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Metformin use is associated with a lower risk of uterine leiomyoma in female type 2 diabetes patients

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Abstract

Background: Whether metformin may reduce the risk of uterine leiomyoma in type 2 diabetes patients has not been investigated. This retrospective cohort study compared the risk of uterine leiomyoma in ever *versus* never users of metformin.

Methods: Female patients with new-onset type 2 diabetes during 1999–2005 were enrolled from the reimbursement database of Taiwan's National Health Insurance and followed up from 1 January 2006 until 31 December 2011. Analyses were conducted in a propensity score (PS) matched-pair cohort of 10,998 ever users and 10,998 never users of metformin. Hazard ratios were estimated by Cox regression incorporated with the inverse probability of treatment weighting using the PS.

Results: A total of 321 never users and 162 ever users developed uterine leiomyoma during follow up, with respective incidence of 704.65 and 329.82 per 100,000 person-years. The overall hazard ratio was 0.467 (95% confidence interval: 0.387–0.564). The hazard ratios for the first (<23.3 months), second (23.3–53.1 months), and third (>53.1 months) tertiles of cumulative duration were 0.881 (0.685–1.132), 0.485 (0.367–0.642), and 0.198 (0.134–0.291), respectively; and were 0.751 (0.576–0.980), 0.477 (0.360–0.632), and 0.277 (0.198–0.386), respectively, for the first (<655,000 mg), second 655,000–1,725,500 mg), and third (>1,725,500) tertiles of cumulative dose. Sensitivity analyses after excluding users of sulfonylurea, users of estrogen, users of insulin, users of incretin-based therapies during follow up, patients with irregular drug refills, patients who discontinued the use of metformin, patients who received metformin prescription less than four times, or redefining uterine leiomyoma by using 'diagnostic code' plus 'procedure codes' consistently supported a lower risk of uterine leiomyoma in ever users of metformin.

Conclusion: Metformin use is associated with a lower risk of uterine leiomyoma.

Keywords: diabetes mellitus, metformin, Taiwan, uterine leiomyoma

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Introduction

Uterine leiomyoma (UL, also known as uterine fibroids) is the most common benign tumor of the uterus that affects women of reproductive age.[1] Age, premenopausal state, hypertension, family history, time since last birth, and food additive and soybean milk consumption are associated with an increased risk,[1] while use of contraceptives, smoking, and low body mass index and parity are associated with a lower risk.[1–3] Epidemiological studies also showed a potential link between metabolic syndrome and UL.[4,5] In addition, serum adiponectin levels are significantly lower in patients with UL.[6] These findings indicate a possibility of shared pathophysiology of insulin resistance leading to clinical development of metabolic syndrome and UL.

UL seems to affect predominantly black ethnicity than other ethnicities, including White, Hispanic

and Asian.[1] It has been reported that up to 70% of women at the time of menopause may have UL, but most patients are asymptomatic, and only approximately 25% may have clinical symptoms at reproductive age.[1] Heavy menstrual bleeding, fatigue, and painful periods are the most common clinical presentations.[1] Because there is no satisfactory medical treatment for UL, prevention of its occurrence may provide an important strategy to reduce the clinical burden of UL.

Metformin, now a first-line oral antidiabetic drug recommended for the treatment of type 2 diabetes mellitus, exerts an insulin sensitizing effect.[7] The early concern of an increased risk of lactic acidosis associated with metformin use is not supported by a recent meta-analysis that included 347 comparative trials and cohort studies.[8] Metformin can inhibit cell proliferation, and recent observational studies suggested that it can prevent the development of various types of cancer,[9–11] including endometrial cancer.[12] However, Suissa and Azoulay called for an attention to potential immortal time bias that might exist in earlier observational studies that have shown a beneficial effect of metformin on cancer.[13]

Previous *in vitro* studies suggested that metformin treatment of leiomyoma cell lines can inhibit cell proliferation *via* an 5'-adenosine monophosphate-activated protein kinase (AMPK)-dependent pathway, with subsequent inhibition of the mammalian target of rapamycin (mTOR) pathway.[14,15] However, to the best of our knowledge, no previous epidemiological studies have ever investigated whether metformin might reduce the risk of UL in patients with type 2 diabetes mellitus, in either the Asian or non-Asian populations. The present population-based retrospective cohort study investigated such a possible effect in a matched cohort by comparing the risk of UL between ever users and never users of metformin in Taiwanese patients.

Materials and methods

This is a population-based retrospective cohort study that used the reimbursement database of the Taiwan's National Health Insurance (NHI). The NHI, a unique and universal healthcare system covering >99% of the population, has been implemented since March 1995. All hospitals, and nearly 93% of all medical settings, have contracts with the Bureau of the NHI. All reimbursement records of disease diagnoses, medication prescriptions, and clinical procedures are kept by the Bureau of the NHI. The database can be used for academic research if approved after ethics review. The present study was granted number 99274 by the Ethics Committee of the National Health Research Institutes. According to local regulations, the National Health Research Institutes deidentified the individuals in the database for the protection of privacy, and the Ethics Committee approved the analyses of the database without the requirement to obtain informed consent from the participants.

The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) was used for disease diagnoses during the study period. Diabetes was coded 250.XX and UL 218.

The database was described in detail in a previously published paper.[9] The present study enrolled a propensity score (PS)-matched cohort following the procedures in Figure 1. At first, 423,949 patients who were newly diagnosed as having diabetes mellitus during 1999–2005 in the outpatient clinics, and who had received two or more times of prescriptions of an antidiabetic drug, or multiple antidiabetic drugs, were identified. The following patients were then excluded: ever users of metformin who had been prescribed other antidiabetic drugs before metformin was initiated ($n = 183,837$); men ($n = 127,145$); patients who held a 'Severe Morbidity Card' as having type 1 diabetes mellitus ($n = 1064$, in Taiwan, patients with type 1 diabetes mellitus were issued a 'Severe Morbidity Card' after certified diagnosis, and many of the copayments are waived); missing data ($n = 172$); diagnosis of UL before entry, or within 6 months of diabetes diagnosis ($n = 10,238$); and follow up for <180 days ($n = 7720$). As a result, 82,724 ever users and 11,049 never users of metformin were identified (unmatched original cohort). PS was created from all characteristics listed in Table 1 plus the date of entry by logistic regression. A matched-pairs cohort of 10,998 ever users and 10,998 never users (matched cohort) was then created by matching the PS based on the Greedy 8→1 digit match algorithm, as detailed elsewhere.[10,16]

The start of follow up was set as 1 January 2006, and all comorbidities and covariates were determined as a status/diagnosis at any time before

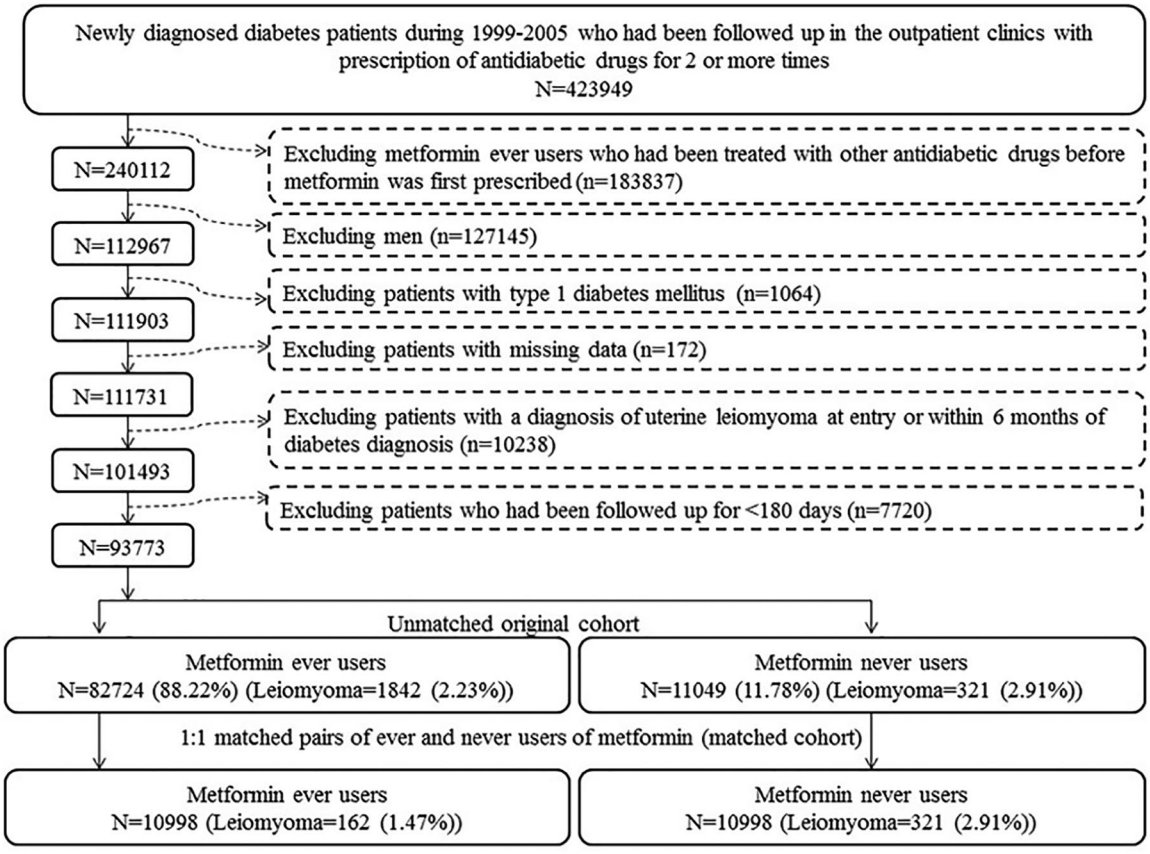

Figure 1. Flowchart showing the procedures in creating a cohort of 1:1 matched-pairs of metformin ever, and never, users from the reimbursement database of the Taiwan's National Health Insurance.

cohort entry. Potential confounders included: demographic data: age, diabetes duration, occupation, and living region; major comorbidities: hypertension, dyslipidemia, and obesity; diabetes-related complications: nephropathy, eye diseases, stroke, ischemic heart disease, and peripheral arterial disease; antidiabetic drugs: insulin, sulfonylurea, meglitinide, acarbose, and thiazolidinediones (rosiglitazone and pioglitazone, respectively); commonly encountered comorbidities: chronic obstructive pulmonary disease (a surrogate for smoking), tobacco abuse, alcohol-related diagnoses, and cancer; commonly used medications in diabetes patients or medications that are potential confounders: angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, calcium channel blocker, statin, fibrate, aspirin, and estrogen; and potential detection examination: receiving Pap smear screening for cervical cancer. The classifications of living region and occupation were detailed previously.[11] In brief, the living region was classified as Taipei, Northern, Central, Southern, and Kao-Ping/Eastern. Occupation was classified as class I (civil servants, teachers, employees of governmental or private businesses, professionals, and technicians), class II (people without a specific employer, self-employed people, or seamen), class III (farmers or fishermen), and class IV (low-income families supported by social welfare, or veterans). The ICD-9-CM codes for the above diagnoses were: hypertension (401–405), dyslipidemia (272.0–272.4), obesity (278), nephropathy (580–589), eye diseases (250.5: diabetes with ophthalmic manifestations, 362.0: diabetic retinopathy, 369: blindness and low vision, 366.41: diabetic cataract, and 365.44: glaucoma associated with systemic syndromes), stroke (430–438), ischemic heart disease (410–414), peripheral arterial disease (250.7, 785.4, 443.81, and 440–448), chronic obstructive pulmonary disease (490–496), tobacco abuse (305.1, 649.0, and 989.84), alcohol-related diagnoses (291, 303, 535.3, 571.0–571.3, and 980.0), and cancer (140–208). The accuracy of disease diagnoses in the NHI database has been studied previously. Agreements

Table 1. Characteristics in never and ever users of metformin in the unmatched and matched cohorts.

	Unmatched original cohort						Matched cohort					
	Never users (*n* = 11,049)		Ever users (*n* = 82,724)		*p* value	SD	Never users (*n* = 10,998)		Ever users (*n* = 10,998)		*p* value	SD
Variable	***n***	**%**	***n***	**%**			***n***	**%**	***n***	**%**		
Demographic data												
Age (years)[a]	67.92	12.72	62.65	11.93	<0.0001	−52.06	67.86	12.70	67.55	11.41	0.0602	−1.87
Diabetes duration (years)[a]	4.50	2.61	5.16	2.25	<0.0001	31.78	4.51	2.61	4.56	2.21	0.1597	1.80
Occupation												
I	3563	32.25	26,461	31.99	<0.0001		3539	32.18	3632	33.02	0.1349	
II	1911	17.30	17,845	21.57		12.39	1907	17.34	1943	17.67		0.70
III	3226	29.20	22,854	27.63		−3.52	3215	29.23	3221	29.29		0.33
IV	2349	21.26	15,564	18.81		−7.35	2337	21.25	2202	20.02		−2.90
Living region												
Taipei	3690	33.40	25,343	30.64	<0.0001		3669	33.36	3606	32.79	0.9246	
Northern	1173	10.62	9905	11.97		5.29	1169	10.63	1178	10.71		0.38
Central	2008	18.17	15,363	18.57		−0.23	2000	18.19	2009	18.27		0.14
Southern	1957	17.71	14,407	17.42		−0.35	1945	17.69	1977	17.98		0.95
Kao-Ping and Eastern	2221	20.10	17,706	21.40		4.70	2215	20.14	2228	20.26		0.32
Major comorbidities												
Hypertension	9158	82.89	64,033	77.41	<0.0001	−16.92	9112	82.85	9052	82.31	0.2861	−1.21
Dyslipidemia	6827	61.79	57,739	69.80	<0.0001	20.52	6808	61.90	6831	62.11	0.7493	0.52
Obesity	267	2.42	3297	3.99	<0.0001	10.04	267	2.43	255	2.32	0.5950	−0.82
Diabetes-related complications												
Nephropathy	3276	29.65	15,536	18.78	<0.0001	−32.73	3248	29.53	3207	29.16	0.5438	−1.16
Eye diseases	886	8.02	14,068	17.01	<0.0001	29.02	886	8.06	830	7.55	0.1592	−2.49
Stroke	3934	35.61	21,868	26.43	<0.0001	−25.32	3900	35.46	3831	34.83	0.3298	−1.11
Ischemic heart disease	5615	50.82	35,511	42.93	<0.0001	−19.86	5580	50.74	5546	50.43	0.6466	−0.56
Peripheral arterial disease	2228	20.16	16,813	20.32	0.6955	−0.41	2220	20.19	2170	19.73	0.3990	−1.06

(Continued)

Table 1. (Continued)

Variable	Unmatched original cohort				p value	SD	Matched cohort				p value	SD
	Never users (n = 11,049)		Ever users (n = 82,724)				Never users (n = 10,998)		Ever users (n = 10,998)			
	n	%	n	%			n	%	n	%		
Antidiabetic drugs												
Insulin	923	8.35	1779	2.15	<0.0001	−37.06	898	8.17	833	7.57	0.1036	−4.78
Sulfonylurea	7757	70.21	57,657	69.70	0.2754	10.60	7745	70.42	8082	73.49	<0.0001	5.23
Meglitinide	1025	9.28	3296	3.98	<0.0001	−25.10	1010	9.18	1016	9.24	0.8887	−0.32
Acarbose	1303	11.79	4365	5.28	<0.0001	−23.81	1287	11.70	1355	12.32	0.1584	−0.90
Rosiglitazone	350	3.17	3727	4.51	<0.0001	7.92	350	3.18	398	3.62	0.0742	1.10
Pioglitazone	237	2.14	1853	2.24	0.5252	2.92	237	2.15	273	2.48	0.1068	1.05
Commonly encountered comorbidities												
Chronic obstructive pulmonary disease	5619	50.86	37,464	45.29	<0.0001	−15.03	5584	50.77	5547	50.44	0.6178	−0.35
Tobacco abuse	29	0.26	305	0.37	0.0783	2.29	29	0.26	24	0.22	0.4917	−1.05
Alcohol-related diagnoses	215	1.95	1908	2.31	0.0167	3.22	215	1.95	222	2.02	0.7352	0.48
Cancer	1681	15.21	8791	10.63	<0.0001	−17.07	1665	15.14	1608	14.62	0.2802	−1.35
Commonly used medications in diabetes patients or medications that are potential confounders												
Angiotensin-converting enzyme inhibitor/ angiotensin receptor blocker	7544	68.28	54,392	65.75	<0.0001	−7.13	7502	68.21	7408	67.36	0.1750	−1.78
Calcium channel blocker	7562	68.44	50,700	61.29	<0.0001	−17.98	7519	68.37	7478	67.99	0.5528	−0.75
Statin	4688	42.43	40,505	48.96	<0.0001	15.83	4676	42.52	4634	42.13	0.5665	−1.02
Fibrate	3042	27.53	26,466	31.99	<0.0001	11.37	3037	27.61	3012	27.39	0.7058	−0.50
Aspirin	6227	56.36	44,480	53.77	<0.0001	−8.00	6195	56.33	6128	55.72	0.3627	−1.12
Estrogen	4342	39.30	36,197	43.76	<0.0001	12.09	4333	39.40	4482	40.75	0.0404	3.00
Potential detection examination												
Pap smear	4760	43.08	39,508	47.76	<0.0001	11.32	4743	43.13	4729	43.00	0.8488	0.03

[a]Age and diabetes duration are expressed as mean and standard deviation. Refer to 'Materials and Methods' for the classification of occupation.
SD, standardized difference.

between claim data and medical records are moderate to substantial, with Kappa values range from 0.55 to 0.86.[17]

The differences between never users and ever users of metformin were compared by Student's *t* test for age and diabetes duration, and by Chi-square test for other variables. Standardized difference was calculated for each covariate as a test of balance diagnostic. A value >10% was used as an indication of potential confounding from the variable.[18]

Cumulative duration of metformin therapy in months, and cumulative dose of metformin therapy in milligrams, were calculated. Their tertiles were used for dose-response analyses. Incidence of UL was calculated for never users, ever users, and the respective tertiles of cumulative duration and cumulative dose of metformin therapy. Follow up started on 1 January 2006, and all patients were free from a diagnosis of UL at the start of follow up. The numerator of incidence was the case number of new-onset UL observed during follow up. The denominator expressed in person-years was the follow-up duration, which ended on 31 December 2011, at the time of a new-onset UL, or on the date of death or the last reimbursement record.

Cox regression incorporated with the inverse probability of treatment weighting (IPTW) using the PS was used to estimate the hazard ratios and their 95% confidence intervals for ever users, and for each tertile of cumulative duration and cumulative dose in comparison to never users. This method was proposed by Austin to reduce the potential confounding from the differences in characteristics.[19]

To further exclude potential residual confounding from variables that happened to differ between ever and never users of metformin, sensitivity analyses were conducted after excluding variables (one at a time) that differed with p values < 0.05 in the matched cohort (i.e. sulfonylurea and estrogen). Because an earlier study suggested that users of insulin was associated with a lower risk of UL,[20] analysis was also conducted after excluding users of insulin. Additional analyses were conducted after excluding patients who received any two consecutive prescriptions of metformin spanning more than 4 months and 6 months, respectively. Because the Bureau of the NHI allows, at most, 3 months of drug prescriptions for patients at each outpatient visit, these analyses might have excluded most of the patients with poor adherence who did not receive a regular drug refill. Incretin-based therapies were not reimbursed by the NHI until after 2009 in Taiwan; to avoid the potential impact of incretin-based therapies, sensitivity analysis was conducted after excluding patients who happened to receive an incretin-based therapy during follow up. Analyses were also conducted by censoring patients at the time when metformin was last prescribed, and censoring patients at the time when metformin was last prescribed plus the duration of that prescription, respectively. These analyses excluded the follow-up time without metformin use in the calculation of person-years in patients who discontinued the use of metformin among ever users. Models were also created by censoring patients from the time 4 months and 6 months, respectively, have elapsed since the last prescription (Models VII and VIII). To address the concern of defining metformin ever users by including patients who had been treated with metformin for only a short period of time, additional sensitivity analyses were conducted after excluding those who had received metformin prescription less than four times. Furthermore, because the presence of UL might not be accurate using only the ICD-9-CM diagnostic code, analysis was also conducted after redefining UL by using the diagnostic code plus 'procedure codes' of 68.XX. These procedure codes included 68.0 (hysterotomy), 68.1 (diagnostic procedures on uterus and supporting structures), 68.2 (excision or destruction of lesion or tissue of uterus), 68.3 (subtotal abdominal hysterectomy), 68.4 (total abdominal hysterectomy), 68.5 (vaginal hysterectomy), 68.6 (radical abdominal hysterectomy), 68.7 (radical vaginal hysterectomy), 68.8 (pelvic evisceration), and 68.9 (other and unspecified hysterectomy).

To further examine whether the effect of metformin on UL could be consistent by using a different approach, and also to check whether the use of other antidiabetic drugs (i.e. insulin, sulfonylurea, meglitinide, acarbose, rosiglitazone, and pioglitazone) might have an effect on UL, traditional Cox regression models were created by including all covariates as independent variables in the unmatched and matched cohorts, respectively.

Analyses were conducted using SAS statistical software, version 9.3 (SAS Institute, Cary, NC, USA).

Table 2. Incidence of uterine leiomyoma and hazard ratios by metformin exposure.

Metformin use	*n*	*N*	Person-year	Incidence rate (per 100,000 person-years)	HR	95% CI	*p* value
Never users	321	10,998	45,554.68	704.65	1.000		
Ever users	162	10,998	49,118.09	329.82	0.467	(0.387–0.564)	<0.0001
Tertiles of cumulative duration of metformin therapy (months)							
Never users	321	10,998	45,554.68	704.65	1.000		
<23.3	76	3619	11,522.91	659.56	0.881	(0.685–1.132)	0.3216
23.3–53.1	58	3636	16,725.99	346.77	0.485	(0.367–0.642)	<0.0001
>53.1	28	3743	20,869.19	134.17	0.198	(0.134–0.291)	<0.0001
Tertiles of cumulative dose of metformin (mg)							
Never users	321	10,998	45,554.68	704.65	1.000		
<655,000	66	3629	11,753.30	561.54	0.751	(0.576–0.980)	0.0349
655,000–1,725,500	57	3629	16,796.52	339.36	0.477	(0.360–0.632)	<0.0001
>1,725,500	39	3740	20,568.26	189.61	0.277	(0.198–0.386)	<0.0001

CI, confidence interval; HR: hazard ratio, *n*, incident case number of uterine leiomyoma, *N*, case number followed.

Results

Table 1 shows the characteristics in never and ever users of metformin before and after matching. Before matching, all variables (except peripheral arterial disease, sulfonylurea, pioglitazone and tobacco abuse) differed between never and ever users of metformin with p values < 0.05. The values of standardized difference were >10% for age, diabetes duration, occupation, hypertension, dyslipidemia, obesity, nephropathy, eye disease, stroke, ischemic heart disease, insulin, sulfonylurea, meglitinide, acarbose, chronic obstructive pulmonary disease, cancer, calcium channel blocker, statin, fibrate, estrogen, and Pap smear examination. However, after matching, none of the variables had a value of standardized difference >10%, but 2 variables (i.e. sulfonylurea and estrogen use) differed with p values < 0.05.

The median follow-up duration was 5 years in the cohort. Table 2 shows the incidence of UL and the hazard ratios by metformin exposure. The overall hazard ratios indicated a lower risk of UL in metformin users, and the tertile analyses suggested a dose–response relationship.

Table 3 shows the results in the sensitivity analyses after excluding users of sulfonylurea (Model I), estrogen (Model II), and insulin (Model III), respectively, excluding two consecutive prescriptions of metformin spanning more than 4 months and 6 months, respectively (Models IV and V), excluding patients treated with incretin-based therapies during follow-up (Model VI), censoring patients from the time 4 months and 6 months, respectively, elapsed since the last prescription (Models VII and VIII), censoring patients at the time when metformin was last prescribed (Model IX), censoring patients at the time when metformin was last prescribed plus the duration of that prescription (Model X), excluding metformin prescription less than four times in ever users (Model XI) and redefining UL by using 'diagnostic code' plus 'procedure codes' (Model XII). All analyses consistently supported a lower risk of UL associated with metformin use.

In the traditional Cox regression models that examined the independent effects of different antidiabetic drugs, both metformin use and insulin use were consistently associated with a lower

Table 3. Sensitivity analyses.

Model/metformin use	*n*	*N*	Person-year	Incidence rate (per 100,000 person-years)	HR	95% CI	*p* value
Excluding users of sulfonylurea (*n* = 6169)							
Never users	77	3253	12,021.77	640.50	1.000		
Ever users	45	2916	11,968.76	375.98	0.586	(0.406–0.847)	0.0044
Excluding users of estrogen (*n* = 13,181)							
Never users	154	6665	27,719.21	555.57	1.000		
Ever users	91	6516	28,963.28	314.19	0.563	(0.435–0.730)	<0.0001
Excluding users of insulin (*n* = 20,265)							
Never users	304	10,100	42,545.6	714.5	1.000		
Ever users	153	10,165	45,633.3	335.3	0.468	(0.385–0.568)	<0.0001
Excluding two consecutive prescriptions of metformin spanning more than 4 months (*n* = 15,178)							
Never users	321	10,998	45,554.68	704.65	1.000		
Ever users	69	4180	17,079.04	404.00	0.568	(0.438–0.737)	<0.0001
Excluding two consecutive prescriptions of metformin spanning more than 6 months (*n* = 16,313)							
Never users	321	10,998	45,554.68	704.65	1.000		
Ever users	88	5315	22,567.91	389.93	0.551	(0.435–0.698)	<0.0001
Excluding patients treated with incretin-based therapies during follow-up (*n* = 19,142)							
Never users	317	10,365	42,808.66	740.50	1.000		
Ever users	153	8777	38,102.50	401.55	0.540	(0.445–0.655)	<0.0001
Censoring patients from the time 4 months have elapsed since the last prescription (*n* = 21,996)							
Never users	254	10,998	40,427.20	628.29	1.000		
Ever users	143	10,998	45,989.82	310.94	0.492	(0.400–0.603)	<0.0001
Censoring patients from the time 6 months have elapsed since the last prescription (*n* = 21,996)							
Never users	279	10,998	41,973.43	664.71	1.000		
Ever users	149	10,998	46,780.10	318.51	0.477	(0.391–0.582)	<0.0001
Censoring patients at the time when metformin was last prescribed (*n* = 21,996)							
Never users	321	10,998	45,554.68	704.65	1.000		
Ever users	138	10,998	41,481.07	332.68	0.470	(0.385–0.574)	<0.0001
Censoring patients at the time when metformin was last prescribed plus the duration of that prescription (*n* = 21,996)							
Never users	321	10,998	45,554.68	704.65	1.000		
Ever users	139	10,998	42,550.89	326.67	0.462	(0.379–0.564)	<0.0001

(Continued)

Table 3. (Continued)

Model/metformin use	*n*	*N*	Person-year	Incidence rate (per 100,000 person-years)	HR	95% CI	*p* value
Excluding metformin prescription less than four times in ever users (*n* = 21,203)							
Never users	321	10,998	45,554.68	704.65	1.000		
Ever users	155	10,205	46,735.45	331.65	0.470	(0.388–0.570)	<0.0001
Redefining uterine leiomyoma by using 'diagnostic code' plus 'procedure codes' (*n* = 21,996)							
Never users	66	10,998	45,554.68	144.88	1.000		
Ever users	32	10,998	49,118.09	65.15	0.447	(0.293–0.681)	0.0002

CI, confidence interval; HR, hazard ratio; *n*, incident case number of uterine leiomyoma, *N*, case number followed.

risk of UL and all the other antidiabetic drugs (i.e. sulfonylurea, meglitinide, acarbose, rosiglitazone, and pioglitazone) showed a neutral association. The hazard ratios for metformin use and insulin use were 0.49 (95% confidence interval: 0.43–0.55) and 0.55 (0.42–0.71), respectively, in the unmatched cohort; and were 0.50 (0.42–0.61) and 0.39 (0.25–0.63), respectively, in the matched cohort.

Discussion

The is the first population-based observational study showing a reduced risk of UL associated with metformin use in patients with type 2 diabetes mellitus in a dose-response pattern (Tables 2 and 3).

The mechanisms of the reduced risk of UL associated with metformin use requires further investigation, but some biological actions of metformin help explain such a beneficial effect. Metformin inhibits the mitochondrial respiratory-chain complex 1, leading to activation of the liver kinase B1/AMPK pathway, which, in turn, inhibits gluconeogenesis in the liver and lowers blood glucose.[21] Previous *in vitro* studies suggested that metformin treatment to leiomyoma cell lines can inhibit cell proliferation *via* an AMPK-dependent pathway, with subsequent inhibition of the mTOR pathway.[14,15] Besides, metformin improves insulin resistance by increasing the expression of insulin receptor and activation of tyrosine kinase.[7] Gut microbiota dysbiosis plays a role in the development of metabolic syndrome,[22] and metformin changes the composition of gut microbiota with an increase in *Akkermansia* species, which is responsible for the improvement in insulin resistance and reduction in tissue inflammation.[23] UL is characterized by inflammation, increased fibrosis with accumulation of extracellular matrix, and high expression of nuclear factor κB and tumor necrosis factor-α.[24–27] Metformin attenuates inflammation not only through improving hyperglycemia, insulin resistance, and dyslipidemia, but also through a direct anti-inflammatory action *via* AMPK-dependent and AMPK-independent pathways, resulting in a suppression of nuclear factor κB and tumor necrosis factor-α.[28,29]

A recent Korean study showed a strong association between metabolic syndrome and UL, and patients with hyperglycemia had a significantly 45% higher risk of UL after multivariate adjustment.[5] This suggested a close link between insulin resistance and UL. However, it should also be pointed out that some studies conducted in the USA (mainly in European Americans and African Americans) have shown that patients with type 2 diabetes mellitus might have a lower risk of UL.[20,30] It remains unknown whether the pathogenesis of UL might differ among different ethnicities because the inverse association between diabetes mellitus and UL, and between insulin use and UL, was observed mainly in European Americans.[20] It is worth pointing out that the US studies might have suffered from some methodological limitations, including cross-sectional design and small sample sizes of UL cases. Because metformin is always considered the

first-line treatment for type 2 diabetes mellitus, it was highly possible that patients who had been treated with metformin at the early stage of diabetes, but who no longer used the drug at the time of their enrollment into the study, would have been misclassified as nonusers in these previous studies, leading to the now commonly cited 'prevalent user bias'. The lower risk of UL associated with inulin use observed in the present study was consistent with the previous US study.[20] However, it remains unknown whether the beneficial effect associated with insulin use could be ascribed to the effect of insulin *per se*, or the use of insulin was only a surrogate for more severe forms of diabetes. Indeed, more indepth investigations are required to clarify the effects of other antidiabetic drugs.

Methodological limitations commonly seen in most pharmaco-epidemiological studies such as selection bias, prevalent user bias, immortal time bias, and confounding by indication have been carefully addressed in the present study.

Selection bias can be avoided by using a nationwide database that covers >99% of the population, and prevalent user bias can be avoided by enrolling patients with new-onset diabetes and new users of metformin.

Immortal time is the follow-up period during which the outcome cannot happen.[31] If the treatment status or the follow-up time is inappropriately assigned, immortal time bias can be introduced. In the present study, treatment status was unlikely mislabeled because prescription information was available during the long follow-up period, and only patients who had received two or more times of prescriptions of antidiabetic drugs were enrolled (Figure 1). The immortal time from diabetes diagnosis to the start of antidiabetic drugs, and in those with a short follow-up period of <180 days was actually not included in the calculation of person-years. Because patients can get all discharge drugs directly from the hospital when they are discharged in Taiwan, the immortal time from the waiting period between drug prescription and dispense during discharge, as pointed out by Lévesque and colleagues,[31] would not happen here.

The PS-matching procedure and the Cox regression incorporated with IPTW were aimed at reducing confounding by indication. Because none of the standardized differences had a value >10% in the matched cohort (Table 1), and the sensitivity analyses did not suggest a discrepant effect (Table 3), a potential risk of residual confounding from the covariates was not likely.

At first glance, one might argue that the mean age at diabetes diagnosis in women as calculated from the matched cohort in Table 1 would be 62 years (aged 67 years at the time of start of follow up, minus the mean duration of diabetes of 5 years). This would seem to be quite a bit older than what was observed in other countries. However, it should be stressed that the general mean age at diabetes diagnosis in a country should not be calculated in this way, especially not from a matched cohort used for investigating specific aims. We calculated the mean age at each step of the flowchart in Figure 1. It was noted that the mean age was 55.14 years in the first box that identified 423,949 newly diagnosed diabetes patients during 1999–2005. This was very similar to the mean age of 55 years at diabetes diagnosis among patients identified during a nationwide population-based survey conducted in Taiwan during a similar period from 1995 to 2002.[32] However, after excluding some ineligible patients in Figure 1, the mean age at diabetes diagnosis of the 93,773 patients enrolled as the unmatched original cohort was 58.18 years. In the unmatched cohort, the age at diabetes diagnosis among ever users of metformin was younger than never users (57.49 *versus* 63.42 years). This was reasonable because older patients with possible impairment of renal function or heart function at the time of diabetes diagnosis would be less prone to be treated with metformin. However, in the matched cohort enrolled for the present study, the age at diabetes diagnosis among ever users was 63.00 years, which was very similar to the age at diabetes diagnosis of 63.35 years among never users. Because never users were older at diabetes diagnosis, and ever users outnumbered never users, the mean age after matching would surely be closer to the mean age of never users (as shown in Table 1). Therefore, if we want to know the general mean age at diabetes diagnosis in Taiwan, it should be calculated from the initial whole cohort, and not from the sample selected for the study after excluding ineligible cases and after matching.

There are some additional merits in the present study. First, by using the medical records, self-reporting bias could be reduced. Second,

detection bias can be a severe problem in some countries because of different socioeconomic status. However, this was less likely in Taiwan because the drug cost-sharing is low in the NHI system, and many expenses can be waived in veterans, in patients with low-income, or when the patients receive prescription refills for chronic disease.

Study limitations may include a lack of measurement data of confounders like biochemical, humoral, and hormonal data, history of menstruation and parity, anthropometric factors, cigarette smoking, alcohol drinking, lifestyle, physical activity, nutritional status, family history, and genetic parameters. Because UL is a benign condition that is always symptomless in its early stage,[1] patients labelled with a diagnostic code might represent those who had clinical symptoms or signs. Furthermore, because most patients with UL do not need medical treatment or surgical intervention, the attending doctors might not have included the diagnostic code of UL in the claim data. Therefore, the case numbers of UL in the study might have been underestimated, and patients with UL identified in the study might represent those who had clinical presentations.

It is also worthy of note that the misclassification of UL by using only the diagnostic code might not be differential for ever and never users of metformin because nondifferential misclassification would be expected to bias the hazard ratios toward the null,[33] and this was observed in the present study (Table 3). The less stringent criterion for UL diagnosis by using only the diagnostic code (Table 2 and Models I to XI in Table 3) might have led to misclassifications of UL that were random and nondifferential, and, therefore, their hazard ratios moved toward the null when compared with the hazard ratio derived from the model that used a more stringent diagnostic criteria which required the 'diagnostic code' plus 'procedure codes' (Model XII, Table 3). However, the consistency of a risk reduction associated with metformin use in the various models supported the reproducibility of the finding.

It is recognized that 'statistical significance' does not necessarily indicate 'scientific significance or practical importance', and inferences should not be simply based on *p* values derived from statistical analyses.[34] Therefore, the present study should be viewed as exploratory, and future confirmation studies to reproduce the findings in other settings and ethnicities are welcome. If the preventive role of metformin on UL can be confirmed, there are some clinical implications. First, the hazard ratios estimated from various models consistently suggested a 40–50% risk reduction of UL (Tables 2 and 3), and an approximately 55% risk reduction of surgical operations to the uterus (Model XII in Table 3) associated with metformin use after a median follow-up of 5 years implied a potentially substantial reduction of clinical burden of UL, which may have psychological impacts on the patients and increase medical expenses incurred by the treatment of UL. Second, because UL may increase the risk of infertility,[35] and cause some complications during pregnancy (e.g. recurrent pain, miscarriage, uterine bleeding, maternal-fetal incommunicability, incorrect fetal position, and fetal deformities),[36] and metformin can be used safely during pregnancy,[37] the prescription of metformin to female patients with type 2 diabetes mellitus during childbearing age may provide extra benefits to these women. Third, because UL has been reported to affect female sexual dysfunction in postmenopausal women but not in premenopausal women,[38] continuous use of metformin in diabetes women ever since the diagnosis of diabetes mellitus may potentially improve their sexual function when they progress into menopause.

In summary, this population-based retrospective cohort study supports a reduced risk of UL associated with metformin use in Taiwanese female patients with type 2 diabetes mellitus. However, confirmation with prospective cohort study design or clinical trials is necessary. Because metformin is inexpensive and safe, and would not cause hypoglycemia when used as monotherapy, its benefit for the prevention of UL provides an additional bonus for the use of metformin as a first-line therapy in the treatment of women with type 2 diabetes mellitus. However, its usefulness and cost-effectiveness in the nondiabetic women remain to be investigated. Because this study was conducted in Taiwan, mainly in patients of Asian descent, future studies in different populations and ethnicities are needed to confirm the findings.

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Author contributions

C.H. researched data and wrote manuscript. The guarantor of this paper is Tseng CH.

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Conflict of interest statement

The author declare that there is no conflict of interest.

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