Accepted Manuscript

Metformin and skin cancer risk in Taiwanese patients with type 2 diabetes

Chin-Hsiao Tseng

PII: S0190-9622(17)32816-5

DOI: 10.1016/j.jaad.2017.12.016

Reference: YMJD 12176

To appear in: *Journal of the American Academy of Dermatology*

Received Date: 3 October 2017

Revised Date: 27 November 2017

Accepted Date: 3 December 2017

Please cite this article as: Tseng C-H, Metformin and skin cancer risk in Taiwanese patients with type 2 diabetes, *Journal of the American Academy of Dermatology* (2018), doi: 10.1016/j.jaad.2017.12.016.

Metformin and skin cancer risk in Taiwanese patients with type 2 diabetes

Running title: Metformin and skin cancer

Chin-Hsiao Tseng[1,2,3]

[1]Department of Internal Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

[2]Division of Endocrinology and Metabolism, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

[3]Division of Environmental Health and Occupational Medicine of the National Health Research Institutes, Zhunan, Taiwan

Corresponding author:

Chin-Hsiao Tseng, MD, PhD

Department of Internal Medicine, National Taiwan University Hospital

No. 7 Chung-Shan South Road, Taipei (100), Taiwan

Telephone and Fax: 886-2-2388-3578; E-mail: ccktsh@ms6.hinet.net

Word count of the abstract: 200

Word count of the text: 1830

Number of tables: 3

Number of figures: 1

Number of references: 23

Conflict of interest: None

Funding: The study was supported by the Ministry of Science and Technology (MOST 103-2314-B-002-187-MY3) of Taiwan. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Capsule summary

* What is already known on this topic

The association between skin cancer and metformin use is not known.

* What this article adds to our knowledge

Metformin use is associated with a decreased risk of skin cancer in a dose-response pattern.

* How this information impacts clinical practice and/or changes patient care

Although prospective studies of metformin's effect on skin cancer are needed, it may be a good choice for patients with type 2 diabetes at high risk of skin cancer.

Abstract

BACKGROUND: Metformin, an antidiabetic drug, is associated with decreased cancer risk, but its effect on skin cancer is unknown.

OBJECTIVE: To evaluate skin cancer risk associated with metformin use.

METHODS: Matched pairs of 16237 ever and never metformin users were retrospectively enrolled from patients with new-onset type 2 diabetes diagnosed between 1999 and 2005 from Taiwan's National Health Insurance database, and followed until December 31, 2011. Hazard ratios (HRs) were estimated using Cox regression weighted for propensity scores.

RESULTS: Skin cancer incidence was 45.59 and 83.90 per 100,000 person-years in ever and never users, respectively (HR 0.540, 95% confidence interval: 0.357–0.819). In ever users, the HRs (95% confidence intervals) for the first (<21.00 months), second (21.00–45.83 months), and third (>45.83 months) cumulative duration tertiles were 0.817 (0.448–1.489), 0.844 (0.504–1.412), and 0.114 (0.036–0.364), respectively; and 1.006 (0.579–1.748), 0.578 (0.317–1.051), and 0.229 (0.099–0.530), respectively, for the first, second, and third cumulative dose tertiles. HRs were 0.523 (0.175–1.562) for melanoma and 0.496 (0.319–0.772) for non-melanoma skin cancer.

LIMITATIONS: Few patients with skin cancer and lack of information on ultraviolet exposure and tumor histopathology.

CONCLUSION: Metformin use is associated with decreased skin cancer risk.

Keywords: diabetes mellitus, metformin, skin cancer, Taiwan

Introduction

Skin cancer rates are increasing worldwide, with increases in rates of both melanoma and non-melanoma skin cancer (NMSC) [1,2]. In Taiwan, skin cancer affects more men than women, and the incidences of both melanoma and NMSC are increasing steadily [3].

Ultraviolet (UV) sunlight exposure is a risk factor for both melanoma and NMSC [2]. Other risk factors include obesity [4], human papillomavirus [5], family history of skin cancer, light-colored skin and eyes, and immunosuppression [6]. Consumption of coffee and tea may protect against NMSC [7].

Epidemiological studies suggest that metformin may demonstrate a preventive effect against cancer [8], but its effect on skin cancer remains unknown. Metformin exerts anticancer effects in melanoma cells in *in vitro* studies [9] and metformin administered through drinking water significantly reduces the risk of skin cancer induced by carcinogens in female mice [10]. Therefore, the present study evaluated whether metformin use may be associated with a lower risk of skin cancer by using the reimbursement National Health Insurance (NHI) database.

Materials and Methods

The NHI, implemented since March 1995, is a compulsory healthcare system in Taiwan. It covers >99% of Taiwan's residents and has contracts with >98% of the hospitals nationwide. The reimbursement databases are handled by the National Health Research Institutes and can be used for academic research after proposal review and approval by an ethical review board. This study was granted approval number 99274.

Individuals were anonymized for the protection of privacy. Diabetes was coded 250.XX and skin cancer included 172 (melanoma) and 173 (NMSC), based on the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM).

Figure 1 shows the procedures performed to select a cohort of 1:1 propensity score

(PS)-matched pairs of metformin ever and never users using the Greedy 8 → 1 digit match algorithm [11]. The PS was derived from all characteristics listed in Table 1 and the date of entry. The patients presented with new-onset diabetes between 1999 and 2005 and followed up in the outpatient clinics with prescription of antidiabetic drugs 2 or more times (n=423949). To assure that diabetes was first diagnosed after 1999, patients diagnosed between 1996 and 1998 were excluded. In Taiwan, patients with type 1 diabetes can be waived of most medical co-payments after a certified diagnosis with issuance of a so-called "Severe Morbidity Card". These type 1 diabetes patients (n=2400) were first excluded because metformin was not indicated for them. Patients with missing data (n=338), a diagnosis of any cancer before entry (n=44260), aged <25 (n=21086) or ≥ 75 (n=43348) years, or followed up for <180 days (n=7960) were then excluded.

Age, sex, occupation, living region, and factors correlated with metformin use, diabetes severity, or cancer risk were considered as potential confounders. Living regions and occupations were classified as described previously [12]. In brief, living regions were classified as Taipei, Northern, Central, Southern, or Kao-Ping/Eastern. Occupations were 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), or class IV (low-income families supported by social welfare, or veterans).

Other confounding variables included 1) major comorbidities associated with diabetes, including hypertension (ICD-9-CM code: 401–405), dyslipidemia (272.0–272.4), and obesity (278); 2) diabetes-related complications, including nephropathy (580–589), eye disease (250.5, 362.0, 369, 366.41, and 365.44), stroke (430–438), ischemic heart disease (410–414), and peripheral arterial disease (250.7, 785.4, 443.81, and 440–448); 3) antidiabetic drugs, including insulin, sulfonylurea, meglitinide, acarbose, rosiglitazone, and pioglitazone; 4)

potential risk factors of cancer, including ocular pterygium (a surrogate of UV sunlight exposure [13]; 372.40–372.44), chronic obstructive pulmonary disease (a surrogate of smoking; 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), gallstones (574.00, 574.01, 574.10, 574.11, 574.20, 574.21, and A348), history of *Helicobacter pylori* infection (defined below), diagnoses of Epstein–Barr virus infection (075, 710.3, and 710.4), hepatitis B virus infection (070.22, 070.23, 070.32, 070.33, and V02.61), and hepatitis C virus infection (070.41, 070.44, 070.51, 070.54, and V02.62); and 5) medications that may potentially affect cancer risk and are commonly administered in diabetes patients, including angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, calcium channel blockers, statins, fibrate, and aspirin. One of the following two criteria defined *Helicobacter pylori* infection: 1) diagnosis of *Helicobacter pylori* infection (041.86) and/or 2) having received eradication therapy, which was detailed previously and defined as combined administration of proton pump inhibitors or H2 receptor blockers, plus clarithromycin, metronidazole, or levofloxacin, plus amoxicillin or tetracycline, with or without bismuth, in the same prescription order for 7–14 days [12].

The characteristics between never and ever users were compared using the Student's *t* test for age and using the Chi-square test for other variables. The standardized differences for all covariates were calculated and a value >10% indicated potential confounding [14].

Analysis of a dose-response relationship was determined a priori using the tertiles of cumulative duration (months) and cumulative dose (mg) of metformin treatment. The incidence density of skin cancer was calculated for never users, ever users, and the tertiles of cumulative duration and cumulative dose. The numerator was the number of cases of incident skin cancer, and the denominator was the person-years of follow-up. Follow-up initiated on the first day of the administration of antidiabetic drugs and ended on December

31, 2011, at the time of a new diagnosis of skin cancer, or on the date of death or the last reimbursement record, whichever occurred first.

Hazard ratios comparing ever users and the tertiles of cumulative duration and dose to never users were estimated using Cox regression incorporated with the inverse probability of treatment weighting using the PS [14]. Overall hazard ratios for the melanoma and NMSC subtypes were also estimated. Additional models were created after excluding patients with obesity, eye diseases, gallstones, insulin use, meglitinide use, or any of the above to avoid potential confounding because they differed significantly between ever and never users.

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

Results

There were 16237 never users and 16237 ever users included in this study (Figure 1). Although several factors (age; obesity; diagnosis of eye diseases; insulin, sulfonylurea, and meglitinide treatment; and presence of gallstones) differed significantly between the two groups, none had a standardized difference >10% except for insulin and sulfonylurea treatment.

Table 2 shows the incidence of skin cancer and the hazard ratios following metformin exposure. The total number of incident skin cancers among ever and never users was 35 and 61, respectively, and the incidence was 45.59 and 83.90 per 100,000 person-years, respectively. The overall hazard ratio suggested a significantly lower risk associated with metformin treatment. In the tertile analyses, there was a trend of decreasing incidence with longer duration or higher dose. A significantly reduced risk was observed in the third tertiles.

Table 3 shows the overall hazard ratios for melanoma and NMSC and for all skin cancers after excluding patients with obesity, eye diseases, gallstones, insulin or meglitinide treatment, or any of the above.

Discussion

This study suggested a lower skin cancer risk associated with metformin treatment in a dose-response pattern (Table 2) and independent of potential confounders (Tables 2 and 3).

In the subtype analyses (Table 3), a lower risk associated with metformin treatment was favored for both melanoma (although not statistically significant) and NMSC (statistically significant). The lack of statistical significance for melanoma could possibly be because of the small number of incident cases in this specific subtype. Because the incidence of skin cancer is increasing in Taiwan, as well as globally, larger studies in higher risk populations are needed to determine whether metformin is associated with lower risk of each subtype of skin cancer. In addition, prospective studies of metformin for skin cancer are essential to show a cause and effect relationship.

Older age and higher proportion of sulfonylurea treatment in ever users of metformin (Table 1) might only underestimate the beneficial effect of metformin, because aging is a risk factor of skin cancer [15] and sulfonylurea may increase cancer risk [16]. Residual confounding effects from the lower proportions of insulin and meglitinide treatment, obesity, eye diseases, and gallstones in ever users was not likely because analyses after excluding patients using these medications or with these diagnoses did not change the results (Table 3).

The mechanisms of how metformin may reduce skin cancer risk remains to be answered. Metformin reduces inflammation either through the improvement of metabolic disturbances or through its inhibitory effects on the proinflammatory cancer-promoting nuclear factor κB and STAT3 pathways [17]. Metformin may also exert an immune-mediated antitumor effect by increasing the number of $CD8^+$ tumor-infiltrating lymphocytes [18]. In animals, metformin inhibits skin tumor growth in overweight and obese mice through the activation of epidermal 5'-adenosine monophosphate-activated protein kinase (AMPK), resulting in the attenuation of downstream signaling of mammalian target of rapamycin [19]. Topical or

systemic administration of metformin prevents UVB-induced DNA damage and suppresses skin cancer cell proliferation through a mechanism involving AMPK in mice [20]. Metformin may also inhibit melanoma cell proliferation via *p53*-dependent pathways [9].

Human papillomavirus, especially the β genus, plays a role in skin cancer [21]. Metformin suppresses viral replication in hepatitis B [22] and C [23] infection, but whether it may suppress the replication of human papillomavirus requires further investigation.

The present study has several strengths that may indicate high generalizability of the findings. First, all claims records and diagnoses of outpatient visits and hospital admissions were included. Second, most medical co-payments can be waived by the NHI for patients with cancer, and there is a low drug cost-sharing for patients with low incomes, veterans, or patients receiving prescription refills for chronic disease. Therefore, the detection rate of skin cancer would be less biased by different social classes. Third, bias related to self-reporting could be reduced using medical records.

There are some limitations. First, there was lack of data on UV sunlight exposure and ocular pterygium might be a rough surrogate. Second, the study was conducted in a population with relatively low risk of skin cancer and only a small number of patients with skin cancers could be found. Third, because only one case (metformin user) was given a diagnosis of human papillomavirus infection, it was not possible to evaluate its impact. Fourth, there were no actual measurement data for some confounders, such as biochemical data, anthropometric factors, consumption of coffee and tea, family history, and genetic parameters. Fifth, the information on the pathology, grading, and staging of skin cancer was not available.

In summary, this study supports a protective effect of metformin on skin cancer in patients with type 2 diabetes, especially when it has been used for >4 years or when the cumulative dose is >1,594,000 mg. However, confirmatory epidemiologic studies with larger

sample sizes and higher risk populations, as well as prospective studies, will be necessary to elucidate the potential role of metformin in protection against skin cancer.

Author Contributions

C.H. researched data and wrote manuscript.

Acknowledgments

The study is based on data from the National Health Insurance Research Database provided by the Bureau of National Health Insurance, Department of Health and managed by National Health Research Institutes. The interpretation and conclusions contained herein do not represent those of Bureau of National Health Insurance, Department of Health or National Health Research Institutes.

Conflict of Interests: None

References

1. Lomas A, Leonardi-Bee J, Bath-Hextall F. A systematic review of worldwide incidence of nonmelanoma skin cancer. Br J Dermatol. 2012;166:1069-1080.
2. Gandhi SA, Kampp J. Skin Cancer Epidemiology, Detection, and Management. Med Clin North Am. 2015;99:1323-1335.
3. Chiang CJ, Chen YC, Chen CJ, You SL, Lai MS; Taiwan Cancer Registry Task Force. Cancer trends in Taiwan. Jpn J Clin Oncol. 2010;40:897-904.
4. Karimi K, Lindgren TH, Koch CA, Brodell RT. Obesity as a risk factor for malignant melanoma and non-melanoma skin cancer. Rev Endocr Metab Disord. 2016;17:389-403.
5. Galloway DA, Laimins LA. Human papillomaviruses: shared and distinct pathways for pathogenesis. Curr Opin Virol. 2015;14:87-92.
6. Pelucchi C, Di Landro A, Naldi L, La Vecchia C; Oncology Study Group of the Italian Group for Epidemiologic Research in Dermatology (GISED). Risk factors for

histological types and anatomic sites of cutaneous basal-cell carcinoma: an Italian case–control study. J Invest Dermatol. 2007;127:935–944.

7. Caini S, Cattaruzza S, Bendinelli B, Tosti G, Masala G, Gnagnarella P, et al. Coffee, tea and caffeine intake and the risk of non-melanoma skin cancer: a review of the literature and meta-analysis. Eur J Nutr. 2017;56:1-12.

8. Yu X, Mao W, Zhai Y, Tong C, Liu M, Ma L, et al. Anti-tumor activity of metformin: from metabolic and epigenetic perspectives. Oncotarget. 2017;8:5619-5628.

9. Snima KS, Pillai P, Cherian AM, Nair SV, Lakshmanan VK. Anti-diabetic drug metformin: challenges and perspectives for cancer therapy. Curr Cancer Drug Targets. 2014;14:727-736.

10. Checkley LA, Rho O, Angel JN, Cho J, Blando J, Beltran L, Hursting SD, DiGiovanni J. Metformin inhibits skin tumor promotion in overweight and obese mice. Cancer Prev Res (Phila) 2014;7:54-64.

11. Parsons LS. Performing a 1:N case-control match on propensity score. http://www.google.com.tw/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CBsQFjAAahUKEwibi7HllcnIAhUDoJQKHVeZA9A&url=http%3A%2F%2Fwww2.sas.com%2Fproceedings%2Fsugi29%2F165-29.pdf&usg=AFQjCNFOHGWYu8E8Bn4-Bo1TUiJKtT987Q (last accessed November 10, 2017).

12. Tseng CH. Diabetes, insulin use and Helicobacter pylori eradication: a retrospective cohort study. BMC Gastroenterology. 2012,12:46.

13. Yu HC, Lin CL, Chen ZT, Hu FR, Sung FC, Wang IJ. Risk of skin cancer in patients with pterygium: a nationwide population-based cohort study in Taiwan. Ocul Surf. 2014;12:69-76.

14. Austin PC, Stuart EA. Moving towards best practice when using inverse probability of treatment weighting (IPTW) using the propensity score to estimate causal treatment effects in observational studies. Stat Med. 2015;34:3661-3679.

15. Al-Dawsari NA, Amra N. Pattern of skin cancer among Saudi patients attending a tertiary care center in Dhahran, Eastern Province of Saudi Arabia. A 20-year retrospective study. Int J Dermatol. 2016;55:1396-1401.

16. Hsieh MC, Lee TC, Cheng SM, Tu ST, Yen MH, Tseng CH. The influence of type 2 diabetes and glucose-lowering therapies on cancer risk in the Taiwanese. Exp Diabetes Res. 2012;2012:413782.

17. Chu NJ, Armstrong TD, Jaffee EM. Nonviral oncogenic antigens and the inflammatory signals driving early cancer development as targets for cancer immunoprevention. Clin Cancer Res. 2015;21:1549-1557.

18. Eikawa S, Nishida M, Mizukami S, Yamazaki C, Nakayama E, Udono H. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. Proc Natl Acad Sci U S A. 2015;112:1809-1814.

19. Anisimov VN. Metformin for cancer and aging prevention: is it a time to make the long story short? Oncotarget. 2015;6:39398-39407.

20. Wu CL, Qiang L, Han W, Ming M, Viollet B, He YY. Role of AMPK in UVB-induced DNA damage repair and growth control. Oncogene. 2013;32:2682-2689.

21. McLaughlin-Drubin ME. Human papillomaviruses and non-melanoma skin cancer. Semin Oncol. 2015;42:284-290.

22. Xun YH, Zhang YJ, Pan QC, Mao RC, Qin YL, Liu HY, et al. Metformin inhibits hepatitis B virus protein production and replication in human hepatoma cells. J Viral Hepat. 2014;21:597-603.

277 23. del Campo JA, García-Valdecasas M, Rojas L, Rojas Á, Romero-Gómez M. The
278 hepatitis C virus modulates insulin signaling pathway in vitro promoting insulin
279 resistance. PLoS One. 2012;7:e47904.

280 Table 1. Characteristics of metformin never users and ever users

Variable	Never users (*n*=16237)		Ever users (*n*=16237)		*P*	Standardized difference
	n	%	*n*	%		
Demographic data						
Age* (years)	59.15±10.38		59.41±9.65		0.0184	3.66
Sex (men)	9306	57.31	9371	57.71	0.4656	0.53
Occupation						
I	6340	39.05	6352	39.12	0.7994	
II	3233	19.91	3175	19.55		-0.89
III	3411	21.01	3467	21.35		1.10
IV	3253	20.03	3243	19.97		-0.31
Living region						
Taipei	5459	33.62	5504	33.90	0.8679	
Northern	1657	10.21	1644	10.13		-0.28
Central	2844	17.52	2807	17.29		-0.57
Southern	2815	17.34	2768	17.05		-0.56
Kao-Ping and Eastern	3462	21.32	3514	21.64		0.97
Major comorbidities						
Hypertension	11984	73.81	12129	74.70	0.0657	2.42
Dyslipidemia	9832	60.55	9686	59.65	0.0980	-1.32
Obesity	361	2.22	298	1.84	0.0132	-2.79
Diabetes-related complications						
Nephropathy	4133	25.45	4081	25.13	0.5068	-1.36
Eye disease	1527	9.40	1354	8.34	0.0007	-4.21
Stroke	4021	24.76	4006	24.67	0.8470	-0.05
Ischemic heart disease	6207	38.23	6268	38.60	0.4865	1.04
Peripheral arterial disease	2513	15.48	2413	14.86	0.1219	-1.96
Antidiabetic drugs						
Insulin	1352	8.33	1003	6.18	<0.0001	-10.18
Sulfonylurea	11807	72.72	12439	76.61	<0.0001	10.25
Meglitinide	1338	8.24	1215	7.48	0.0112	-2.68
Acarbose	1833	11.29	1797	11.07	0.5261	-1.98
Rosiglitazone	479	2.95	450	2.77	0.3344	-1.49
Pioglitazone	400	2.46	419	2.58	0.5013	0.25
Potential risk factors of cancer						
Ocular pterygium	635	3.91	587	3.62	0.1616	-1.44
Chronic obstructive pulmonary disease	6518	40.14	6496	40.01	0.8033	-0.02
Tobacco abuse	264	1.63	254	1.56	0.6578	-0.36
Alcohol-related diagnoses	1038	6.39	997	6.14	0.3479	-1.56
Gallstones	1641	10.11	1494	9.20	0.0057	-3.19

History of *Helicobacter pylori* infection	3653	22.50	3543	21.82	0.1416	-1.82
Epstein-Barr virus-related diagnoses	95	0.59	93	0.57	0.8837	-0.14
Hepatitis B virus infection	340	2.09	328	2.02	0.6390	-0.65
Hepatitis C virus infection	720	4.43	692	4.26	0.4461	-0.98
Medications that are commonly used in diabetes patients or may affect cancer risk						
Angiotensin converting enzyme inhibitor/angiotensin receptor blocker	9599	59.12	9658	59.48	0.5051	0.89
Calcium channel blocker	9032	55.63	9140	56.29	0.2273	1.59
Statin	6427	39.58	6374	39.26	0.5473	-0.29
Fibrate	4435	27.31	4403	27.12	0.6899	-0.11
Aspirin	7655	47.15	7629	46.99	0.7725	0.08

*Age is expressed as mean ± standard deviation

Refer to “Materials and Methods” for the classification of occupation

283 Table 2. Incidence rates of skin cancer and hazard ratios by metformin exposure

Metformin use	Incident cases	Cases followed	Person-years	Incidence rate (per 100,000 person-years)	Hazard ratio	95% Confidence interval	*P* value
Never users	61	16237	72709.64	83.90	1.000		
Ever users	35	16237	76766.89	45.59	0.540	(0.357-0.819)	0.0037
Tertiles of cumulative duration of metformin therapy (months)							
Never users	61	16237	72709.64	83.90	1.000		
＜21.00	13	5329	18788.23	69.19	0.817	(0.448-1.489)	0.5085
21.00-45.83	19	5387	26642.68	71.31	0.844	(0.504-1.412)	0.5175
>45.83	3	5521	31335.98	9.57	0.114	(0.036-0.364)	0.0002
Tertiles of cumulative dose of metformin therapy (mg)							
Never users	61	16237	72709.64	83.90	1.000		
＜642,000	16	5358	19058.52	83.95	1.006	(0.579-1.748)	0.9831
642,000-1,594,000	13	5358	26627.68	48.82	0.578	(0.317-1.051)	0.0724
>1,594,000	6	5521	31080.68	19.30	0.229	(0.099-0.530)	0.0006

284

285 Table 3. Sensitivity analyses estimating hazard ratios for metformin ever users vs. never users

Model	Incident cases	Cases followed	Person-years	Incidence rate (per 100,000 person-years)	Hazard ratio	95% confidence interval	*P*-value
Melanoma							
Never users	9	16237	72709.64	12.38	1.000		
Ever users	5	16237	76766.89	6.51	0.523	(0.175-1.562)	0.2456
Non-melanoma skin cancer							
Never users	57	16237	72709.64	78.39	1.000		
Ever users	30	16237	76766.89	39.08	0.496	(0.319-0.772)	0.0019
All skin cancer							
Excluding patients with obesity							
Never users	59	15876	71207.47	82.86	1.000		
Ever users	33	15939	75392.47	43.77	0.526	(0.343-0.805)	0.0031
Excluding patients with eye disease							
Never users	54	14710	65682.78	82.21	1.000		
Ever users	30	14883	65682.78	45.67	0.516	(0.330-0.807)	0.0037
Excluding patients with gallstones							
Never users	55	14596	65899.29	83.46	1.000		
Ever users	32	14743	69913.10	45.77	0.546	(0.353-0.844)	0.0065
Excluding users of insulin							
Never users	59	14885	67342.87	87.61	1.000		
Ever users	31	15234	72390.33	42.82	0.486	(0.315-0.751)	0.0012
Excluding users of meglitinide							
Never users	60	14899	67071.46	89.46	1.000		
Ever users	33	15022	70911.18	46.54	0.518	(0.338-0.792)	0.0024
Excluding patients with obesity, eye disease, gallstone, insulin use or meglitinide use							
Never users	45	11052	50728.29	88.71	1.000		
Ever users	23	11667	55513.23	41.43	0.466	(0.282-0.770)	0.0029

286

Figure 1. Flowchart showing the procedures performed to select a cohort of 1:1 propensity score-matched pairs of metformin ever and never users from the reimbursement database of the National Health Insurance.