Causal effects of gut microbiota on renal tumor: a Mendelian randomization study

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Dear Editor,

In 2020, kidney cancer is the 14th most common malignancy with an estimated 431 288 new cases globally, according to the Global Cancer Observatory[1]. Currently, prognostic studies on renal cell carcinoma are conducted extensively. Newly developed prognostic models by Wang *et al.*[2] at the *International Journal of Surgery* significantly increase our confidence in clinical decisions and offer practical assistance in the management of patients with renal cell carcinoma. However, the etiologic research on renal neoplasms including benign tumors is limited. It is reported that gut microbiota could be a preventive or risk factor for cancers, especially for gastrointestinal malignancies[3]. Thus, whether intestinal microbiota as a potentially modifiable factor is causally associated with renal neoplasms still needs further investigation.

Theoretically, randomized controlled trial (RCT) is regarded as the greatest approach to establishing a causal link between intestinal microbiota and renal neoplasms. Nevertheless, it is not pragmatic to determine the causal effect of gut microbiota on renal neoplasms using an RCT. Mendelian randomization (MR) is an epidemiological approach in which the exposures and outcomes are replaced by single nucleotide polymorphisms (SNPs) and corresponding instrumental variables (IVs). This method has been widely applied for causal inferences to avoid confounding and reverse causal biases originating from small sample sizes and cross-sectional design[4]. Here, we performed this two-sample MR analysis to determine the causal links between gut microbiota and renal neoplasms based on the summary statistics of a genome-wide association study (GWAS).

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Summary statistics of gut microbiota in humans were retrieved from the MiBioGen consortium, in which 18 340 individuals from 24 cohorts were included. Meanwhile, summary statistics of cases with renal neoplasms (malignant and benign) were retrieved from FinnGen (https://r7.finngen.fi/). For benign neoplasm of the kidney, 581 cases and 308 573 controls were included. For malignant neoplasm of the kidney, 1631 cases and 307 523 controls were enrolled. All participants were of European descent. Of note, cancer cases (in other organs of any type) in the controls were all removed in the sensitivity analyses for further verification. Finally, 248 356 controls for benign renal tumor and 238 678 controls for renal malignancy were enrolled in the sensitivity analyses. Six methods were applied to investigate the effects of gut microbiota on kidney neoplasms, including inverse variance weighted (IVW), maximum likelihood (ML), weighted median, MR-Egger, pleiotropy residual sum and outlier (MR-PRESSO), and robust adjusted profile score (MR.RAPS). All IVs were assumed to be valid IVs in IVW models and then were merged using meta-technique. Cochran Q was selected to explore the heterogeneity of adopted IVs, and P-value <0.05 represented the existence of heterogeneity, and the random-effects IVW method was applied. MR-Egger and MR-PRESSO global tests were utilized to detect the horizontal pleiotropy. The SNPs strength was quantified by F-statistics of bacterial taxon[5]. The F-statistic greater than 10 indicated the existence of bias. All statistical analyses in this study were conducted using R 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

In this two-sample MR study, we found that gut microbiota might have causal effects on kidney neoplasms. Significantly protective effects of *Class Actinobacteria* (OR: 0.58), *Family XI* (OR: 0.70), *Intestinimonas* (OR: 0.65), and *Veillonella* (OR: 0.47) against renal malignancy were detected (all $P < 0.05$). Moreover, *Intestinibacter* showed causality on the risk of renal malignancy (OR: 1.88, $P < 0.001$). The protective effects against benign renal tumor were identified in the *Holdemanella* (OR: 0.55), *Ruminiclostridium9* (OR: 0.31), and *Sellimonas* (OR: 0.61) (all $P < 0.05$). Additionally, *Catenibacterium* (OR: 2.12), *Eubacterium eligens group* (OR: 3.17), *Intestinibacter* (OR: 2.72), and *Oscillospira* (OR: 2.58) revealed a causal association with increased risk of benign renal tumor (all $P < 0.05$) (Fig. 1). The effect sizes and direction remained consistent in the other five methods. No heterogeneity and pleiotropy were identified by Cochran's Q test and global test (all $P > 0.05$) (Supplementary Figs S1–S8, Supplemental Digital Content 1, http://links.lww.com/JS9/B686, Supplemental Digital Content 2, http://links.lww.com/JS9/B687, Supplemental Digital Content 3, http://links.lww.com/JS9/B688, Supplemental Digital Content 4, http://links.lww.com/JS9/B689, Supplemental Digital Content 5, http://links.lww.com/JS9/B690, Supplemental Digital Content 6, http://links.lww.com/JS9/B691, Supplemental Digital Content 7, http://links.lww.com/JS9/B692, Supplemental Digital Content 8,

A

Actinobacteria (Class)

Methods	Malignant neoplasm of kidney OR (95% CI)	P values	Malignant neoplasm of kidney (Controls excluding all cancers) OR (95% CI)	P values
IVW	0.58 (0.39-0.87)	0.008	0.59 (0.40-0.89)	0.011
MR Egger	0.96 (0.30-3.05)	0.944	0.97 (0.30-3.12)	0.965
Weighted median	0.53 (0.30-0.93)	0.028	0.52 (0.30-0.89)	0.017
Maximum likelihood	0.59 (0.39-0.88)	0.01	0.60 (0.40-0.90)	0.015
RAPS	0.56 (0.37-0.86)	0.008	0.57 (0.37-0.88)	0.01
MR-PRESSO	0.58 (0.42-0.81)	0.007	0.59 (0.43-0.82)	0.007

Family XI (Family)

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	0.70 (0.54-0.92)	0.009	0.70 (0.54-0.92)	0.01
MR Egger	0.35 (0.06-1.90)	0.269	0.32 (0.06-1.74)	0.235
Weighted median	0.66 (0.47-0.92)	0.013	0.65 (0.46-0.91)	0.014
Maximum likelihood	0.70 (0.54-0.92)	0.012	0.70 (0.53-0.92)	0.011
RAPS	0.70 (0.53-0.93)	0.013	0.70 (0.53-0.93)	0.014
MR-PRESSO	0.70 (0.60-0.82)	0.003	0.70 (0.59-0.83)	0.005

Butyricimonas

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	-	-	1.53 (1.01-2.32)	0.049
MR Egger	-	-	3.47 (0.78-15.51)	0.131
Weighted median	-	-	1.31 (0.73-2.33)	0.364
Maximum likelihood	-	-	1.56 (1.01-2.40)	0.046
RAPS	-	-	1.59 (0.98-2.55)	0.058
MR-PRESSO	-	-	1.53 (1.01-2.31)	0.07

Coprococcus1

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	-	-	0.51 (0.26-0.99)	0.046
MR Egger	-	-	0.73 (0.13-4.07)	0.729
Weighted median	-	-	0.86 (0.41-1.79)	0.677
Maximum likelihood	-	-	0.50 (0.30-0.84)	0.008
RAPS	-	-	0.51 (0.26-1.01)	0.05
MR-PRESSO	-	-	0.51 (0.26-0.99)	0.074

Intestinibacter

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	1.88 (1.28-2.74)	0.001	1.93 (1.32-2.83)	0.001
MR Egger	2.05 (0.60-7.07)	0.275	2.15 (0.62-7.48)	0.251
Weighted median	2.22 (1.30-3.80)	0.004	2.36 (1.34-4.16)	0.003
Maximum likelihood	1.91 (1.29-2.83)	0.001	1.94 (1.31-2.89)	0.001
RAPS	1.98 (1.31-2.99)	0.001	2.05 (1.35-3.11)	0.001
MR-PRESSO	1.88 (1.32-2.66)	0.003	1.93 (1.35-2.76)	0.003

Intestinimonas

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	0.65 (0.45-0.93)	0.018	0.64 (0.45-0.93)	0.018
MR Egger	1.22 (0.45-3.35)	0.703	1.25 (0.45-3.45)	0.674
Weighted median	0.64 (0.39-1.06)	0.082	0.64 (0.40-1.05)	0.077
Maximum likelihood	0.65 (0.45-0.95)	0.024	0.64 (0.44-0.92)	0.018
RAPS	0.65 (0.44-0.95)	0.027	0.64 (0.44-0.95)	0.026
MR-PRESSO	0.65 (0.50-0.84)	0.006	0.64 (0.49-0.84)	0.006

Veillonella

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	0.47 (0.27-0.81)	0.007	0.48 (0.28-0.82)	0.008
MR Egger	0.22 (0.01-16.8)	0.535	0.23 (0.01-17.79)	0.546
Weighted median	0.49 (0.25-0.97)	0.04	0.51 (0.25-1.03)	0.062
Maximum likelihood	0.47 (0.27-0.82)	0.008	0.47 (0.27-0.83)	0.009
RAPS	0.47 (0.26-0.84)	0.011	0.47 (0.26-0.85)	0.012
MR-PRESSO	0.47 (0.32-0.69)	0.012	0.48 (0.32-0.70)	0.013

B

Acidaminococcaceae (Family)

Methods	Benign neoplasm of kidney OR (95% CI)	P values	Benign neoplasm of kidney (Controls excluding all cancers) OR (95% CI)	P values
IVW	-	-	2.24 (1.01-4.95)	0.047
MR Egger	-	-	3.13 (0.28-34.66)	0.395
Weighted median	-	-	1.84 (0.66-5.08)	0.242
Maximum likelihood	-	-	2.27 (1.01-5.13)	0.049
RAPS	-	-	2.27 (0.98-5.28)	0.057
MR-PRESSO	-	-	2.24 (1.20-4.18)	0.045

Catenibacterium

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	2.12 (1.12-4.00)	0.021	2.08 (1.09-3.96)	0.026
MR Egger	1.75 (0.01-29969.28)	0.921	1.84 (0.01-35273.43)	0.915
Weighted median	1.77 (0.81-3.85)	0.153	1.71 (0.76-3.85)	0.196
Maximum likelihood	2.17 (1.12-4.19)	0.022	2.13 (1.10-4.12)	0.025
RAPS	2.17 (1.07-4.37)	0.031	2.13 (1.06-4.30)	0.035
MR-PRESSO	2.12 (1.12-4.00)	0.104	2.08 (1.09-3.96)	0.112

Eubacterium eligens group

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	3.17 (1.07-9.40)	0.037	3.21 (1.10-9.40)	0.033
MR Egger	0.13 (0.01-6.37)	0.36	0.13 (0.01-6.61)	0.367
Weighted median	3.33 (0.79-14.14)	0.102	3.35 (0.78-14.43)	0.104
Maximum likelihood	3.42 (1.12-10.43)	0.031	3.46 (1.14-10.56)	0.029
RAPS	3.87 (1.22-12.29)	0.021	3.89 (1.23-12.32)	0.021
MR-PRESSO	3.17 (1.07-9.40)	0.092	3.21 (1.10-9.40)	0.086

Holdemanella

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	0.55 (0.33-0.91)	0.021	0.55 (0.33-0.92)	0.023
MR Egger	0.50 (0.11-2.19)	0.383	0.49 (0.11-2.11)	0.365
Weighted median	0.53 (0.26-1.09)	0.085	0.54 (0.26-1.11)	0.094
Maximum likelihood	0.54 (0.32-0.92)	0.023	0.55 (0.32-0.92)	0.024
RAPS	0.57 (0.33-0.99)	0.046	0.57 (0.33-0.99)	0.048
MR-PRESSO	0.55 (0.34-0.90)	0.038	0.55 (0.34-0.89)	0.036

Intestinibacter

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	2.72 (1.39-5.35)	0.004	2.76 (1.40-5.47)	0.004
MR Egger	6.50 (0.7-60.63)	0.125	6.56 (0.68-63.00)	0.127
Weighted median	2.10 (0.82-5.41)	0.123	2.13 (0.85-5.30)	0.105
Maximum likelihood	2.84 (1.52-5.28)	0.001	2.90 (1.55-5.42)	0.001
RAPS	2.87 (1.34-6.17)	0.007	2.96 (1.36-6.41)	0.006
MR-PRESSO	2.72 (1.39-5.35)	0.011	2.76 (1.40-5.47)	0.011

Oscillospira

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	2.58 (1.22-5.47)	0.013	2.66 (1.25-5.63)	0.011
MR Egger	0.68 (0.03-15.99)	0.819	0.77 (0.03-18.27)	0.876
Weighted median	2.23 (0.88-5.63)	0.091	2.20 (0.79-6.10)	0.131
Maximum likelihood	2.64 (1.22-5.72)	0.014	2.72 (1.25-5.91)	0.012
RAPS	2.63 (1.19-5.85)	0.017	2.71 (1.22-6.05)	0.015
MR-PRESSO	2.58 (1.57-4.25)	0.007	2.66 (1.58-4.46)	0.008

Ruminiclostridium9

Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	0.31 (0.11-0.92)	0.035	0.31 (0.11-0.92)	0.035
MR Egger	0.10 (0.01-13.32)	0.398	0.09 (0.01-12.17)	0.380
Weighted median	0.28 (0.07-1.09)	0.067	0.30 (0.08-1.16)	0.081
Maximum likelihood	0.31 (0.10-0.94)	0.039	0.31 (0.10-0.94)	0.038
RAPS	0.31 (0.10-0.97)	0.044	0.31 (0.10-0.96)	0.043
MR-PRESSO	0.31 (0.17-0.58)	0.010	0.31 (0.17-0.57)	0.009

Sellimonas

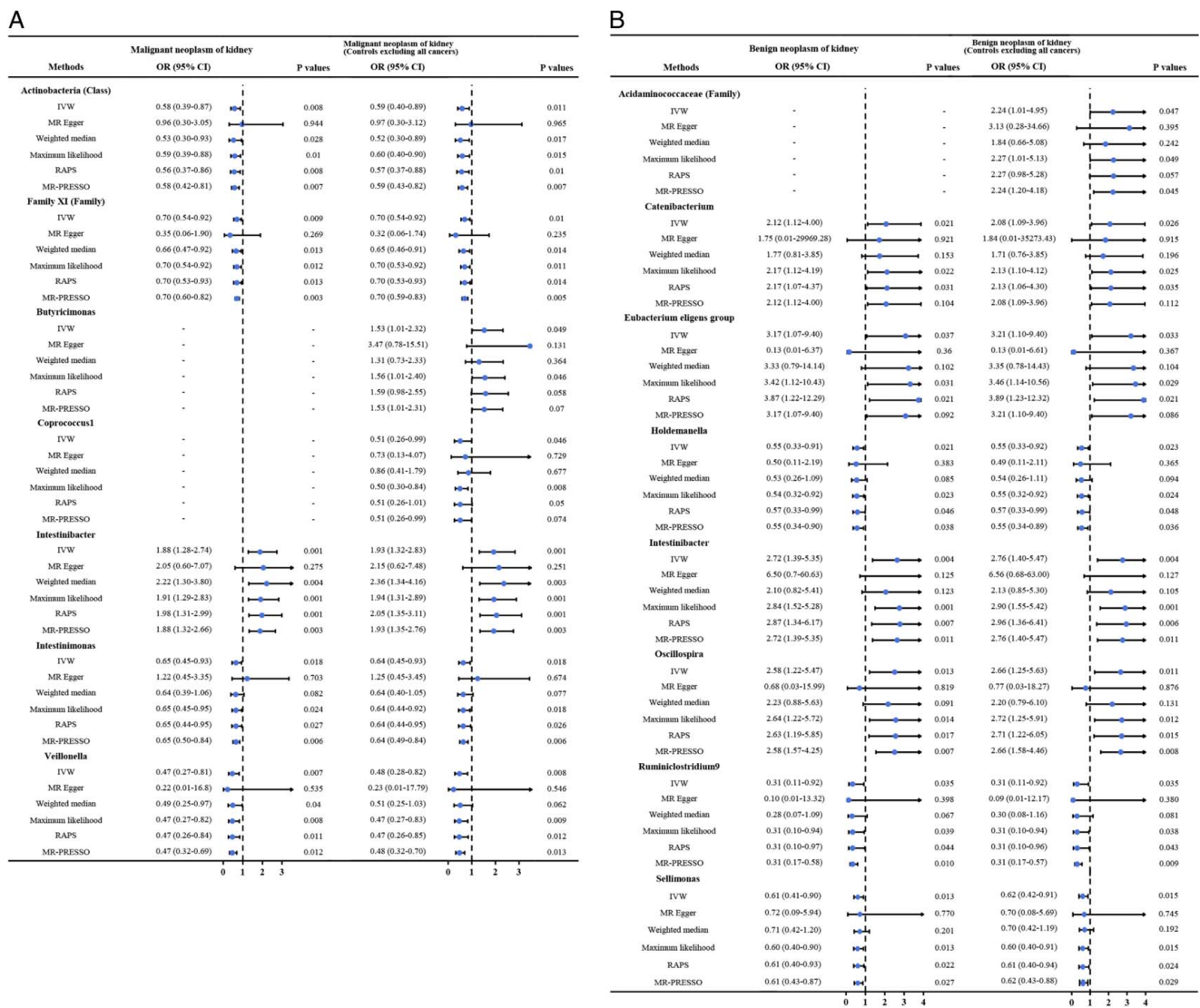| Methods	OR (95% CI)	P values	OR (95% CI)	P values
IVW	0.61 (0.41-0.90)	0.013	0.62 (0.42-0.91)	0.015
MR Egger	0.72 (0.09-5.94)	0.770	0.70 (0.08-5.69)	0.745
Weighted median	0.71 (0.42-1.20)	0.201	0.70 (0.42-1.19)	0.192
Maximum likelihood	0.60 (0.40-0.90)	0.013	0.60 (0.40-0.91)	0.015
RAPS	0.61 (0.40-0.93)	0.022	0.61 (0.40-0.94)	0.024
MR-PRESSO	0.61 (0.43-0.87)	0.027	0.62 (0.43-0.88)	0.029

Figure 1. (A) The results of MR estimating the causal association between intestinal microbiota and malignant neoplasm of the kidney. (B) The results of MR estimating the causal association between intestinal microbiota and benign neoplasm of the kidney. CI, confidence interval; IVW, inverse variance weighted; MR, Mendelian randomization; OR, odds ratio; PRESSO, pleiotropy residual sum and outlier; RAPS, robust adjusted profile score.

http://links.lww.com/JS9/B693) (Supplementary Tables S1–S4, Supplemental Digital Content 9, http://links.lww.com/JS9/B694, Supplemental Digital Content 10, http://links.lww.com/JS9/B695, Supplemental Digital Content 11, http://links.lww.com/JS9/B696, Supplemental Digital Content 12, http://links.lww.com/JS9/B697). We further evaluated whether there were bacteria that can simultaneously affect benign and malignant kidney neoplasms. In all, only one genus, *Intestinibacter*, could increase the risks of developing both benign (OR: 2.72) and malignant renal neoplasm (OR: 1.93), indicating the core role of *Intestinibacter* in the onset of kidney neoplasms (Supplementary Figure S9, Supplemental Digital Content 13, http://links.lww.com/JS9/B698). More efforts are necessary to clarify the molecular mechanisms underlying these links.

Ethics statement

Ethical approval is not needed in this study.

Consent

Consent is not applicable in this study.

Sources of funding

Author contribution

F.Z., Y.X., and B.Z.: conception, design, and manuscript writing; B.Z.: administrative support; F.Z. and Y.X.: provision of study materials or patients, collection and assembly of data, and data analysis and interpretation. All authors were involved in the final approval of the manuscript.

Conflicts of interest disclosure

All authors report no conflicts of interest in this work.

Research registration unique identifying number (UIN)

Not applicable.

Guarantor

Bo Zhang.

Data availability statement

The datasets are available from the corresponding author on reasonable request.

Provenance and peer review

Our paper was not invited.

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