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ORIGINAL ARTICLE

Metformin may reduce bladder cancer risk in Taiwanese patients with type 2 diabetes

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Abstract Whether metformin therapy affects bladder cancer risk in patients with type 2 diabetes mellitus (T2DM) has not been extensively investigated. The reimbursement databases of all Taiwanese patients with a new diagnosis of T2DM between 1998 and 2002 ($n = 940{,}708$) were retrieved from the National Health Insurance for follow-up of bladder cancer up to the end of 2009. Metformin was treated as a time-dependent variable, and of these patients, 532,519 were never-users and 408,189 were ever-users of metformin. A time-dependent approach was applied in the calculation of bladder cancer incidence and in the estimation of hazard ratios by Cox regression for ever-users, never-users, and subgroups of metformin exposure (using tertile cutoffs of cumulative duration of therapy and cumulative dose). During the study period, 1,847 (0.45 %) metformin ever-users and 6,213 (1.17 %) metformin never-users developed bladder cancer, representing an incidence of 72.03 and 189.22 per 100,000 person-years, respectively. The age-sex-adjusted and multivariable-adjusted hazard ratios (95 % confidence intervals) for ever- versus never-users were 0.382 (0.360–0.405) and 0.600 (0.564–0.638), respectively. The multivariable-adjusted hazard ratios for the first, second, and third tertiles of cumulative duration of metformin therapy were 1.034 (0.954–1.120), 0.696 (0.632–0.766), and 0.258 (0.229–0.291), respectively (P trend <0.0001). Similarly, the multivariable-adjusted hazard ratios for the first, second, and third tertiles of cumulative dose of metformin were 0.997 (0.920–1.080), 0.615 (0.559–0.677), and 0.285 (0.253–0.321), respectively (P trend <0.0001). This study suggests that metformin use is associated with a decreased risk of bladder cancer in patients with T2DM.

Keywords Bladder cancer · Diabetes · Epidemiology · Metformin · Taiwan

Introduction

Patients with type 2 diabetes mellitus (T2DM) have a significantly higher risk of various types of cancer [1–9], including bladder cancer [10–12]. For instance, among Taiwanese patients, the risk of bladder cancer is approximately 50 % higher in those with T2DM than among non-diabetics [10]. Smoking is the most important risk factor for bladder cancer in the general population, but exposure to aromatic amine or arsenic and chronic infection with *Schistosoma hematobium* may be responsible in some specific occupations or geographical regions [13–15]. The increased risk of bladder cancer in patients with T2DM is not yet well characterized, but in addition to the well-recognized risk factors as mentioned above, benign prostatic hyperplasia (in diabetic men), nephropathy and urinary

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tract diseases such as stones or infection may increase the risk by twofold to 2.5-fold in the Taiwanese patients with T2DM [10, 16]. Recent studies suggested that the use of anti-diabetic drugs may also affect the risk of bladder cancer in patients with T2DM. For example, pioglitazone use may increase the risk in some population-based observational studies [17–19], and a recently published paper suggests a joint effect of smoking and insulin use in predicting bladder cancer mortality in a large prospective cohort of Taiwanese patients with T2DM [20].

Metformin is an oral anti-diabetic agent that has been used to treat hyperglycemia in patients with T2DM for several decades. The United Kingdom Prospective Diabetes Study has demonstrated that patients with T2DM treated with metformin may have a lower risk of developing microvascular and macrovascular complications [21]. Recent observational studies suggested that patients with T2DM using metformin may also have a lower risk of developing cancer, including those affecting the colon [22–24], breast [25, 26], prostate [27, 28], and pancreas [23]. A series of studies conducted in Taiwan also suggested that metformin use in patients with T2DM is associated with a lower risk of all cancers [29], and specifically, colorectal [22, 30], lung [30, 31], liver [32, 33], pancreatic [32], and breast cancer [30].

To our knowledge, whether metformin use has an impact on the risk of bladder cancer in patients with T2DM has not been extensively investigated. A recent meta-analysis by Noto et al. [34] showed a pooled risk ratio for bladder cancer (95 % confidence interval) of 0.94 (0.64–1.38) in association with metformin treatment. This pooled risk ratio was derived from two clinical trials [35, 36] and one population-based observational cohort study [10]. The two clinical trials [35, 36] included in the meta-analysis were limited by a small number of bladder cancer cases, meanwhile the observational study [10] did not evaluate a dose–response relationship between metformin and bladder cancer.

Although randomized controlled trials are best for controlling confounders (either known or not yet clarified) in evaluating the efficacy of metformin on bladder cancer, it may not be feasible in the near future because of the very high cost and the long duration required for investigating cancer risk which has low incidence in the general population [37]. The risk of all cancers is only approximately 20 per 1,000 per year in the population aged around 70 [37], and the incidence of bladder cancer in patients with T2DM in different ethnicities is generally below 1 per 1,000 patients per year [19, 37]. Therefore, a further clarification of the use of metformin and bladder cancer risk in patients with T2DM may rely on a well-designed observational study with a sufficient sample size for evaluating a dose–response relationship.

The present study sought to further evaluate whether metformin therapy in patients with T2DM would affect the risk of bladder cancer. Using Taiwan's National Health Insurance (NHI) reimbursement databases, the present retrospective study analyzed data from a cohort of patients who were newly diagnosed with T2DM between 1998 and 2002 and followed up to the end of 2009. Specifically, we used a new-user design and a time-dependent approach for metformin use in data analyses in order to minimize the potential confounding and biases commonly seen in observational studies using administrative database analyses. Additionally, because a previous Hong Kong study showed that the use of sulfonylureas in the Chinese population with T2DM was associated with a lower risk of cancer and an interaction with metformin could be observed [38], the present study also evaluated the potential interaction between metformin and sulfonylureas on bladder cancer risk.

Materials and methods

The planned analysis of the reimbursement databases of all patients with a diagnosis of T2DM during the period from 1996 to 2009 was approved by the ethic review board of the National Health Research Institutes (registered approval number: 99274).

Since March 1995, a compulsory and universal system of NHI was implemented in Taiwan. According to this system, all contracted medical institutes must submit computerized and standard claim documents for reimbursement. More than 99 % of citizens are enrolled in the NHI, and over 98 % of the hospitals nationwide are under contract with the NHI. The average number of annual physician visits in Taiwan is one of the highest around the world, at approximately 15 visits per year per capita in 2009.

The National Health Research Institutes is the only organization approved, as per local regulations, for handling the NHI reimbursement databases for academic research. The databases contain detailed records of every visit for each patient, including outpatient visits, emergency department visits, and hospital admission. The databases also include principal and secondary diagnostic codes, prescription orders, and claimed expenses.

The identification information of the individuals was scrambled for the protection of privacy. Diabetes was coded as 250.1–250.9 and bladder cancer as 188, based on the *International Classification of Diseases, Ninth Revision, Clinical Modification* (ICD-9-CM).

To create a cohort of patients newly diagnosed with T2DM within the period of 1998–2002, patients with a previous diagnosis of diabetes at outpatient clinics before

January 1, 1998, in the available NHI databases were first excluded. The data of all patients with a first diagnosis of diabetes and/or an initiation of therapy with either oral anti-diabetic agents or insulin during the period of 1998–2002 were then accessed ($n = 971,224$). After further excluding patients with type 1 diabetes ($n = 2,416$), those with a diagnosis of bladder cancer before the diagnosis of diabetes ($n = 2,102$), those with a duplicated identification number ($n = 72$), unclear information on date of birth or sex ($n = 7,463$), or a follow-up duration of less than 6 months ($n = 23,296$), a total of 940,708 patients with a diagnosis of new onset of T2DM during 1998–2002 were identified.

Patients who had ever been prescribed metformin after entry were defined as ever-users ($n = 408,189$, 43.4 %); never-users ($n = 532,519$, 56.6 %) were defined as those who had never been prescribed metformin. Cumulative duration (months) and cumulative dose (mg) of metformin use were calculated from the reimbursement databases. To evaluate a potential dose–response relationship between metformin and bladder cancer, tertiles of cumulative metformin duration and dose were used for analyses. Exposure to other oral anti-diabetic drugs (sulfonylurea, acarbose, pioglitazone, and rosiglitazone) and insulin was also similarly defined.

A number of comorbidities and covariates were determined as a status/diagnosis at the time of entry, including nephropathy (ICD-9-CM code: 580–589), hypertension (401–405), chronic obstructive pulmonary disease (a surrogate for smoking; 490–496), stroke (430–438), ischemic heart disease (410–414), peripheral arterial disease (250.7, 785.4, 443.81, and 440–448), eye disease (250.5, 362.0, 369, 366.41, and 365.44), obesity (278), dyslipidemia (272.0–272.4), urinary tract disease (590–599), benign prostatic hyperplasia (in men only; 600), and other cancers (140–208; excluding 188). Other medications included statins, fibrate, angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker, calcium channel blocker, aspirin, ticlopidine, clopidogrel, dipyridamole, and nonsteroidal anti-inflammatory drugs.

Follow-up started on the first day of diabetes diagnosis and ended on December 31, 2009, at the time of a new diagnosis of bladder cancer, or at the date of the last reimbursement record. Exposure to metformin was treated as a time-dependent variable. Therefore, the metformin ever-users contributed person-years to the non-metformin group until they started using metformin, and after starting metformin, to the metformin group.

The baseline characteristics of metformin never-users and ever-users were compared by chi-square test. The crude incidence density of bladder cancer was calculated for metformin ever-users and never-users and for the different exposure subgroups. The numerator for the incidence was the number of patients with incident bladder cancer during follow-up, and the denominator was the person-years of follow-up. Time-dependent Cox proportional hazards regression was performed to estimate the hazard ratios for bladder cancer among metformin ever-users versus never-users, and for the various dose–response parameter subgroups. The following models were created: (1) adjusted for age and sex and (2) adjusted for all variables compared previously as baseline characteristics between ever-users and never-users (fully adjusted). Age was adjusted for as a continuous variable in the models.

To take full advantage of the large sample size of the study, a further model was created to evaluate the potential interaction between metformin and sulfonylureas on bladder cancer risk by stratifying the patients into one of the following four groups: (1) patients not using metformin or sulfonylureas (referent group), (2) patients using metformin but not using sulfonylureas, (3) patients using sulfonylureas but not using metformin, and (4) patients using both drugs. The combined use of metformin and sulfonylureas was defined as a concurrent prescription of a sulfonylurea within 6 months of metformin use. Two-sided P value for the interaction between metformin and sulfonylureas was assessed with the Wald test of the cross-product term of metformin and sulfonylureas in a model that included the two separate variables of metformin and sulfonylureas together with their cross-product term. In these models, all baseline characteristics were adjusted.

Analyses were conducted using SAS statistical software, version 9.3 (SAS Institute, Cary, NC, USA). $P < 0.05$ was considered statistically significant.

Results

Table 1 summarizes the baseline characteristics and the incidence of bladder cancer among metformin ever-users ($n = 408,189$) and never-users ($n = 532,519$). A smaller proportion of metformin ever-users versus never-users developed bladder cancer during follow-up (0.45 vs. 1.17 %). With the exception of rates of aspirin and clopidogrel use, all baseline characteristics differed significantly between the two groups. Metformin ever-users were characterized by a smaller proportion of patients aged $\geq$70 years, higher proportion of males, lower frequency of most comorbidities (except hypertension, eye disease, and obesity), lower frequency of other cancers, and higher rates of using other medications (except ticlopidine and nonsteroidal anti-inflammatory drugs).

Table 2 lists bladder cancer incidence among metformin ever-users and never-users, and among the different tertiles of the dose–response parameters for metformin exposure. The incidences of bladder cancer among metformin

Table 1 Baseline characteristics and incident cases of bladder cancer in metformin never-users and ever-users

Variables	Use of metformin				*P* value
	Never-users		Ever-users		
	n	(%)	*n*	(%)	
N	532,519		408,189		
Incident cases of bladder cancer	6,213	1.17	1,847	0.45	<0.0001
Age at entry (years)					
<40	94,357	17.72	43,303	10.61	<0.0001
40–49	98,987	18.59	101,944	24.97	
50–59	102,612	19.27	112,708	27.61	
60–69	109,822	20.62	95,757	23.46	
≥70	126,741	23.80	54,477	13.35	
Sex (men)	246,505	46.29	214,484	52.55	<0.0001
Hypertension	70,418	13.22	67,442	16.52	<0.0001
Chronic obstructive pulmonary disease	19,944	3.75	9,134	2.24	<0.0001
Stroke	18,166	3.41	10,168	2.49	<0.0001
Nephropathy	24,589	4.62	7,285	1.78	<0.0001
Ischemic heart disease	18,482	3.47	13,478	3.30	<0.0001
Peripheral arterial disease	7,015	1.32	4,763	1.17	<0.0001
Eye disease	1,571	0.30	1,591	0.39	<0.0001
Obesity	1,545	0.29	1,952	0.48	<0.0001
Dyslipidemia	76,466	14.36	50,351	12.34	<0.0001
Urinary tract disease	24,797	4.66	18,499	4.53	0.0043
Benign prostatic hyperplasia (men only)	6,148	2.49	3,990	1.86	<0.0001
Other cancer	37,150	6.98	14,752	3.61	<0.0001
Statin	4,912	0.92	7,278	1.78	<0.0001
Fibrate	5,535	1.04	13,005	3.19	<0.0001
Angiotensin-converting enzyme inhibitor/ angiotensin receptor blocker	22,607	4.25	30,028	7.36	<0.0001
Calcium channel blocker	23,745	4.46	23,530	5.76	0.0129
Sulfonylurea	70,251	13.19	372,380	91.23	<0.0001
Insulin	12,237	2.30	97,064	23.78	<0.0001
Acarbose	6,820	1.28	117,749	28.85	<0.0001
Pioglitazone	1,990	0.37	73,002	17.88	<0.0001
Rosiglitazone	2,833	0.53	80,566	19.74	<0.0001
Aspirin	14,441	2.71	14,486	3.55	0.5452
Ticlopidine	899	0.17	512	0.13	<0.0,001
Clopidogrel	72	0.01	58	0.01	0.7783
Dipyridamole	12,476	2.34	10,494	2.57	<0.0001
Non-steroidal anti-inflammatory drugs (excluding aspirin)	92,901	17.45	60,547	14.83	<0.0001

never-users and ever-users were 189.22 and 72.03 per 100,000 person-years, respectively. In the models evaluating the overall hazard ratios for metformin ever-users versus never-users and in the models evaluating the dose–response exposure to metformin, all analyses showed a significantly lower risk of bladder cancer among metformin users, with the exception of the first tertiles of cumulative duration and cumulative dose in the fully adjusted models. All the *P* trends suggested a dose–response relationship.

The hazard ratios for bladder cancer for patients who had used metformin only, patients who had used sulfonylureas only, and patients who had used both drugs in comparison with a referent group who had neither used metformin nor sulfonylureas are shown in Table 3. Users of metformin only and users of sulfonylureas only were both associated with a significantly lower risk of bladder cancer with respective hazard ratios (95 % confidence interval) of 0.662 (0.562–0.779) and 0.489 (0.458–0.522). Users of sulfonylureas only seemed to have a lower risk of bladder cancer than users of metformin only. Furthermore, an even lower risk was observed for users of both metformin and sulfonylureas [hazard ratio (95 % confidence interval) 0.460 (0.430–0.492)], although the confidence interval for these patients was overlapping with that for patients using sulfonylureas only. The *P* values for trend and for the interaction term of metformin and sulfonylureas were both significant ($P < 0.0001$).

Discussion

This is the first population-based study aimed at specifically evaluating whether the use of metformin in patients with T2DM impacts the risk of bladder cancer. The findings consistently suggested a significantly lower risk of bladder cancer among metformin users than nonusers, even after multivariable adjustment (Table 2) or considering the possible interaction with sulfonylureas (Table 3).

Metformin has been demonstrated to exert anticancer effects on several types of cancer, including those affecting the liver, colon, breast, pancreas, and prostate [22, 30, 34]. Several randomized clinical trials are ongoing in evaluating the effects of metformin together with other chemotherapeutic agents on the treatment of some solid tumors including breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, and prostate cancer [39, 40]. However, the utility of metformin in the prevention and treatment of bladder cancer has not been specifically investigated. The present study provided a strong rationale for further in-depth investigation, including randomized controlled trials, of the potential effect of metformin on the prevention and treatment of bladder cancer.

While the mechanisms underlying the effect of metformin on bladder cancer are under-explored, these may involve AMP-activated protein kinase (AMPK)-dependent and AMPK-independent pathways [40, 41]. The activation

Table 2 Exposure to metformin and risk of bladder cancer

Exposure to metformin	Cases followed	Cases with incident bladder cancer	Incidence rate (per 100,000 person-years)	Model 1			Model 2		
				HR	95 % CI	*P* value	HR	95 % CI	*P* value
Never-users	866,981	6,213	189.22	1.000			1.000		
Ever-users	408,189	1,847	72.03	0.382	(0.360, 0.405)	<0.0001	0.600	(0.564–0.638)	<0.0001
Cumulative duration (months)									
<14.13	134,573	946	160.83	0.841	(0.777–0.910)	<0.0001	1.034	(0.954–1.120)	0.4196
14.13–45.17	134,881	591	72.68	0.445	(0.406–0.489)	<0.0001	0.696	(0.632–0.766)	<0.0001
>45.17	138,735	310	26.66	0.152	(0.136–0.171)	<0.0001	0.258	(0.229–0.291)	<0.0001
P trend						<0.0001			<0.0001
Cumulative dose (mg)									
<406,000	134,511	941	160.11	0.826	(0.763–0.894)	<0.0001	0.997	(0.920–1.080)	0.9413
406,000–1,525,000	134,886	587	71.40	0.419	(0.381–0.460)	<0.0001	0.615	(0.559–0.677)	<0.0001
>1,525,000	138,792	319	27.64	0.160	(0.143–0.180)	<0.0001	0.285	(0.253–0.321)	<0.0001
P trend						<0.0001			<0.0001

Referent group: metformin never-users

HR hazard ratio, *CI* confidence interval

Model 1 adjusted for age and sex

Model 2 adjusted for all variables in Table 1

Table 3 Interaction between metformin and sulfonylureas on bladder cancer risk

Metformin	Sulfonylureas	Cases of bladder cancer	Total case number	Hazard ratio (95 % confidence interval)
No	No	5,164	484,782	1.000
Yes	No	84	7,179	0.662 (0.562–0.779)
No	Yes	2,439	382,199	0.489 (0.458–0.522)
Yes	Yes	373	66,548	0.460 (0.430–0.492)
				P trend <0.0001
				P interaction <0.0001

Models are adjusted for all covariates in Table 1

of AMPK is indirect and appears to act via inhibition of mitochondrial complex I in the respiratory chain resulting in an increase in the AMP:ATP ratio [40]. Metformin may inhibit cell cycle progression and cell proliferation through down-regulating cyclin D1 as an AMPK-dependent effect in breast cancer cells, or activating HIF target gene REDD1 leading to the inhibition of the mammalian target of rapamycin (mTOR) pathway and cell cycle arrest in prostate cancer cells [40]. Furthermore, metformin inhibits hepatic glucose output and improves insulin sensitivity with lowering of circulating levels of insulin and glucose, reduces insulin-like growth factors (IGFs), decreases Akt phosphorylation, and inhibits the crosstalk between receptors of insulin/IGF1 and G protein-coupled receptor signaling pathways [40]. It has been recently demonstrated that activation of AMPK with a resulting mTOR inhibition by ursolic acid [42] or by Rhodiola rosea extracts and salidroside [43] in bladder cancer cell lines can inhibit the growth and induce apoptosis of the cancer cells. Furthermore, it is well established that IGF receptors and binding proteins are overexpressed in bladder cancer cells and may play crucial roles in its development and clinical invasiveness [44–47]. Although it is reasonable to speculate that metformin may prevent the development of bladder cancer through these molecular mechanisms, clearly, more basic in vitro and in vivo studies are warranted.

Metformin is generally recommended as the first-line therapy for patients with T2DM because of its low cost, relative safety, and beneficial effects on microvascular and macrovascular complications [48]. Taiwanese physicians generally follow such international guidelines. However, if patients have a relatively high glucose level at time of diabetes diagnosis, if metformin is contraindicated (e.g., patients with chronic kidney disease with deteriorating renal function, indicating a high risk of lactic acidosis), or if metformin monotherapy is unlikely to adequately control the glucose level, sulfonylureas are always considered for use in combination therapy with metformin. Metformin is therefore usually initiated at an early stage of T2DM, but may be used in combination with other oral anti-diabetic drugs (usually sulfonylureas) or insulin at a late stage of the disease. It is less used in patients with deteriorating renal function or in patients with organ failure when the risk of lactic acidosis is considered high. On the other hand, although insulin is necessary for patients with type 1 diabetes mellitus, it is only used in approximately 12 % of the

patients with T2DM in Taiwan (Table 1) [49]. The lower rate of insulin use in patients with T2DM in Taiwan could be due to the requirement of injection for its administration, which is not easily accepted by the patients. Therefore, insulin is generally the last resort for glycemic control and its use may indicate a prolonged period of uncontrollable hyperglycemia under available oral anti-diabetic drugs. For these reasons, the existence of indication bias in the study can not be completely excluded.

If prevalent cases of diabetes had been used in the study, it is possible that metformin users may have represented those who survive with a less severe clinical disease and the propensity to develop cancer in these patients may not be similar to nonusers of metformin. Because the present study included only incident cases of T2DM and new users of metformin, such a potential "prevalent user bias" [50–52] should have been minimized.

Diabetes [10–12] as well as the use of thiazolidinediones, especially pioglitazone [6, 53], may both increase the risk of bladder cancer. Because a much higher proportion of metformin users also used pioglitazone (Table 1), confounding by pioglitazone use is unlikely to explain a decreased bladder cancer risk in metformin users. Insulin use predicts bladder cancer mortality in patients with T2DM in a cohort study in Taiwan [20]. For similar reason, the potential link between the beneficial effect of metformin on bladder cancer risk in the present study can not be ascribed to a confounding effect of insulin use because a higher rate of insulin use was observed in the ever-users of metformin (Table 1). Furthermore, the uses of pioglitazone and insulin have also been adjusted for in the full models (Table 2).

Nephropathy, urinary tract disease, and benign prostatic hyperplasia have also been identified as important risk factors for bladder cancer in the Taiwanese diabetic patients [10, 16]. It was observed that metformin users had slightly lower rates of these comorbidities at baseline (Table 1). Because these factors only differed to a small extent between ever-users and never-users of metformin and all of them were considered for adjustment in the full models (Table 2), the possible residual confounding from these risk factors should be trivial.

Smoking is an important risk factor for bladder cancer in the general population [13, 14]. It is not known whether smoking could be a stronger risk factor for bladder cancer in diabetic versus non-diabetic patients. Even though a surrogate of smoking (chronic obstructive pulmonary disease) has been considered for adjustment in the fully adjusted models (Table 2), such a confounding effect of smoking could not be completely excluded in the present study.

Most of the other baseline characteristics also differed significantly between metformin never-users and ever-users (Table 1), thereby suggesting confounding related to these factors or a potential bias related to indication. The confounding effects can be reduced by adjusting for the baseline characteristics in the full models (Table 2). On the other hand, indication bias is probably minimal because the hazard ratios are similar in secondary analyses when the models are either created after adjustment for propensity scores derived from these baseline characteristics or after adjusting for these characteristics redefined at the end of follow-up (data not shown). However, it should be stressed that residual confounding could not be excluded because other anti-diabetic medications and age that are associated with cancer are also very differently distributed (Table 1).

The findings of the present study conducted in the Chinese patients with T2DM in Taiwan (Table 3) were consistent with another earlier study conducted in the Chinese population with T2DM in Hong Kong, which showed a lower risk of cancer associated with the use of sulfonylureas and an interaction between metformin and sulfonylureas [38]. It has been shown in early in vitro studies that sulfonylurea receptor is expressed in the urinary bladder [54, 55] and that administration of a sulfonylurea (i.e., glibenclamide) significantly inhibits the proliferation and growth of bladder cancer cells [56]. By inhibiting the opening of the K_{ATP} channel, sulfonylureas can inhibit protein accumulation and reduce the percentage of cells in the S phase of the cell cycle in human bladder cancer cells [56]. Therefore, the findings of the present study are supportive for a protective effect of sulfonylureas on the development of bladder cancer as observed in the early in vitro study [56]. Such a protection of sulfonylureas on bladder cancer risk is probably a class effect because the findings did not remarkably change when specific drugs (i.e., glibenclamide, glipizide, gliclazide, glimepiride, and gliquidone) in the sulfonylurea class were analyzed separately (data not shown). However, because the risk of bladder cancer associated with the use of sulfonylureas has not been extensively investigated in other ethnicities, the findings of the present study should be viewed as preliminary. Future studies are required to confirm the lower risk of bladder cancer associated with sulfonylureas by treating each specific sulfonylurea as a time-dependent variable and analyzing a dose–response relationship. Whether the protective effect of sulfonylureas was specific to bladder cancer and could not be extended to other types of cancer is worthy of further investigation.

The interactions between metformin use and other anti-diabetic drugs were not investigated in the present study because it was recognized that at the inception of the study, only metformin, sulfonylureas, acarbose, and human insulin were available for the treatment of hyperglycemia in Taiwan. However, the reimbursement of acarbose had been restricted by the NHI for patients who remained to have an elevated fasting plasma glucose level $\geq$200 mg/dL after the use of maximum doses of both metformin and

sulfonylureas in Taiwan. This restriction was not extinguished until after July 1, 2002. With regards to insulin, it is always used in a late stage in the Taiwanese patients with T2DM as discussed earlier. In Taiwan, rosiglitazone and pioglitazone were not available until after 2001 and 2002, respectively, and the reimbursement for both of them were also restricted to patients who failed on other medications as regulated by the NHI. Therefore, the effects of other types of anti-diabetic medications might not be independent of the use of metformin and/or sulfonylureas because there would be a significant period of time between the use of metformin and/or sulfonylureas and the other anti-diabetic medications, allowing sufficient time for the impact of metformin and/or sulfonylureas to occur.

Because the databases were derived from the whole population without a sampling procedure and they spanned the whole period from the earliest available databases of the NHI since 1996, there was no concern of potential selection bias related to sampling error. Although misclassification of bladder cancer might occur, such a probability was low because labeled diagnoses should be printed on all prescriptions handed to the patients. Mislabeling of a cancer diagnosis would undoubtedly be unacceptable to patients and thus would be promptly corrected.

This study has several strengths. The databases included all claim records on outpatient visits, emergency department visits, and hospital admission. We derived the diagnoses of bladder cancer from all these sources. Cancer is considered a severe morbidity by the NHI, and most medical co-payments can be waived. Furthermore, there is a low drug cost-sharing required by the NHI and patients from a low-income household, veterans, or patients with prescription refills for chronic disease are exempted from the drug cost-sharing. Therefore, the detection rate of bladder cancer would tend not to differ among different social classes. The use of medical records also reduced the potential bias related to self-reporting.

The study limitations included a lack of actual measurement data for confounders such as obesity, smoking, alcohol drinking, family history, lifestyle, diet, water intake, hair dye use, occupational exposure, and genetic parameters. In addition, we could not consider the potential impact of biochemical data such as levels of glucose, insulin, C-peptide, and IGFs. Another limitation is the lack of information on the pathology, grading, and staging of bladder cancer. Finally, it is worth to stress that the interpretations of the findings of the present study should be cautious because it is an observational study and bias or residual confounding may not be completely excluded.

In summary, the present study suggests that metformin use is associated with a decreased risk of bladder cancer in patients with T2DM. However, a confounding effect of smoking or other factors on this relationship cannot be completely excluded. Confirmation of the findings through additional studies is necessary.

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Conflict of interest None.

Human and Animal Rights The study was conducted according to local regulations.

Informed Consent The identification information of the persons has been scrambled and handled by the National Health Research Institutes. No indentifying details are available in the databases provided for statistical analyses and personal information is not shown in the manuscript.

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