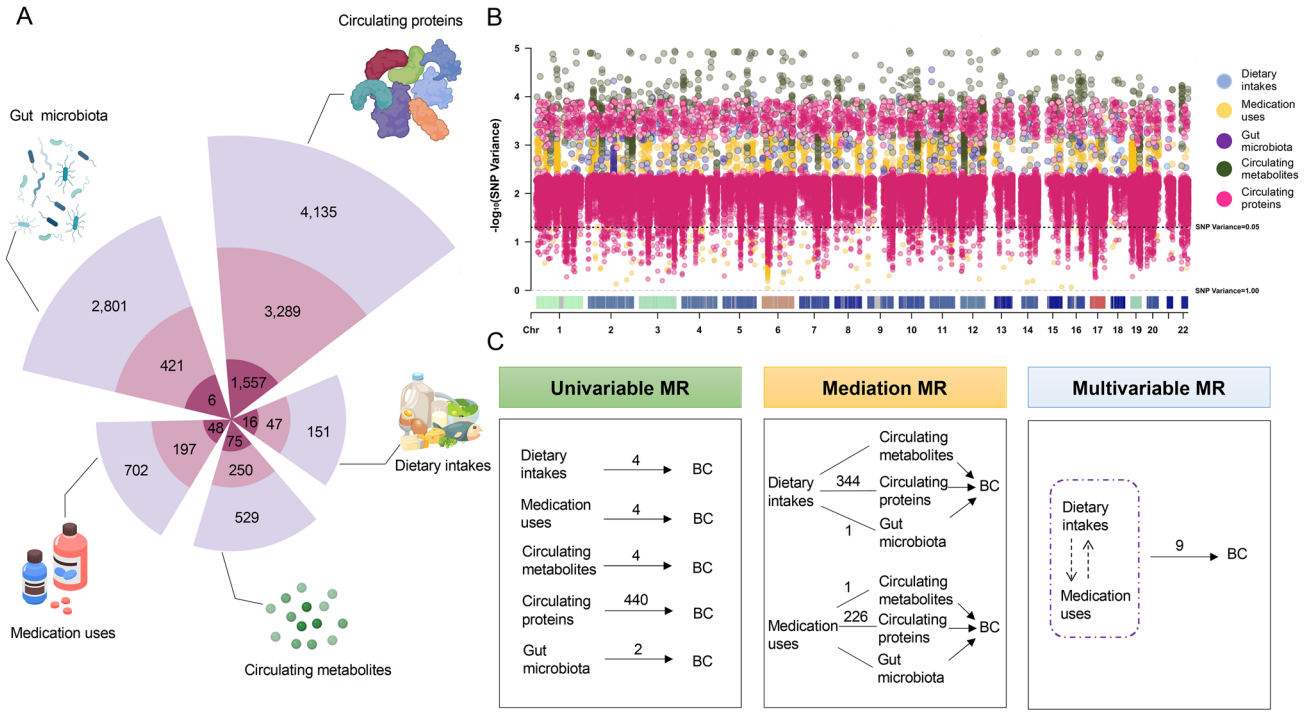

Figure 2. Characteristics of included SNPs.
A, Nightingale Rose Charts showing the instrumental variables. Each segment on this chart represents an exposure or mediator (i.e., dietary intakes, medication uses, circulating metabolites, circulating proteins, and gut microbiota). The lightest purple rings (outer ring) represent the original number of traits in the included GWASs. The medium purple ring (middle ring) represents the number of traits with one or more than one SNPs reporting in GWASs reaching 5×10^{-8} and can be found in the outcome (BC) datasets. The darkest purple ring (inner ring) represents the number of traits with IVs not less than five SNPs among those selected in the last step; B, distribution of explained variance that the selected genetic variants contributed to the corresponding traits. The horizontal coordinate represents the position of the SNP, the vertical coordinate indicates the $-\log_{10}$ of the variance of the SNP. The dashed line represents a variance of 0.05 for SNP. Different colors indicate SNPs from different exposures and mediators: i.e., blue color represents dietary intakes; yellow color represents medication uses; purple color represents gut microbiota; green color represents circulating metabolites; pink color represents circulating proteins; C, the overall results obtained by different MR approaches. The arrows indicate the MR analysis from exposures (or via the mediators) to the outcome (BC). The numbers upon the arrows indicate the number of statistically significant associations suggested by IVW. The dashed boxes represent co-intake and interaction of dietary intakes and medication uses.
Abbreviations: MR: Mendelian randomization; BC: bladder cancer; GWAS: genome-wide association studies; SNP: single nucleotide polymorphism; IV: instrumental variants; IVW: inverse variance weighted.

of exposures or mediators without any genetic instruments with significant genome-wide association ($P < 5 \times 10^{-8}$), a total of 47 dietary intakes, 197 medication uses, 250 circulating metabolites, 3,289 circulating proteins and 421 gut microbes were used for further analyses (Figure 2A). The F statistic of the used genetic instruments ranged from 15.86 to 9,672.34. The obtained variance values explained by the genetic instruments and corresponding exposures, or mediators were between 1.1×10^{-5} and 2.42, of which 94.8% were <0.05 (Figure 2B). Overall, 572 associations and 9 associations were found to have causal evidence through mediation MR and multivariable MR, respectively (Figure 2C).

Univariable MR Estimates for Exposures/ Mediators on Bladder Cancer Risk

By making use of univariable MR analyses, 4 food items, i.e., 1) instant coffee consumption vs. no instant coffee consumption, 2) major dietary changes in the last 5 years because of illness vs. because of other reasons or no major changes, 3) cereal consumption (e.g., cornflakes, Frosties) vs. bran/biscuit/oat/muesli cereal consumption, and 4) non-wheat products consumption vs. wheat products consumption, were found to be associated with a decreased BC risk ($OR_{\text{-IVW}}$ 0.54, 95% CI 0.36–0.83, $P_{\text{-fdr}}$=0.042; $OR_{\text{-IVW}}$ 0.48, 95% CI 0.30–0.77, $P_{\text{-fdr}}$=0.040; $OR_{\text{-IVW}}$ 0.58, 95% CI 0.44–0.77, $P_{\text{-fdr}}$=0.003; $OR_{\text{-IVW}}$ 0.93, 95% CI 0.89–0.97, $P_{\text{-fdr}}$=0.040, respectively) (Figure 3A; Table S7). In addition, 4 medications (i.e., calcipotriol, dovobet ointment, thyroxine product, and cholesterol lowering medication) were observed to have a protective effect on BC risk ($OR_{\text{-IVW}}$ 0.95, 95% CI 0.93–0.98, $P_{\text{-fdr}}$=0.001; $OR_{\text{-IVW}}$ 0.96, 95% CI 0.94–0.97, $P_{\text{-fdr}}=2.15 \times 10^{-5}$; $OR_{\text{-IVW}}$ 0.91, 95% CI 0.86–0.96, $P_{\text{-fdr}}$=0.007; $OR_{\text{-IVW}}$ 0.86, 95% CI 0.78–0.95, $P_{\text{-fdr}}$=0.038, respectively) (Figure 3A; Table S8).

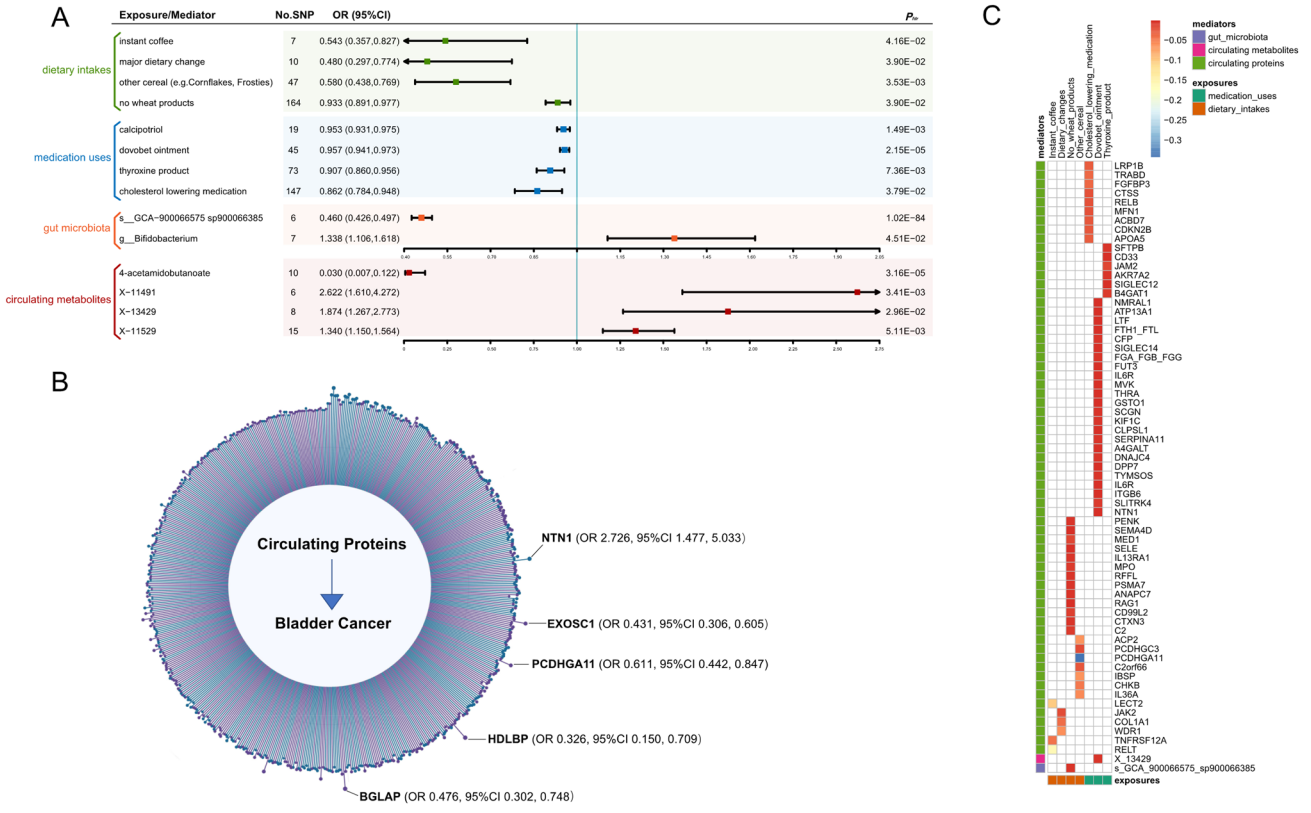

Figure 3. Associations of univariable and mediation MR.
A, forest plot showing univariable MR analyses of dietary intakes, medication uses, circulating metabolites, and gut microbiota on BC risk. The odds ratios (ORs) were shown by solid square and horizontal lines representing the 95% confidence intervals (CIs). Colors are used to distinguish between different exposures: i.e., green color represents dietary intakes; blue color represents medication uses; orange color represents gut microbiota; red color represents circulating metabolites; B, lollipop plot for associations between circulating proteins and bladder cancer risk. The 440 associations suggested to be statistically significant by IVW between circulating proteins and BC are shown on the graph. Each line with a solid circle indicates the beta effect of the association. Blue lines indicate positive beta effect, while purple lines indicate negative beta effect; C, heatmap of identified associations for exposures through identified mediators on bladder cancer risk. Columns represent exposures, rows represent mediators, and the outcome is BC. Exposures were indicated by two colors, i.e., green for medication uses, and orange for dietary intakes. Mediators were indicated by three colors, i.e., purple for gut microbiota, pink for circulating metabolites, and green for circulating proteins.
Abbreviations: No.SNP: number of single-nucleotide polymorphism; OR: odds ratio; CI: confidence Interval; fdr: false discovery rate; IVW: inverse variance weighted; BC, bladder cancer.

Four circulating metabolites were shown to be associated with BC risk based on IVW method. Specifically, 4-acetamidobutanoate, as the only one which chemical identity was determined at the time of the GWAS analysis, was estimated to broadly decrease the risk of BC (OR_{-IVW} 0.03, 95% CI 0.01–0.12, $P_{-fdr}=3.16\times10^{-5}$) (Figure 3A; Table S9). Furthermore, 440 circulating proteins were obtained to have an effect on BC risk based on IVW, with 186 showing a positive association and 254 showing a negative association. Amongst, 23 key proteins showing a P value $<1.0\times10^{-5}$, after multiple correction (Figure 3B; Table S10). Lastly, an unclassified species of gut microbes (i.e., *GCA-900066575 sp900066385*) and a genus of *Bifidobacterium* were identified to be causally associated with BC risk, showing a decreased OR_{-IVW} of 0.46 (95% CI 0.43–0.50, $P_{-fdr}=1.02\times10^{-84}$) and an increased OR_{-IVW} of 1.34 (95% CI 1.11–1.62, $P_{-fdr}=0.045$) per increment of relative abundance, respectively (Figure 3A; Table S11).

Mediation MR Estimates for Exposures on Bladder Cancer Risk via Mediators

By assessing the effects of the dietary intakes and medication uses shown to significantly affecting BC risk, on circulating metabolites, circulating proteins or gut microbiota, we found that 29.34% of mediators were affected by the exposures according to IVW and MR-PRESSO methods (Tables S12–S17). Through a mediation test, 572 analytes showed a consistent mediation effect between the dietary intakes or medication uses and BC risk, where the total effect, direct effect and indirect effect all act in the same effect direction. Amongst, increase of the gut microbe *s_GCA-900066575 sp900066385* abundance was observed to mediate the negative association between non-wheat products consumption vs. wheat products consumption and BC risk, and a lower metabolite X-13429 was observed to mediate the negative association between dovobet ointment and BC risk. Other mediators showed to be circulating proteins ($n=243$), with an indirect mediation effect ranging from 2% to 74%. There were 159 (46.2%) associations of dietary intakes with a decreased risk of BC was mediated by an increase of circulating proteins, while 117 (51.8%) associations of medication uses with a decreased risk of BC were mediated by an increase of circulating proteins. Of those proteins, 59 (24.3%) circulating proteins were found to be uniquely mediated between an exposure and BC risk (Figure 3C; Table S18).

Multivariable MR Estimates for Joint Effects of Dietary Intakes and Medication Uses on Bladder Cancer Risk

Six-teen diet-medication pairs showed to be eligible for multivariable MR analyses, of which 9 were observed to significantly interact between each other based on the IVW method through multivariable MR. The co-intakes of instant coffee and calcipotriol showed no associations with BC risk, while the cholesterol lowering medication and dovobet ointment maintained the protective effect (OR 0.86, 95% CI 0.77–0.96, $P_{-fdr}=0.006$; OR 0.97, 95% CI 0.95–0.99, $P_{-fdr}=0.025$, respectively) on BC risk although the effect of instant coffee eliminated when consumed jointly. In addition, our results showed that major dietary changes in the last 5 years because of illness would attenuate the effects of medication uses (i.e., calcipotriol, dovobet ointment, thyroxine and cholesterol lowering medication) on BC, which no evidence of associations was found when consumed jointly (Figure 4; Table S19).

Sensitivity Analysis

Results obtained by the Weighted-Median or MR-Egger were broadly consistent with results obtained from the IVW and MR-PRESSO methods, thereby strengthening our estimates. No horizontal pleiotropy was detected in MR-Egger regression. Neither heterogeneity was detected using Cochran's Q statistics. In addition, the included number and F statistics for the included genetic instruments were consistent with an absence of weak instrument bias.

Discussions

In the present study, we found evidence for individual dietary intakes (i.e., instant coffee, cereal consumption, non-wheat products consumption, or major dietary change due to illness) and medication uses (i.e., calcipotriol, dovobet ointment, thyroxine product, or cholesterol lowering medication) in affecting BC risk, in which an unspecified gut microbe (i.e., *s_GCA-900066575 sp900066385*), an unknown circulating metabolite (i.e., X-13429) and several circulating proteins showed a mediating effect between diets/medications and BC risk. In addition, the individual effect of either a dietary intake or medication use on BC risk might change when consumed together, thereby suggesting that future studies should take the diet-medication interaction into account when assessing their associations with BC risk.

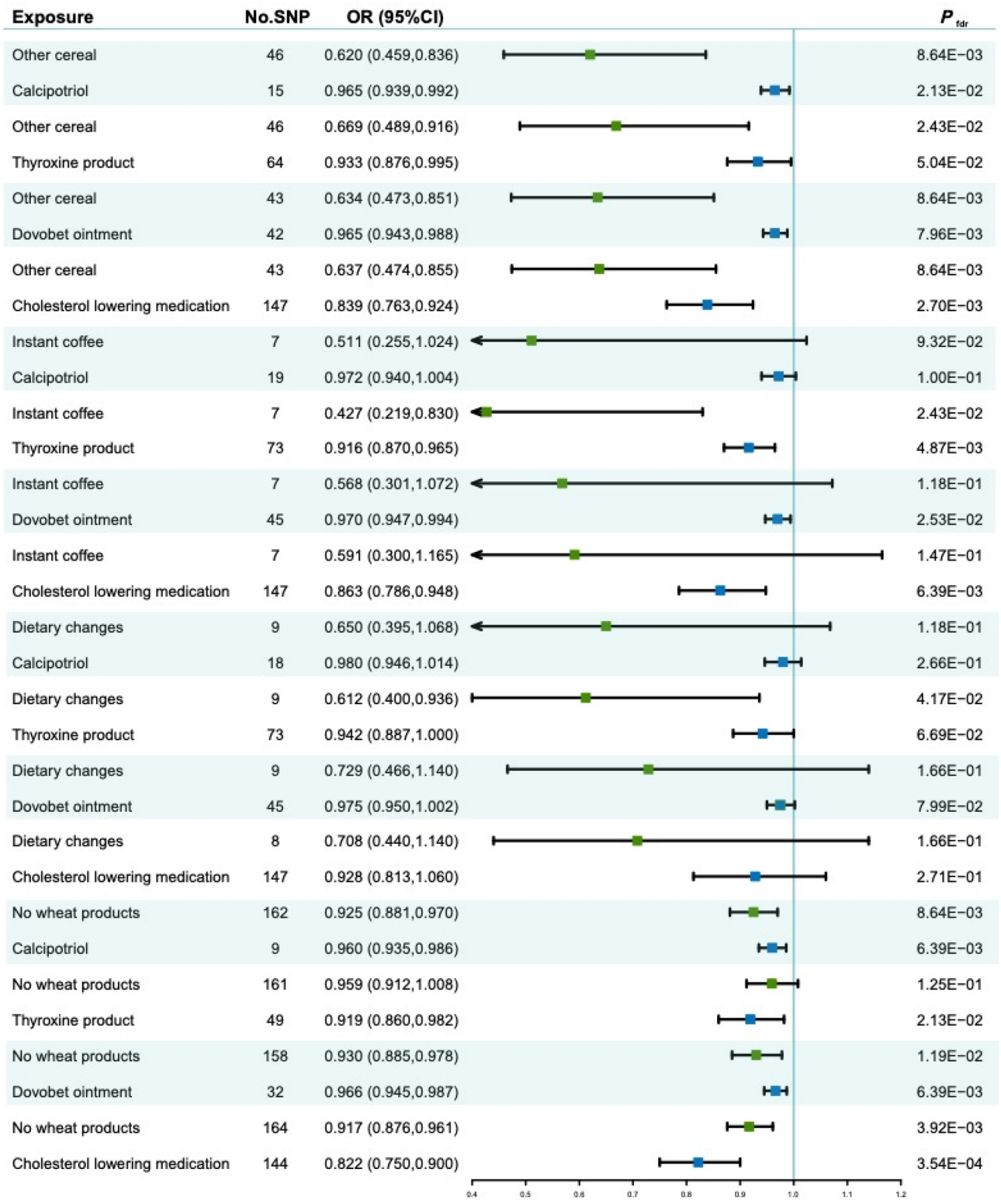

Figure 4. Forest plot for multivariable MR of identified diet-medication pairs and bladder cancer risk.
Square dots denote the odds ratios (ORs); horizontal lines represent the 95% confidence intervals (CIs); Green squares indicate dietary intakes, while blue squares indicate medication uses. A light green color, along with the white color was used to distinguish each diet-medication pair.
Abbreviations: No.SNP: number of single-nucleotide polymorphism; OR: odds ratios; CI: confidence Interval; fdr: false discovery rate.

Many observational studies investigated how diet relates to BC risk, including our previous studies based on large-scale cohorts that showed coffee consumption (23) to be significantly associated with BC risk. Like regular coffee, instant coffee contains many powerful antioxidants; according to Niseteo

et al. (24), the amount of certain antioxidants are thought to be even higher in instant coffee compared to other brewed coffee, thereby possible contributing to the inhibition of BC development.

Grain products, particularly whole grains, are enriched with dietary fiber and have been hypothesized to have several health benefits, including their effect on cancer prevention (25). Results of the present study are in line with this hypothesis, in that it is shown that whole grains (e.g., corns or Frosties), but no refine grains (e.g., wheat products), decreases BC risk. In addition, although the species of *GCA-900066575 sp900066385* is unclassified, it belongs to the genus of *Lachnospiraceae,* which is reported to be involved in the digestion of dietary fiber (26). According to the latest evidence that dietary fiber is mainly metabolized by gut microbiota (27), this study provided a mechanistic insight that the effect of wheat products on BC might be through certain gut microbiota and warrants further investigation.

Due to the increase in use and misuse of various medications, medication uses became a serious public health issue in recent years. However, studies on the impact of medication uses on BC risk are mainly lacking. In the current study we identified several medications causally associated with BC risk, of which the cholesterol lowering medication showed to be protective. A growing body of evidence suggest that high levels of cholesterol and its metabolites are associated with cancer initiation, progression, and metastasis (28). Cholesterol lowering medication (e.g., statin) can affect the urothelial bladder cells by regulating metabolic changes in the mitochondria through blood circulation, and therefore, yielded the effects of inhibiting inflammatory effects on bladder (29).

Results of the present study showed strong interactions in several diet-medication pairs, e.g., instant coffee with calcipotriol, dovobet ointment, and cholesterol lowering medication, in which the effects of instant coffee eliminated when consumed together. In addition, major dietary changes in the last 5 years due to illness weakened the effects of calcipotriol, dovobet ointment, thyroxine product, and cholesterol lowering medication. This finding clearly indicates that diet can alter the effects of medications and subsequently affect human health (30), and should, therefore, be taken into account by both physicians and patients during the development of a treatment strategy.

The majority of the identified mediators were proteins, making them to be the main molecules in linking exposures and BC risk. However, although proteins have been used as important biomarkers for various cancers (31), their function on distinguishing and predicting BC risk has yet to be well established. This current study, through multi-stages MR analyses, enabled a greater understanding of the effects of lifestyle changes on BC risk through circulating levels of protein and thereby, may improve pharmaceutical interventions and clinical trials for BC.

As smoking is indeed a well-established risk factor in relation to bladder cancer development, which was hypothesized to affect the results with regards to assessment of various etiologies on bladder cancer risk as a conventional covariate. Therefore, as a sensitivity analysis for those exposures with significant MR associations, additional multivariable MR studies with adjustment of smoking on bladder cancer risk were conducted. The results remained largely similar, which indicated the influence of smoking on the MR associations assessed in the current study is minimal, thereby suggested the primary analyses without smoking was valid.

Here are some limitations that require acknowledgement in our study; firstly, the included studies were obtained in mostly European studies, the interpretation of results obtained from current study upon other ethnicity should be cautious. Secondly, as a result of the extensive MR analysis conducted in this study, we were unable to examine all of the selected genetic instrumental variables, including their biological functions. Thirdly, each dietary intake or medication use was a binary variable, we were therefore unable to assess the potential dose-dependent changes in relation to BC risk. Finally, with consideration of the separateness and availability of GWAS cohorts for two-sample MR analysis, we only included the GWASs relatively meet all the criteria with largest sample size, most recent and containing most traits, thereby the comprehensiveness of genetic instrumental variables was believed to be sufficient. However, due to the inherent nature of GWAS and MR, the missing of residual genetic instrumental variables are inevitable. Still, we need to acknowledge, some cutting-edge GWAS summary data was unavailable, where analyses of the current study might, therefore, impeded by some uncovered SNPs. Updates based on the current study are warranted.

Conclusion

In summary, the current study extends observational literature in showing the importance of diets and medications on BC prevention, which might be through circulating metabolites, circulating proteins

and gut microbiota. These findings have important implications for prevention strategies development that point to potential lifestyle interventions or medication prescriptions to reduce the risk of developing BC.

Ethics Approval and Consent to Participate

All studies have existing ethical permissions from their respective institutional review boards and include participant written informed consent and rigorous quality control. Because all analyses herein are based on publicly available summary data, no ethical approval from an institutional review board was required for the current study.

Authors' Contributions

Y.T.C, E.Y.W.Y: conceived and designed the study; E.Y.W.Y: supervision; Y.T.C, Q.Y.T: conducted data analyses and interpretation and drafted the manuscript; Y.T.C, Q.Y.T: data curation; Y.X.Z, S.Z.W, A.W, W.C.L, M.P.Z: critical revision of the manuscript. All authors read and approved the final manuscript.

Disclosure Statement

No potential conflicts of interest relevant to this article were reported.

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ORCID

Evan Yi-Wen Yu http://orcid.org/0000-0001-7825-5087

Data Availability Statement

This work has been conducted using multiple publicly available resources. The data that support the current study and findings could be accessed according to each resource's guideline. Further information is available from the corresponding author upon request. Analysis code is available *via*: https://github.com/TeamEYu/MR-of-bc. Supplementary materials with detailed data and additional information are available online. To access these files, please visit https://doi.org/10.6084/m9.figshare.24745152.

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