Cohort Study

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Dissecting the role of metformin in urogenital malignancies

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Abstract

Metformin has shown great potential for anti-tumor therapy according to laboratory results, but there is currently no consensus on the role it playing in the pathogenesis of urogenital malignancies. Initially, a systematic review was conducted on clinical research examining the association between the use of metformin and the incidence and prognosis of prevalent urogenital malignancies. Then, a retrospective analysis of the participants in NHANES was performed to strengthen the study. Given the distinct methodological advantages of Mendelian randomization (MR) in research design, whereby genetic variations influencing the exposure of interest are independent of potential confounders, available GWAS data were thoroughly collected and utilized in a two-sample MR analysis to assess the causal relationships between metformin use and various urogenital malignancies. The review of literature demonstrated inconsistencies and ambiguities in clinical findings. The retrospective analysis of 20,527 participants in the NHANES did not reveal strong evidence in the four urogenital malignancies. The current MR study indicates that metformin use is unlikely to be a causal factor in the development of five urogenital malignancies ($P > 0.05$), either does the reverse MR analysis ($P > 0.05$). Nevertheless, the results were reliable to some extent since neither heterogeneity nor pleiotropy was detected in most cases. This study suggests that metformin use does not demonstrate a protective effect on the studied urogenital malignancies, contradicting the positive results observed in laboratory settings. Additional evidence from clinical studies is required to validate this conclusion.

Introduction

Urologists frequently encounter prostate cancer (PC), urothelial carcinoma, renal cell carcinoma (RCC), testicular cancer (TC), and penile cancer as prevalent neoplastic conditions within their clinical practice. To prevent and manage these cancers, it is necessary to explore protective factors.

Metformin is a classic first-line drug for the treatment of type 2 diabetes and is mainly excreted from urine in its prototype form. As more research conducted, it has been found to play some role in anti-tumor effects[1]. Mechanistically, metformin may target JNK pathway, adenosine monophosphate-activated protein kinase (AMPK) pathway and mammalian target of rapamycin (mTOR) pathway, reducing glucose metabolism and inhibiting oxidative phosphorylation[2]. Clinically, the current research is contradictory in metformin use for urogenital malignancies risk and survival. One recent retrospective study indicates that metformin use is beneficial for reducing the incidence and all-cause mortality of PC[3], while the meta-analysis showed that the use of metformin did not significantly alter the risk of prostate cancer[4]. The majority of the included studies, however, were observational in nature and had small sample sizes. In contrast, Mendelian randomization (MR) analysis uses genetic variation as an instrumental variable to evaluate the impact of exposure factors on outcomes, while minimizing the impact of confounding factors and reverse causality. Considering the conflicting findings in the literature, this study aims to resolving these inconsistencies.

This study made a combination of literature review, cross-sectional and longitudinal research methods and genetic epidemiological method. In detail, this study conducted a systematic review of clinical research examining the relationship between metformin use and the incidence and survival rates of prevalent urogenital malignancies. Then, a retrospective analysis of the participants in National Health and Nutrition Examination Survey (NHANES) was performed to enhance credibility of results in statistics. Upon identifying the uncertain nature of

HIGHLIGHTS

- Systematic review plus NHANES study plus Mendelian randomization study
- Metformin use does not demonstrate a protective effect on the studied urogenital malignancies.
- Temper our expectations regarding the efficacy of metformin.

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the correlation between metformin use and common urogenital malignancies, we employed MR to investigate the causal link between metformin and the risk of prevalent urogenital malignancies in diverse populations using publicly available genome-wide association study (GWAS) data, in order to elucidate the protective effects of metformin medication in suffering from urogenital malignancies.

Method

Literature review

A thorough review was conducted to evaluate the clinical implications of metformin in the treatment of common urogenital malignancies, specifically prostate cancer, urothelial carcinoma, renal cancer, testicular cancer, and penile cancer. PubMed, Embase, and Cochrane Central Register of Controlled Trials databases were searched for randomized clinical trials, prospective studies, retrospective studies, and meta-analyses published in SCIE-indexed journals falling within the Q1 and Q2 JCR categories from 1 January 2000 to 31 December 2023. The terms utilized in this study included "metformin" in conjunction with "neoplasms," "kidney," "renal pelvis," "upper urinary tract," "bladder," "testis," "prostate" and "penis." Following the literature search, duplicate publications and those predating the most recent meta-analysis were excluded (Figure S1, available at: http://links.lww.com/MS9/A777). Statistical odds ratios (ORs) and 95% confidence intervals (CIs) were selected as the primary measures for assessing the impact of metformin use on cancer risk. The hazard ratios (HRs) were utilized as an estimate of the OR due to the infrequency of the target outcomes. The endpoints selected for this study included recurrence-free survival (RFS), disease-free survival (DFS), cancer-specific survival (CSS), and overall survival (OS). Adjusted HRs were extracted from studies that reported them, and the findings from these studies were incorporated into our analysis.

Retrospective study based on NHANES database

The analysis included a cohort of 91,351 adult participants from the NHANES spanning from 2001 to 2017. The methodology and data collection process of NHANES are delineated in Figure S2 (available at: http://links.lww.com/MS9/A778), with all findings adjusted for sampling probabilities. Details regarding metformin usage and past occurrences of urogenital malignancies were gathered through a questionnaire administered via computer-assisted personal interviewing during the physical examination segment of the survey. Univariate and multivariable logistic regression analyses were conducted to evaluate the relationship between metformin usage and urogenital malignancies, while controlling for variables such as age, gender, race/ethnicity, level of education, smoking status, and other relevant factors (Supplementary Excels 1–4). The selection of variables was informed by academic consensus on the one hand, while being constrained by the availability of variable entries in the database on the other. The work has been reported in line with the STROCSS criteria[5].

Study design for MR

This study was performed by two-sample MR based on the genetic data obtained from global genetic consortia, which was based on three assumptions[6]: in the first place, we should expect single-nucleotide polymorphisms (SNPs) to be strongly associated with exposure when they are considered instrumental variables (IVs); a second requirement is that selected SNPs should be independent of confounding factors, and a third requirement is that the association between IVs and outcomes must be through exposure rather than a direct relationship.

GWAS summary data sources

This study utilized raw data from the genome-wide association study (GWAS) database. We search the IEU OpenGWAS for all datasets of exposures and outcomes originated from different study in population of the same ancestry. As a general practice, datasets containing more than 1000 cases are typically included for analysis. However, in cases where this criterion is not met, datasets with a minimum of 100 cases are considered, it is important to acknowledge that the findings from such analyses may not robust. Regrettably, we were unable to locate publicly accessible GWAS data for penile cancer patients that met the required sample size. The dataset pertaining to metformin treatment and urogenital malignancies, including renal cell carcinoma, renal clear cell carcinoma, renal pelvis cancer, bladder cancer, prostate cancer and testis tumor are presented in Table S1 (available at: http://links.lww.com/MS9/A781). Participants were categorized as either cases or controls irrespective of age or gender, based on their receipt of metformin treatment. Gender, age, and pathology were also not taken into account when selecting the urogenital malignancies cases. Due to the collection of GWAS data by various consortiums or organizations, there is no sample overlap. The initial stage involved identifying IVs that demonstrated independent significance at the genome-wide level ($P < 1 \times e^{-5}$). Subsequently, linkage disequilibrium was assessed ($r^2 < 0.001$ and distance >10 000 kb) to mitigate potential biases stemming from linkage disequilibrium[7]. Harmonization across various databases was performed to maintain uniform effects of IV alleles. IVs with an F-statistic <10 were excluded as "weak instruments," while those with an F-statistic of 10 were chosen for subsequent MR analysis[8].

Statistical analysis

A two-sample MR analysis was performed utilizing R software (version 4.2.1, http://www.r-project.org) and the "Two-Sample MR" package (version 0.5.6)[9]. The MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) parameters were computed using the "MRPRESSO" R package. Significant causality was defined as P <0.05.

Primary analysis

This study employs two-sample bidirectional MR to evaluate the mutual causality between metformin and urogenital malignancies. A meta-analysis is conducted to combine Wald ratios for causal effects of each single SNP using inverse variance weighting (IVW)[10]. In addition to IVW, alternative MR methods such as MR-Egger, weighted-median and Weighted Modes are utilized as complements[11]. The MR estimates are derived using various methods tailored to different assumptions of validity.

Sensitivity and heterogeneity analysis

The directionality of causation between each identified SNP for both the exposure and outcome variables was evaluated through

the application of MR Steiger filtering. This method entails the calculation and comparison of the variance in exposure explained by instrumental SNPs with the variance in outcome. A "TRUE" MR Steiger result signifies a causal relationship in the expected direction, while a "FALSE" result suggests causality in the opposite direction. SNPs yielding "FALSE" results, indicating a substantial influence on the outcome rather than the exposure, were excluded from subsequent analysis.

In order to evaluate the impact of individual SNPs on causal estimates, a leave-one-out sensitivity analysis is a valuable tool. Our assessment of heterogeneity as a measure of causal relationships, utilizing Cochran's Q statistic and associated *P* values, revealed that lower levels of heterogeneity are indicative of more dependable MR estimates when $P > 0.05$.

Results

Review of previous clinical studies

We listed the literature reviews of clinical studies concerning metformin use on urogenital malignancies in Table 1, both therapeutic and preventive effect were reviewed here[3,4,12-21]. In our analysis of RCC, we incorporated six retrospective studies that examined both localized and metastatic disease. None of the studies examined the specific correlation between the utilization of metformin and the development of RCC. While one study suggested a potential improvement in OS among individuals with metastatic RCC patients who used metformin (HR = 0.42 [0.26, 0.69]), the other five studies found no statistically significant effect of metformin administration on OS in cases of localized or metastatic RCC. Moreover, the findings of all six studies consistently indicate that the utilization of metformin does not have a significant impact on patients' PFS, DFS, or CSS.

A recent meta-analysis on urothelial carcinoma indicated that metformin may reduce the incidence and recurrence risk of non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC) patients. However, the study did not observe a significant impact of metformin on PFS, DFS, CSS, or OS. Additionally, a retrospective study indicated that metformin use did not significantly influence postoperative PFS, DFS, or CSS in patients with upper urinary tract urothelial carcinoma (UTUC).

Recent meta-analyses have indicated that metformin does not have a significant effect on the incidence or OS of prostate cancer. However, subsequent retrospective studies and

Table 1

Literature review of clinical studies concerning metformin use on urogenital malignancies

Cancers	Study design	Year	Population	Sample size	Tumor stage	Outcomes	PMID
RCC	Retrospective study	2022	Italy, Spain, and USA	304	Metastatic	OS: HR = 1.05 (0.56–1.96) PFS:HR = 0.80 (0.50–1.30)	35947324
RCC	Retrospective study	2021	Czech Republic	343	Metastatic	OS: HR = 0.45 (0.256–0.794) PFS: HR = 0.55(0.343–0.883)	34054309
RCC	Retrospective study	2017	Canada	158	M0	DFS: HR = 0.99 (0.36–2.74) CSS: HR = 0.38 (0.08–1.86) OS: HR = 0.86 (0.40–1.85)	27424258
RCC	Retrospective study	2017	USA	4736	Metastatic	PFS: HR = 0.979 (0.806–1.189) OS: HR = 1.053 (0.837–1.324)	27460432
RCC	Retrospective study	2016	Singapore	1528	Localized/ metastatic	Localized/DFS: HR = 0.47 ($P < 0.01$) CSS: HR = 0.21 ($P < 0.01$) Metastatic/CSS: HR = 0.78 ($P = 0.286$)	26794391
RCC	Retrospective study	2016	Israel and USA	108	Metastatic	PFS: HR = 0.71 (0.77–1.40) OS: HR = 0.42 (0.26–0.69)	27211307
UTUC	Retrospective study	2014	Europe, Japan, Canada, Austria, and USA	2492	Resectable	RFS: HR = 1.03 (0.47–1.08) DFS: HR = 0.81 (0.57–1.16) OS: HR = 0.81 (0.62–1.07)	24113620
BC	Meta-analysis	Not mentioned	China, USA, Canada, UK, Korea, and Singapore	1 552 773	NMIBC/MIBC	Incidence: OR = 0.45 (0.37–0.56) OS: HR = 0.93 (0.67–1.68) DSS: HR = 0.73 (0.47–1.16) RFS: HR = 0.56 (0.41–0.76) PFS: HR = 0.78 (0.53–1.15)	35462910
PC	Meta-analysis	2022	Europe, North America, and Asian	3 094 152	Not mentioned	Incidence: OR = 0.92 (0.78–1.09)	35074527
PC	Meta-analysis	2022	80–90% White	4277	CRPC	OS: HR = 0.83 (0.67–1.03)	35643841
PC	Cohort study	2022	Israeli	145 617	Not mentioned	1 year of metformin exposure on PC risk: HR = 1.53 (1.19–1.96) 2–7 year of metformin exposure on PC risk: HR = 0.58 (0.37–0.93)	34893792
PC	Retrospective study	2022	China	66 411	Not mentioned	PC risk: HR = 0.80 (0.69–0.93) All-cause mortality: HR = 0.89 (0.86–0.92)	35714677
PC	RCT	2022	Multi-center	2283	mCRPC	OS: HR = 0.77 (0.62–0.95)	35568679

RCC = renal cell carcinoma; UTUC = upper urinary tract urothelial carcinoma; BC = bladder cancer; PC = prostate cancer; TT = testis tumor; OR = odds ratio; HR = hazard ratio; RFS = recurrence-free survival; DFS = disease-free survival; CSS = cancer-specific survival; OS = overall survival.

randomized controlled trials have presented conflicting evidence, suggesting that metformin may actually reduce the incidence of prostate cancer (HR = 0.80 [0.69-0.93]) and lower the risk of all-cause mortality (HR = 0.89 [0.86–0.92] and HR = 0.77 [0.62–0.95], respectively). Additionally, a cohort study revealed that the duration of metformin use may play a role in its effects, with shorter durations potentially increasing the risk of prostate cancer (HR = 1.53 [1.19, 1.96]) and longer durations potentially increasing the risk of prostate cancer (HR = 0.58 [0.37, 0.93]).

However, we did not retrieve any clinical studies related to metformin and penile or testicular cancer. Overall, the research findings on the association between metformin use and urogenital malignancies risk and survival outcomes exhibit inconsistencies and ambiguities.

Association between metformin use and urogenital malignancies in NHANES

The NHANES database contains data exclusively on PC, BC, RCC, and TC. The overall weighted prevalence rates were 2.19% (450 cases) for PC, 0.33% (68 cases) for BC, 0.287% (59 cases) for RCC, and 0.07% (14 cases) for TC. Characteristics of the study subjects are detailed in Table S2 (available at: http://links.lww.com/MS9/A781), using PC as an illustrative example. Our study included a total of 20,527 participants, comprising 450 cases and 20,077 controls. Significant differences in age (72.45 vs. 56.10, $P < 0.001$), race (3.3% of cases were Mexican American vs. 7.1% of controls), family income (2.85 vs. 2.68, $P = 0.035$), smoking (12.7% of cases were current smokers compared to 22.9% of controls; $P < 0.001$), drinking (74.0% of cases were drinking compared to 66.5% of controls, $P = 0.001$), BMI (28.98 vs. 29.75, $P = 0.019$), diabetes (56.4% of cases were not diabetes compared to 65.7% of controls; $P < 0.001$), and hypertension rates (82.0% of cases were hypertension compared to 67.8% of controls; $P < 0.001$) were observed between individuals with PC and those without PC (Table S2, available at: http://links.lww.com/MS9/A781). The findings from multivariable logistic regression analyses examining the relationship between metformin use and urogenital malignancies are presented in Fig. 1. No statistically significant associations were observed between metformin use and the risks of bladder cancer (BC), renal cell carcinoma (RCC), and testicular cancer (TC) across the three models ($P > 0.05$). Notably, in Model 1, a significant positive association was identified between metformin use and prostate cancer (PC) risk (OR = 2.88 [1.99, 4.16]; $P < 0.05$); however, this association was no longer significant after adjusting for other covariates ($P > 0.05$). Therefore, a retrospective analysis of the NHANES did not reveal strong evidence suggesting a link between the metformin use and the risk of the four urogenital malignancies.

Causal relationship between metformin and urogenital malignancies

Following the exclusion of palindromic and ambiguous SNPs, as well as SNPs lacking proxy information, target SNPs were identified as IVs for metformin. Various methods, including IVW, MR-Egger, weighted-median, and Weighted Modes, were employed to assess the potential causal relationship between genetically predicted metformin exposure and urogenital malignancies outcomes (see Table 2 and Figure S3, available at: http://links.lww.com/MS9/A779). Across all four MR approaches, consistent evidence was found supporting a negative association between metformin use and urogenital malignancies outcomes ($P > 0.05$). Additionally, the reverse MR analysis also indicated no evidence of reverse causality for genetically predicted metformin and urogenital malignancies ($P > 0.05$) (see Table S3, available at: http://links.lww.com/MS9/A781, and Figure S4, available at: http://links.lww.com/MS9/A780).

Sensitivity & heterogeneity analysis

The study conducted sensitivity analyses to assess and correct for pleiotropy in causal estimates, as indicated in Table S4 (available at: http://links.lww.com/MS9/A781). Results from Cochran's Q-test did not show any evidence of heterogeneity or asymmetry among the SNPs analyzed, and the MR-Egger intercept analysis did not detect pleiotropy at the directional level of metformin in most cases ($P > 0.05$). A leave-one-out analysis was conducted to validate the impact of each SNP on the overall causal estimates, demonstrating that all SNPs contributed to the statistical significance of the causal relationship.

Discussion

Metformin, a primary pharmacological agent for managing hyperglycemia in individuals with type 2 diabetes, has garnered attention for its potential therapeutic effects in cancer treatment[1]. Initially, following an extensive review of the literature, it remains uncertain as to whether metformin exhibits anti-tumor properties within the urinary and reproductive systems, with conflicting findings reported in various sources. The retrospective analysis of the NHANES did not reveal strong evidence suggesting a link between the metformin use and the risk of the four urogenital malignancies either. Utilizing a two-sample MR analysis with summary statistics from extensive GWAS, we investigated the causal relationship between metformin use and the risk of developing urogenital malignancies, revealing no causal association. A notable gap exists in the literature regarding preclinical and clinical findings.

At present, the anti-urogenital malignancies effects of metformin mainly come from laboratory results. A systematic review has determined that metformin may exert direct anti-cancer effects through various mechanisms, including activation of liver kinase B1 (LKB1) and AMPK, inhibition of mTOR activity and protein synthesis, induction of apoptosis and autophagy by p53 and p21, and reduction of blood insulin levels[22]. Additionally, metformin has been shown to mitigate the negative effects of NKX3.1 deficiency under conditions of oxidative stress, leading to decreased tumorigenicity and restoration of mitochondrial function both in vivo and in vitro[23]. For urothelial carcinoma, the findings from both in vitro and in vivo studies indicate that metformin, when used as a standalone treatment, effectively suppressed the metabolic activity and cell proliferation[24,25]. Preliminary results from laboratories indicate that the combination of gefitinib effectively induced a potent anti-proliferative and anti-colony forming effect, as well as apoptosis, in BC cell lines[26]. Additionally, metformin enhanced panobinostat-induced apoptosis, and the combined treatment cooperatively inhibited the growth of BC cells[27]. The laboratory findings for RCC indicated that treatment with metformin resulted in the activation of AMPK, leading to the

Table 2

Summary-level MR results demonstrating causal estimates from metformin usage on the risk of five urogenital malignancies

		Exposure: metformin (ukb-a-159)			Exposure: metformin (ukb-b-14609)		
Outcome	**Method**	**OR**	**95% interval**	***P* value**	**OR**	**95% interval**	***P* value**
RCC	IVW	3.80	0.25–56.78	0.792	1.30	0.06–26.46	0.971
	MR Egger	18.26	0.03–12822.69	0.919	0.18	0.00–549.11	0.919
	Weighted median	7.92	0.08–778.34	0.832	1.46	0.01–233.31	0.943
	Weighted mode	6.99	0.02–2809.45	0.805	0.63	0.00–715.07	0.978
RCCC	IVW	1.01	0.01–115.11	0.998	0.17	0.00–20.49	0.883
	MR Egger	113.6	0–1.08 E+7	0.919	0.10	0.00–31592.20	0.919
	Weighted median	1.87	0.00–3370.77	0.943	0.65	0.00–1717.15	0.943
	Weighted mode	1.01	0.00–11599.48	0.999	0.49	0.00–33509.80	0.978
RPC	IVW	0.14	0.00–7234.99	0.915	0.01	0.00–2346.25	0.883
	MR Egger	0.00	0.00–1599.84	0.919	0.00	0.00–8.23E+10	0.919
	Weighted median	0.52	0–8.67 E+6	0.943	0.00	0.00–99.40	0.703
	Weighted mode	12.13	0.00–3.73E+13	0.978	0.00	0.00–1.67E+5	0.636
BC	IVW	0.90	0.04–18.30	0.985	0.44	0.02–9.87	0.895
	MR Egger	0.13	0.00–197.72	0.919	0.01	0.00–28.85	0.919
	Weighted median	0.78	0.01–62.47	0.943	0.25	0.00–32.33	0.859
	Weighted mode	0.00	0.00–3.73	0.55	0.00	0.00–0.36	0.517
BC	IVW	1.18	0.68–2.04	0.883	0.75	0.42–1.32	0.792
	MR Egger	0.76	0.2–2.92	0.919	0.66	0.15–2.87	0.919
	Weighted median	0.95	0.41–2.20	0.943	0.42	0.16–1.10	0.666
	Weighted mode	0.43	0.12–1.57	0.636	0.34	0.09–1.29	0.550
PC	IVW	0.62	0.18–2.22	0.883	0.79	0.18–3.40	0.915
	MR Egger	0.96	0.05–20.11	0.977	0.49	0.01–22.45	0.919
	Weighted median	0.42	0.05–3.38	0.832	0.36	0.03–3.87	0.832
	Weighted mode	0.38	0.02–6.26	0.782	0.19	0.01–3.28	0.636
PC	IVW	0.65	0.24–1.74	0.849	0.76	0.30–1.90	0.883
	MR Egger	0.52	0.05–5.62	0.919	1.60	0.15–16.88	0.919
	Weighted median	0.80	0.24–2.63	0.921	1.54	0.46–5.18	0.846
	Weighted mode	1.26	0.16–9.75	0.978	2.30	0.36–14.80	0.736
PC	IVW	0.89	0.16–5.07	0.985	0.71	0.17–3.07	0.985
	MR Egger	1.00	0.02–63.46	1.00	2.46	0.06–95.15	0.842
	Weighted median	2.99	0.48–18.75	0.55	2.40	0.50–11.55	0.550
	Weighted mode	1.52	0.21–10.88	0.683	2.35	0.31–17.52	0.655
TT	IVW	0.96	0.00–412.51	0.998	0.02	0.00–19.46	0.792
	MR Egger	0.00	0.00–619.3	0.919	0.00	0.00–14.29	0.919
	Weighted median	0.01	0.00–146.71	0.832	0.00	0.00–0.12	0.490
	Weighted mode	0.00	0.00–31.32	0.57	0.00	0.00–14.87	0.550

RCC = renal cell carcinoma; RCCC = renal clear cell carcinoma; RPC = renal pelvis cancer; BC = bladder cancer; PC = prostate cancer; TT = testis tumor; OR = odds ratio; identified instrumental variables (IVs) that demonstrated independent significance at the genome-wide level ($P < 1 \times e^{-5}$).

suppression of cell proliferation under normal conditions, but enhancing cell proliferation under glucose deprivation conditions[28]. Furthermore, the combination of AMPK activation and PKM2 depletion or inhibition was shown to produce a more effective therapeutic outcome[28]. A separate study provided additional support for the inhibitory effects of metformin on cell growth and cell cycle progression in renal cancer cells, partially through the upregulation of miRNA34a[29]. In the context of testicular cancer, an in vitro study demonstrated that metformin inhibited tumor cell progression through the G1 phase by upregulating phosphorylated YAP1 and downregulating the expression of cyclin D1, CDK6, CDK4, and RB[30]. This action resulted in increased sensitivity to cisplatin chemotherapy and activation of cleaved caspase 3 expression[29]. Additionally, research has shown that metformin exerts anti-proliferative and anti-migratory effects in the male germ tumor SEM-1 cell line by modulating HMGA1 and its downstream targets, including cyclin D1, the IGFs system, and MMP-11[31]. In summary, extant laboratory findings substantiate the anti-tumor properties of metformin on urogenital malignancies.

Nevertheless, the association between metformin and urogenital tumors remains ambiguous in clinical investigations, with instances of synergistic interactions observed when co-administered with specific medications. The potential reasons for the discrepancies between laboratory and clinical data should be explored in greater depth. First, differences between in vitro/animal models and humans: (1) The tumor microenvironment, immune system, and metabolic pathways in cell experiments (in vitro) or animal models (e.g., mice) significantly differ from those in humans, potentially leading to an overestimation of metformin's efficacy. (2) Laboratory studies often use a single type of tumor cell or genetically engineered animal models, whereas clinical patients exhibit high tumor heterogeneity (e.g., mutation profiles, microenvironment, drug-resistant subclones, etc.). (3) Laboratory conditions cannot simulate the impact of patient age, comorbidities, drug interactions, dietary

habits, and other factors on drug efficacy. Second, differences in pharmacokinetics and pharmacodynamics: Laboratory studies may use high doses of metformin, whereas the safe doses used clinically may be insufficient. Third, Complexity of tumor biology: (1) Patient tumors may exhibit heterogeneity between primary and metastatic sites or develop resistance mutations during treatment. (2) Tumor-associated fibroblasts, immunosuppressive cells, or hypoxic environments may weaken the drug's effects. (3) After metformin inhibits a specific pathway, tumors may survive through compensatory signaling pathways, Forth, issues in clinical study design: (1) Population selection bias (e.g., different ethnic groups may exhibit varying responsiveness to metformin's anti-tumor effects). (2) Insufficient sample size or low statistical power. (3) The efficacy of metformin appears to be contingent upon dosage and the glucose-deprived state, as indicated by laboratory data. However, the variables of metformin dosage, treatment duration, and patient blood glucose levels are not properly controlled in clinical studies. Fifth, experimental data bias: (1) Laboratories may exhibit publication bias (reporting only positive results) or methodological flaws (e.g., lack of blinding, insufficient replication). (2) The "anti-tumor effects" observed in laboratories may result from non-specific toxicity (e.g., cellular stress) rather than true targeted effects. This may have contributed to the absence of a significant correlation between metformin use and the occurrence of urogenital malignancies in the present study.

The current study offers several noteworthy advantages. Firstly, it utilized large-scale GWAS summary statistics in conjunction with MR analysis methods that are resilient to confounding variables. Furthermore, the robust estimation of each IV with all F-statistics >10 mitigated the risk of bias stemming from weak IVs. Finally, to enhance the reliability and stability of the observed association between metformin treatment and urogenital malignancies, multiple sensitivity tests were conducted. Our findings suggests that metformin use does not demonstrate a protective effect on the studied urogenital malignancies. It may be advisable to temper our expectations regarding the efficacy of metformin.

However, three main limitations should be considered. First, the data extracted by this study does not include information on the dosage and duration of metformin, patient demography, tumor pathology type, etc. This omission limits our ability to assess dose-dependent effects or distinguish between transient and sustained outcomes, etc. Second, to ascertain the consistency of genetic background, all participants included in the study were of European population, which limits the extrapolation of findings to other populations. Genetic polymorphisms, cultural factors, and socioeconomic variables may differentially influence drug efficacy and safety across ethnicities. Third, the cases of diabetic patients who are taking metformin drugs were not excluded from the cases collected due to lack of diabetes status. Thus, the causal inference is not rigorous in this case.

Until robust clinical evidence emerges, caution is warranted, but these findings underscore the need to re-evaluate metformin's anti-tumor role in large sample clinical research in multi ethnicities. Besides, this study informs future research directions that:1)Translational Medical Research: Develop clinically relevant models (e.g., patient-derived xenografts or organoids) to better replicate human disease progression and therapeutic responses, 2)Biomarker Development: Identify treatment-sensitive patient subpopulations through genomic and proteomic profiling to guide personalized therapeutic strategies, 3) Adaptive Clinical Trial Design: Dynamically adjust dosing regimens while rigorously controlling variables such as metformin dosage, treatment duration, tumor pathology type, patient demographics, and blood glucose levels, 4)Integrated Multi-Omics Analysis: Elucidate metformin's mechanism of action in urologic tumors by integrating molecular profiling, pharmacokinetic data, and clinical outcomes to establish robust mechanistic insights, 5)Strengthening Mendelian Randomization (MR) Validity: Future studies should prioritize (i) instruments specific to metformin response or (ii) diabetes-free populations where metformin is used off-label.

Conclusion

In conclusion, while laboratory findings tend to corroborate the potential anticancer properties of metformin within the urogenital system, our findings underscore metformin's potential in oncology. Large-scale, randomized controlled trials with rigorous design are urgently needed to confirm its efficacy and safety in diverse populations. Future research should prioritize Translational Medical Research and Integrated Multi-Omics Analysis. Additionally, exploring metformin's effects within molecularly defined urogenital malignancies subtypes may unlock personalized applications. Collaborative efforts to address these gaps will be essential to translate preclinical insights into clinically actionable advancements.

Ethical approval

In view of the retrospective nature of the study and the use of publicly available datasets, ethical approval was waived by the Ethics Committee of the Second Xiangya Hospital of Central South University.

Consent

This study was based on publicly available datasets, written informed consent has been obtained from all the individuals in the original studies to publish this paper.

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Author's contribution

Y.H.W., X.C.: conceptualization; X.C., Z.B.D.: methodology; Z. B.D., X.C.: writing – original draft preparation; X.C., Y.J.L.: writing – review and editing; X.C., Y.J.L., Y.H.W.: formal analysis and investigation; X.C., Z.B.D.: data collection and analysis.

Conflicts of interest disclosure

The authors declare no conflict of interest.

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None.

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None.

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None.

Data availability statement

The original contributions presented in the study are included in the article, and further inquiries can be directed to the corresponding author.

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