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Dose dependent relationship of metformin use and diabetic peripheral neuropathy risk in patients with type 2 diabetes mellitus

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This study investigated the correlation between metformin use and diabetic peripheral neuropathy (DPN) risk in patients with type 2 diabetes mellitus (T2DM) and its dose-dependent relationship. The study included new-onset T2DM patients from 2002 to 2013. Patients were divided into two groups based on metformin treatment, and DPN risk was assessed at 2- and 5-year follow-ups. After adjusting for various factors, two logistic models, metformin cumulative defined daily dose (cDDD) and metformin treatment intensity (defined daily dose [DDD]/month), evaluated the metformin-DPN risk association. Results showed that patients with metformin cDDD < 300, 300–500, and > 500 had higher DPN risk at both follow-ups. Odds ratios (ORs) and confidence intervals (CIs) for DPN were 1.74 (1.69–1.79), 2.05 (1.81–2.32), and 2.36 (1.34–4.16) at 2 years and 1.63 (1.60–1.65), 1.82 (1.69–1.96), and 2.17 (1.56–3.03) at 5 years. Similarly, patients with < 10, 10–25, and > 25 DDD/month had higher DPN risk at both follow-ups. Metformin use correlated with DPN risk in T2DM patients, with a dose-dependent relationship. Higher metformin cDDD or treatment intensity increased DPN risk. However, the absence of vitamin B12 data limits the understanding of the underlying mechanisms. Well-designed, large-scale studies are required to evaluate the potential risks of metformin therapy for DPN in patients with T2DM.

Keywords Diabetes mellitus, Diabetic peripheral neuropathy, Metformin, Cumulative defined daily dose, Risk factors

Peripheral neuropathy (PN) comprises a wide range of clinical syndromes potentially presenting with peripheral nervous system disorder[1]. Some individuals with diabetes mellitus (DM) develop diabetic neuropathy, which is characterized by both positive symptoms (e.g., burning, pain, and tingling in the extremities) and negative symptoms (e.g., numbness and dysesthesia in the extremities and stumbling)[2]. Diabetic PN (DPN) is the most common complication of DM. Its prevalence increases with the disease duration of DM, and approximately 50% of patients with type 2 DM (T2DM) develop neuropathy in their lifetime[2]. In patients with T2DM, the DPN prevalence ranges from 21.3% to 34.5%, and up to 45% of patients with T2DM with DPN may be asymptomatic[3].

Hyperglycemia is the most common risk factor for nerve cell damage through several physiological mechanisms, including the oxidative stress and polyol accumulation pathways[4]. Metformin has been noted to attenuate diabetic neuropathic pain in a rodent model. It was reported may also reduce serum vitamin B12 levels, potentially leading to nerve injury[5]. However, the correlation between metformin use and the risk of DPN remains unclear.

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Because relevant epidemiological studies thus far have reported inconsistent findings[6,7], we conducted a study to determine whether metformin use is associated with increased odds of DPN development by using data from the Taiwan National Health Insurance (NHI) Research Database (NHIRD), a large-scale, cross-sectional, nationwide database; we also investigated whether this association is dose-dependent.

Results

Table 1 presents the baseline characteristics of all patients. The average age of all patients was 56.20 ± 12.46 years, and 47.24% of all patients were women. Moreover, 29.00%, 16.00%, 15.90%, 12.64%, and 25.60% were aged 20–49, 50–54, 55–59, 60–64, and > 65 years, respectively.

In the patients treated with metformin, the average age was 54.94 ± 12.19 years. Among all patients, 3,412 (0.71%) had hyperuricemia, 16,181 (3.39%) had CVD, 2,973 (0.62%) had RA, 85,803 (17.96%) had sleep disturbance, 228 (0.05%) had SLE, 2,237 (0.47%) had migraine, 28,447 (5.96%) had CAD, 2,328 (0.49%) had PAD, 4,122 (0.86%) had depression, 3,328 (0.70%) had obesity, 5,450 (1.14%) had diabetic retinopathy, and 1,525 (0.32%) had CKD. The distribution of each comorbidity, with the exception of alcoholism, differed significantly between the patients treated and not treated with metformin ($P < 0.05$).

Table 2 presents the DPN incidence rates and risk at the 2-year follow-up. In total, 22,137 (2.48%) patients developed DPN 2 years after their initial DM diagnosis. The incidence rate of DPN in patients not treated with metformin was 1.79%. In the patients treated with metformin at a cDDD of < 300, 300–500, and > 500, the DPN incidence rates were 3.07%, 3.56%, and 4.26%, respectively; moreover, in those treated with < 10, 10–25, and > 25 DDDs/month of metformin, the rates were 3.03%, 3.18%, and 3.59%, respectively. Furthermore, at the 2-year follow-up, the ORs (95% CIs) for DPN after treatment with metformin at a cDDD of < 300, 300–500, and > 500 were 1.82 (1.72–1.93), 2.22 (1.90–2.59), and 2.48 (1.39–4.46), respectively. Moreover, the ORs (95% CIs) for DPN after treatment with > 25, 10–25, and < 10 DDDs/month of metformin were 1.79 (1.69–1.90), 1.90 (1.78–2.02), and 2.22 (1.90–2.59), respectively—indicating that the DPN risk was higher after 2 years of metformin treatment at > 25 DDDs/month than at ≤ 25 DDDs/month.

Table 3 presents the DPN risk at the 5-year follow-up. After adjusting for the relevant variables, we discovered that the ORs (95% CIs) for DPN after treatment with metformin at a cDDD of < 300, 300–500, and > 500 DPN were 1.66 (1.61–1.71), 1.86 (1.70–2.04), and 2.07 (1.47–2.92), respectively. Moreover, the ORs (95% CIs) for DPN after treatment with < 10, 10–25, and > 25 DDDs/month of metformin were 1.64 (1.59–1.70), 1.69 (1.63–1.75), and 1.87 (1.71–2.05), respectively. Compared with patients aged 20–49 years, those aged ≥ 65 years had a higher DPN risk (OR: 1.27; 95% CI: 1.22–1.31). Moreover, patients with comorbid CVD (OR: 1.05, 95% CI: 1.01–1.09) had a higher DPN risk, whereas those with comorbid hyperuricemia (OR: 0.73, 95% CI: 0.72–0.75), CAD (OR: 0.94, 95% CI: 0.91–0.97), and obesity (OR: 0.83, 95% CI: 0.75–0.93) had a lower DPN risk. Finally, patients with comorbid alcoholism, RA, sleep disturbance, SLE, migraine, PAD, depression, sarcopenia, and diabetic retinopathy did not exhibit a notable DPN risk.

Table 4 presents the association between metformin use and DPN in different cohorts. At the 2-year follow-up, there was a higher risk of developing DPN in female patients using metformin at a cDDD of < 300 (OR: 1.99, 95% CI: 1.85–2.15) and 300–500 (OR: 2.21, 95% CI: 1.77–2.77). The ORs (95% CIs) for DPN after treatment with < 10, 10–25, and > 25 DDDs/month of metformin were 1.96 (1.82–2.12), 2.08 (1.92–2.26), and 2.16 (1.73–2.71), respectively. Similarly, in male patients, the risk of DPN was higher with metformin at a cDDD of < 300 (OR: 1.57, 95% CI: 1.48–1.67), 300–500 (OR: 1.86, 95% CI: 1.55–2.23), and > 500 (OR: 2.84, 95% CI: 1.50–5.38). Among patients aged 20–49, the risk of developing DPN was higher with metformin at a cDDD of < 300 (OR: 1.85, 95% CI: 1.68–2.03), 300–500 (OR: 2.45, 95% CI: 1.92–3.14) and > 500 (OR: 3.29, 95% CI: 1.34–8.11). Furthermore, in patients with a DCSI score 0, the risk of developing DPN was also higher with metformin at a cDDD of < 300 (OR: 1.83, 95% CI: 1.71–1.96), 300–500 (OR: 2.39, 95% CI: 1.98–2.88), and > 500 (OR: 3.96, 95% CI: 1.95–8.05). The results of the risk of developing DPN obtained from the 5-year follow-up are similar to those observed at the 2-year follow-up.

Discussion

Few large-scale retrospective cohort epidemiology studies have evaluated the association between T2DM patients who are receiving metformin and the risk of DPN. In our study, we can find that metformin use was associated with DPN risk among T2DM patients in a dose-dependent association manner. The results suggest that T2DM patients received < 300, 300–500, ≥ 300 cDDD of metformin or use intensity of < 10, 10 ~ 25, > 25 DDD/month experienced higher risk of DPN at 2 and 5 years. The higher cDDD of metformin or use intensity, the higher DPN risk was found in this study. Our findings also indicated that among T2DM patients receiving metformin, being elderly and having a higher DCSI score were associated with an increased risk of DPN. In addition, T2DM patients comorbid with cerebrovascular disease also had a higher risk for the development of DPN.

The mechanisms underlying the pathogenesis of DPN are not fully understood. Multiple hypotheses have been proposed. The most generally accepted theory regarding DPN is the multifactorial process that involves several metabolic pathways, induced by hyperglycemia, which associate with nerve injury and dysfunction[8]. Oxidative stress, alternation in mitochondrial dysfunction, neuroinflammation and changes in the patterns of gene expression may be involved in the development of DPN[9]. Hyperglycemia is a major pathophysiologic risk factor that contributes to the development of DPN in T2DM patients. Hyperglycemia acts as an inducer to the endothelial cells through increasing oxidative stress and increasing the production of vasoconstrictor compounds, which lead to hypoxia, which is a strong inducer of VEGF expression[10].

Over the last few years, the clinical symptoms of vitamin B12 deficiency have shown notable trends towards signs and symptoms of nervous system disorders. Vitamin B12 deficiency is associated with multiple neurological

Variables	Total		Metformin				p-value
			Non-users		Users		
	N	%	N	%	N	%	
Total	892,836	100.00	415,192	100.00	477,644	100.00	
Gender							< 0.001
Female	421,754	47.24	204,278	49.20	217,476	45.53	
Male	471,082	52.76	210,914	50.80	260,168	54.47	
Age (year) (Mean ± SD)	56.20 ± 12.46		57.65 ± 12.62		54.94 ± 12.19		< 0.001
20–49	263,024	29	107,126	25.80	155,898	32.64	
50–54	146,495	16	64,478	15.53	82,017	17.17	
55–59	141,936	15.90	65,151	15.69	76,785	16.08	
66–64	112,829	12.64	54,926	13.23	57,903	12.12	
≥ 65	228,552	25.60	123,511	29.75	105,041	21.99	
Income level (NTD)[a]							< 0.001
≤ 21,000	452,076	50.63	213,432	51.41	238,644	49.96	
21,001–33,000	211,447	23.68	92,516	22.28	118,931	24.90	
≥ 33,001	229,313	25.68	109,244	26.31	120,069	25.14	
Urbanization[b]							< 0.001
Level 1	248,461	27.83	123,210	29.68	125,251	26.22	
Level 2	292,116	32.72	134,485	32.39	157,631	33.00	
Level 3	142,897	16.00	62,789	15.12	80,108	16.77	
Level 4	120,517	13.50	54,956	13.24	65,561	13.73	
Level 5	19,125	2.14	9,078	2.19	10,047	2.10	
Level 6	36,381	4.07	16,094	3.88	20,287	4.25	
Level 7	33,339	3.73	14,580	3.51	18,759	3.93	
DCSI score[c]							< 0.001
0	601,698	67.39	270,141	65.06	331,557	69.42	
1	162,506	18.20	78,577	18.93	83,929	17.57	
≥ 2	128,632	14.41	66,474	16.01	62,158	13.01	
Hyperuricemia							< 0.001
No	885,384	99.17	411,152	99.03	474,232	99.29	
Yes	7,452	0.83	4,040	0.97	3,412	0.71	
CVD[c]							< 0.001
No	857,440	96.04	395,977	95.37	461,463	96.61	
Yes	35,396	3.96	19,215	4.63	16,181	3.39	
Alcoholism							0.765
No	892,081	99.92	414,845	99.92	477,236	99.91	
Yes	755	0.08	347	0.08	408	0.09	
RA[c]							< 0.001
No	886,382	99.28	411,711	99.16	474,671	99.38	
Yes	6,454	0.72	3,481	0.84	2,973	0.62	
Sleep disturbance							< 0.001
No	720,323	80.68	328,482	79.12	391,841	82.04	
Yes	172,513	19.32	86,710	20.88	85,803	17.96	
SLE[c]							< 0.001
No	892,277	99.94	414,861	99.92	477,416	99.95	
Yes	559	0.06	331	0.08	228	0.05	
Migraine							< 0.001
No	888,449	99.51	413,042	99.48	475,407	99.53	
Yes	4,387	0.49	2,150	0.52	2,237	0.47	
CAD[c]							< 0.001
No	831,228	93.10	382,031	92.01	449,197	94.04	
Yes	61,608	6.90	33,161	7.99	28,447	5.96	
PAD[c]							< 0.001
No	887,880	99.44	412,564	99.37	475,316	99.51	
Yes	4,956	0.56	2,628	0.63	2,328	0.49	
Depression							< 0.001

Continued

Variables	Total		Metformin				
			Non-users		Users		
	N	%	N	%	N	%	p-value
No	884,263	99.04	410,741	98.93	473,522	99.14	
Yes	8,573	0.96	4,451	1.07	4,122	0.86	
Sarcopenia							0.014
No	892,287	99.94	414,908	99.93	477,379	99.94	
Yes	549	0.06	284	0.07	265	0.06	
Obesity							< 0.001
No	887,186	99.37	412,870	99.44	474,316	99.30	
Yes	5,650	0.63	2,322	0.56	3,328	0.70	
Diabetic retinopathy							< 0.001
No	880,923	98.67	408,729	98.44	472,194	98.86	
Yes	11,913	1.33	6,463	1.56	5,450	1.14	
CKD[c]							< 0.001
No	887,020	99.35	410,901	98.97	476,119	99.68	
Yes	5,816	0.65	4,291	1.03	1,525	0.32	

Table 1. Baseline characteristics of study subjects. [a]The premium-based salary of the patient which is according to the payroll bracket table of the National Health Insurance Administration Taiwan. NTD is New Taiwan Dollar. NTD 1 ≈ USD 0.034). [b]Level 1 denoted the highest degree of urbanization, whereas level 7 denoted the lowest degree of urbanization. [c]DCSI, diabetes complications severity index; CVD, cerebrovascular disease; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; CAD, coronary artery disease; PAD, peripheral arterial disease; CKD, chronic kidney disease.

and neurocognitive symptoms, including peripheral and autonomic neuropathy[11,12]. Peripheral neuropathy can be asymptomatic and could likely exacerbate DPN in T2DM patients[13].

In our large-scale retrospective cohort study, we report that metformin use was associated with DPN risk among T2DM patients in a dose-dependent association manner. The results suggest that T2DM patients received < 300, 300–500, ≥ 300 cDDD of metformin or use intensity of < 10, 10 ~ 25, > 25 DDD/month experienced higher risk of DPN after 2-year and 5-year follow-up period. The higher cDDD of metformin or use intensity, the higher DPN risk was found in this study. Several studies showed that T2DM patients using metformin was associated with higher incidence of DPN development[7,14,15]. The key factors predicting the occurrence of DPN in T2DM patients treated with metformin include a higher dosage and prolonged use of metformin, low vitamin B12 levels, and elevated homocysteine levels[15]. Several studies have generally indicated that metformin use was associated with lower plasma vitamin B12 levels[16,17]. A meta-analysis study also showed that metformin use is a risk factor for vitamin B12 deficiency in T2DM subjects[18]. The dosage of metformin also influences the likelihood of vitamin B12 deficiency. A positive correlation exists between metformin dosage and the risk of DPN, likely because higher metformin doses contribute to more severe vitamin B12 deficiency[14]. Over a period of at least 6 months, patients taking between 1000 and 1500 mg per day had a 72% higher risk of developing vitamin B12 deficiency compared to those taking 1000 mg or less daily. Those consuming between 1500 and 2000 mg per day experienced a 334% increase in risk, while individuals taking more than 2000 mg daily faced an 867% higher risk[19]. A logistic regression analysis also showed that T2DM patients treated with metformin for prolonged duration and higher metformin dose > 2000 mg were associated with lower vitamin B12 level and more severe DPN[15]. Although metformin can attenuate diabetic neuropathic pain via AMPK/NF-κB signaling pathway in diabetic rats model[20]. However, prolonged use of metformin can cause a deficiency in serum vitamin B12, which may exacerbate signs of peripheral nerve damage[14]. The occurrence of DPN in T2DM patients receiving metformin treatment is influenced by factors such as vitamin B12 and homocysteine levels[15]. However, the lack of this data restricts the ability to understand the underlying mechanisms. The relationship between cumulative metformin dosage and DPN risk remains uncertain, emphasizing the need for well-designed, large-scale studies to assess the potential risks of metformin therapy for DPN in patients with T2DM.

Vitamin B12 plays an important role in the conversion of homocysteine to methionine in methionine cycle[21]. Deficiency of vitamin B12 can impair the remethylation of homocysteine, and metformin-induced vitamin B12 deficiency could be associated with hyperhomocysteinemia[22]. While et al. showed that T2DM patients with exposure to metformin more than 6 months had lower serum vitamin B12 and higher serum homocysteine, which may be an iatrogenic cause for exacerbation of peripheral neuropathy. This study highlight that these abnormalities were correlated strongly with cumulative metformin exposure[7]. Plasma homocysteine levels may be independently associated with the prevalence and severity of diabetic neuropathy in T2DM patients[23,24]. A larger and prospective study in larger population would be suitable to clarify the role of homocysteine in the pathogenesis of DPN.

The underlying mechanism by which vitamin B12 deficiency occurs in patients with long-term metformin use is unclear. However, proposed mechanisms accounting for metformin-induced vitamin B12 deficiency have been proposed, include alteration of the small intestine motility leading to small intestinal bacterial overgrowth and

Variables	Two-year follow-up of incident peripheral neuropathy												
	Events			Model 1					Model 2				
	N	%	p-value	OR	95% CI			p-value	OR	95% CI			p-value
Total	22,137	2.48											
cDDD of metformin use			< 0.001										
Non-users	7,422	1.79		1					–		–		–
< 300	14,452	3.07		1.82	1.72	–	1.93	< 0.001	–		–		–
300–500	251	3.56		2.22	1.90	–	2.59	< 0.001	–		–		–
> 500	12	4.26		2.48	1.39	–	4.46	0.002	–		–		–
Intensity of metformin use			< 0.001										
Non-users	7,422	1.79		–		–		–	1				
< 10	10,358	3.03		–		–		–	1.79	1.69	–	1.90	< 0.001
10–25	4,094	3.18		–		–		–	1.90	1.78	–	2.02	< 0.001
> 25	263	3.59		–		–		–	2.22	1.90	–	2.59	< 0.001
Gender			0.081										
Female	10,585	2.51		1					1				
Male	11,552	2.45		1.05	1.01	–	1.10	0.040	1.05	1.01	–	1.10	0.041
Age (year)			< 0.001										
20–49	5,963	2.27		1					1				
50–54	3,660	2.50		1.16	1.11	–	1.21	< 0.001	1.16	1.11	–	1.21	< 0.001
55–59	3,430	2.42		1.16	1.10	–	1.22	< 0.001	1.16	1.10	–	1.22	< 0.001
66–64	2,796	2.48		1.19	1.12	–	1.26	< 0.001	1.19	1.12	–	1.26	< 0.001
≥ 65	6,288	2.75		1.31	1.23	–	1.39	< 0.001	1.31	1.23	–	1.39	< 0.001
Income level (NTD)[a]			< 0.001										
≤ 21,000	12,804	2.83		1					1				
21,001–33,000	4,638	2.19		0.78	0.75	–	0.80	< 0.001	0.78	0.75	–	0.80	< 0.001
≥ 33,001	4,695	2.05		0.76	0.73	–	0.78	< 0.001	0.76	0.73	–	0.78	< 0.001
Urbanization[b]			< 0.001										
Level 1	5,805	2.34		1					1				
Level 2	7,041	2.41		0.99	0.96	–	1.02	0.524	0.99	0.96	–	1.02	0.524
Level 3	3,634	2.54		1.02	0.98	–	1.07	0.274	1.02	0.98	–	1.07	0.271
Level 4	3,212	2.67		1.04	0.99	–	1.08	0.100	1.04	0.99	–	1.08	0.094
Level 5	559	2.92		1.08	0.99	–	1.18	0.071	1.09	1.00	–	1.19	0.065
Level 6	982	2.70		0.99	0.92	–	1.06	0.754	0.99	0.93	–	1.06	0.780
Level 7	904	2.71		1.01	0.94	–	1.08	0.778	1.01	0.94	–	1.09	0.769
DCSI score[c]			< 0.001										
0	14,030	2.33		1					1				
1	4,342	2.67		1.11	1.06	–	1.16	< 0.001	1.11	1.06	–	1.16	< 0.001
≥ 2	3,765	2.93		1.13	1.07	–	1.21	< 0.001	1.13	1.06	–	1.21	< 0.001
Hyperuricemia			0.026										
No	21,982	2.48		1					1				
Yes	155	2.08		0.73	0.70	–	0.76	< 0.001	0.73	0.71	–	0.76	< 0.001
CVD[c]			< 0.001										
No	21,117	2.46		1					1				
Yes	1,020	2.88		1.04	0.97	–	1.11	0.305	1.04	0.97	–	1.11	0.320
Alcoholism			0.764										
No	22,117	2.48		1					1				
Yes	20	2.65		1.07	0.69	–	1.66	0.757	1.07	0.69	–	1.66	0.755
RA[c]			0.229										
No	21,962	2.48		1					1				
Yes	175	2.71		1.09	0.94	–	1.27	0.244	1.09	0.94	–	1.27	0.238
Sleep disturbance			0.010										
No	17,711	2.46		1					1				
Yes	4,426	2.57		1.02	0.98	–	1.05	0.343	1.02	0.98	–	1.05	0.307
SLE[c]			0.970										
No	22,123	2.48		1					1				
Continued													
Yes	14	2.50		1.07	0.64	–	1.81	0.790	1.07	0.63	–	1.81	0.796

Variables	Two-year follow-up of incident peripheral neuropathy												
	Events			Model 1					Model 2				
	N	%	p-value	OR	95% CI			p-value	OR	95% CI			p-value
Migraine			0.138										
No	22,013	2.48		1					1				
Yes	124	2.83		1.14	0.95	–	1.36	0.160	1.14	0.95	–	1.36	0.157
CAD[c]			0.990										
No	20,609	2.48		1					1				
Yes	1,528	2.48		0.91	0.86	–	0.97	< 0.001	0.91	0.86	–	0.97	< 0.001
PAD[c]			0.065										
No	21,994	2.48		1					1				
Yes	143	2.89		1.04	0.88	–	1.22	0.664	1.04	0.88	–	1.23	0.658
Depression			0.314										
No	21,910	2.48		1					1				
Yes	227	2.65		1.08	0.95	–	1.23	0.265	1.08	0.95	–	1.23	0.264
Sarcopenia			0.703										
No	22,122	2.48		1					1				
Yes	15	2.73		1.05	0.63	–	1.74	0.859	1.05	0.63	–	1.74	0.858
Obesity			0.016										
No	22,025	2.48		1					1				
Yes	112	1.98		0.87	0.72	–	1.04	0.128	0.87	0.72	–	1.04	0.126
Diabetic retinopathy			0.144										
No	21,817	2.48		1					1				
Yes	320	2.69		1.04	0.93	–	1.16	0.541	1.04	0.93	–	1.16	0.545
CKD[c]			0.021										
No	22,020	2.48		1					1				
Yes	117	2.01		0.83	0.69	–	0.99	0.047	0.83	0.69	–	0.99	0.048
cDDD of metformin use*Gender	–	–		0.92	0.87	–	0.97	0.002	–		–		–
cDDD of metformin use*Age	–	–		0.98	0.96	–	0.99	0.004	–		–		–
cDDD of metformin use*DCSI	–	–		1.10	1.06	–	1.14	< 0.001	–		–		–
Intensity of metformin use*Gender	–	–		–		–		–	0.92	0.87	–	0.97	0.002
Intensity of metformin use*Age	–	–		–		–		–	0.98	0.96	–	0.99	0.006
Intensity of metformin use*DCSI	–	–		–		–		–	1.10	1.06	–	1.14	< 0.001

Table 2. Two-year follow-up of incident peripheral neuropathy in new-onset diabetes mellitus patients with metformin. [a]The premium-based salary of the patient which is according to the payroll bracket table of the National Health Insurance Administration Taiwan. NTD is New Taiwan Dollar. NTD 1 ≈ USD 0.034). [b]Level 1 denoted the highest degree of urbanization, whereas level 7 denoted the lowest degree of urbanization. [c]DCSI, diabetes complications severity index; CVD, cerebrovascular disease; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; CAD, coronary artery disease; PAD, peripheral arterial disease; CKD, chronic kidney disease.

subsequent inhibition of vitamin B12 calcium-dependent intrinsic factor complex absorption[25,26]. Competitive inhibition of vitamin B12 absorption and alterations of the intrinsic factor and cubilin receptor have also been proposed[27]. This malabsorption basically leads to a decrease of serum vitamin B12 plasma level. Metformin reduces hepatic vitamin B12 storage, eventually leading to vitamin B12 deficiency, which may contribute to distal symmetrical or autonomic neuropathy, spinal subacute combined degeneration, or the progression of pre-existing neuropathies associated with diabetes[28].

Our findings show that T2DM patients receiving metformin, being elderly and having a higher DCSI score were associated with an increased odds of DPN. Several risk factors of DPN have been identified, including aging and duration of DM are correlated with significantly higher risk for DPN in T2DM patients[29]. DPN prevalence increased with age from 11.9% aged below 40 years up to > 50% aged above 70 years[30]. The Diabetes Complications and Severity Index (DCSI) is a useful tool for prediction of risk of mortality and hospitalization in patients with DM[31]. Our study showed that T2DM patients receiving metformin with higher DCSI scores had a higher odds for developing DPN. DCSI was associated with the risk of DPN. DCSI may be used an indicator for estimating the risk of DPN. In our study, we can find that T2DM patients comorbid with cerebrovascular disease was associated with higher risk for the development of DPN. Our study results is consistent with a previous study showed that the risk factors for PAD including: age above 40 years and cerebrovascular disease[32]. Ischemic damage is caused by an interruption in cerebral blood flow, which causes a dramatic alteration of the complex neural network and induces severe neural injuries within the affected area[33]. Persistent changes following stroke

Variables	Five-year follow-up of incident peripheral neuropathy												
	Events			Model 1					Model 2				
	N	%	p-value	OR	95% CI			p-value	OR	95% CI			p-value
Total	68,024	7.62											
cDDD of metformin use			<0.001										
Non-users	24,020	5.79		1					–		–		–
<300	43,256	9.20		1.66	1.61	–	1.71	<0.001	–		–		–
300–500	713	10.12		1.86	1.70	–	2.04	<0.001	–		–		–
>500	35	12.41		2.07	1.47	–	2.92	<0.001	–		–		–
Intensity of metformin use			<0.001										
Non-users	24,020	5.79		–		–		–	1				
<10	31,219	9.14		–		–		–	1.64	1.59	–	1.70	<0.001
10–25	12,037	9.34		–		–		–	1.69	1.63	–	1.75	<0.001
>25	748	10.21		–		–		–	1.87	1.71	–	2.05	<0.001
Gender			0.018										
Female	32,429	7.69		1					1				
Male	35,595	7.56		1.03	1.01	–	1.06	0.013	1.03	1.01	–	1.06	0.014
Age (year)			<0.001										
20–49	18,448	7.01		1					1				
50–54	11,415	7.79		1.18	1.15	–	1.21	<0.001	1.18	1.15	–	1.21	<0.001
55–59	10,583	7.46		1.16	1.13	–	1.19	<0.001	1.16	1.13	–	1.19	<0.001
66–64	8,693	7.70		1.20	1.16	–	1.24	<0.001	1.20	1.16	–	1.24	<0.001
≥65	18,885	8.26		1.27	1.22	–	1.31	<0.001	1.27	1.22	–	1.31	<0.001
Income level (NTD)[a]			<0.001										
≤21,000	40,274	8.91		1					1				
21,001–33,000	13,180	6.23		0.69	0.68	–	0.71	<0.001	0.69	0.68	–	0.71	<0.001
≥33,001	14,570	6.35		0.73	0.72	–	0.74	<0.001	0.73	0.72	–	0.74	<0.001
Urbanization[b]			<0.001										
Level 1	18,119	7.29		1					1				
Level 2	21,590	7.39		0.97	0.95	–	0.99	0.006	0.97	0.95	–	0.99	0.006
Level 3	11,203	7.84		1.01	0.99	–	1.04	0.256	1.01	0.99	–	1.04	0.253
Level 4	9,911	8.22		1.03	1.01	–	1.06	0.018	1.03	1.01	–	1.06	0.016
Level 5	1,673	8.75		1.05	0.99	–	1.10	0.083	1.05	1.00	–	1.10	0.078
Level 6	2,842	7.81		0.92	0.88	–	0.96	<0.001	0.92	0.88	–	0.96	<0.001
Level 7	2,686	8.06		0.96	0.93	–	1.00	0.075	0.96	0.93	–	1.00	0.076
DCSI score[c]			<0.001										
0	43,926	7.30		1					1				
1	13,248	8.15		1.07	1.04	–	1.10	<0.001	1.07	1.04	–	1.10	<0.001
≥2	10,850	8.43		1.03	0.99	–	1.07	0.130	1.03	0.99	–	1.07	0.138
Hyperuricemia			<0.001										
No	67,546	7.63		1					1				
Yes	478	6.41		0.73	0.72	–	0.75	<0.001	0.73	0.72	–	0.75	<0.001
CVD[c]			<0.001										
No	65,011	7.58		1					1				
Yes	3,013	8.51		1.05	1.01	–	1.09	0.024	1.05	1.01	–	1.09	0.026
Alcoholism			0.336										
No	67,974	7.62		1					1				
Yes	50	6.62		0.88	0.67	–	1.16	0.366	0.88	0.67	–	1.16	0.367
RA[c]			0.249										
No	67,508	7.62		1					1				
Yes	516	8.00		1.06	0.97	–	1.16	0.198	1.06	0.97	–	1.16	0.194
Sleep disturbance			0.013										
No	54,634	7.58		1					1				
Yes	13,390	7.76		1.01	0.99	–	1.03	0.207	1.01	0.99	–	1.03	0.184
SLE[c]			0.226										
No	67,989	7.62		1					1				
Continued													
Yes	35	6.26		0.88	0.63	–	1.22	0.436	0.88	0.63	–	1.22	0.433

Variables	Five-year follow-up of incident peripheral neuropathy												
	Events			Model 1					Model 2				
	N	%	p-value	OR	95% CI			p-value	OR	95% CI			p-value
Migraine			0.117										
No	67,662	7.62		1					1				
Yes	362	8.25		1.10	0.99	–	1.22	0.078	1.10	0.99	–	1.22	0.077
CAD[c]			0.661										
No	63,358	7.62		1					1				
Yes	4,666	7.57		0.94	0.91	–	0.97	< 0.001	0.94	0.91	–	0.97	< 0.001
PAD[c]			0.297										
No	67,627	7.62		1					1				
Yes	397	8.01		0.98	0.89	–	1.08	0.686	0.98	0.89	–	1.08	0.690
Depression			0.648										
No	67,382	7.62		1					1				
Yes	642	7.49		1.00	0.92	–	1.08	0.955	1.00	0.92	–	1.08	0.956
Sarcopenia			0.538										
No	67,986	7.62		1					1				
Yes	38	6.92		0.88	0.64	–	1.21	0.434	0.88	0.64	–	1.21	0.435
Obesity			< 0.001										
No	67,691	7.63		1					1				
Yes	333	5.89		0.83	0.75	–	0.93	< 0.001	0.83	0.75	–	0.93	< 0.001
Diabetic retinopathy			0.307										
No	67,087	7.62		1					1				
Yes	937	7.87		1.01	0.95	–	1.08	0.808	1.01	0.95	–	1.08	0.812
CKD[c]			< 0.001										
No	67,710	7.63		1					1				
Yes	314	5.40		0.76	0.68	–	0.85	< 0.001	0.76	0.68	–	0.85	< 0.001
cDDD of metformin use*Gender	–	–		0.94	0.91	–	0.97	< 0.001	–		–		–
cDDD of metformin use*Age	–	–		0.98	0.97	–	0.99	< 0.001	–		–		–
cDDD of metformin use*DCSI	–	–		1.12	1.10	–	1.15	< 0.001	–		–		–
Intensity of metformin use*Gender	–	–		–		–		–	0.94	0.91	–	0.97	< 0.001
Intensity of metformin use*Age	–	–		–		–		–	0.98	0.97	–	0.99	< 0.001
Intensity of metformin use*DCSI	–	–		–		–		–	1.12	1.10	–	1.15	< 0.001

Table 3. Five-year follow-up of incident peripheral neuropathy in new-onset diabetes mellitus patients with metformin. [a]The premium-based salary of the patient which is according to the payroll bracket table of the National Health Insurance Administration Taiwan. NTD is New Taiwan Dollar. NTD 1 ≈ USD 0.034). [b]Level 1 denoted the highest degree of urbanization, whereas level 7 denoted the lowest degree of urbanization. [c]DCSI, diabetes complications severity index; CVD, cerebrovascular disease; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; CAD, coronary artery disease; PAD, peripheral arterial disease; CKD, chronic kidney disease.

can occur in lower motor neurons when central pathways are interrupted by stroke[34]. Regarding the association between chronic kidney disease (CKD) and DPN, available data do not demonstrate a clear correlation[35]. Some studies showed that patients with CKD are at higher risk of diabetic foot disease, and lower extremity amputation is at least two to six times greater among patients with both CKD and DM than DM alone[36,37]. However, our study discovered that DM patients comorbid with CKD had a lower risk for developing DPN. Further research is needed to better understand the relationship between CKD and DPN among DM patients.

This study has several strengths. First, the primary strength of our study is its population-based design performed using Taiwan's NHIRD. We included the entire Taiwanese population in our study; thus, the qualitative sample sizes are large enough that can provide high-quality database to reduce the selection bias. Second, the characteristics of the database can provide sufficient statistical power to investigate the association between metformin use and DPN risk among patients with T2DM. Third, the follow-up period of metformin use in our study is divided into 2 years and 5 years. cDDD of metformin use is divided into ≤ 300, > 300, > 500, intensity of metformin use (DDD/month) is divided into ≤ 10, 10–25, > 25/month to investigate the relationship between T2DM patients and the risk of DPN development.

However, there were also several limitations in this population-based cohort study. First, we had no information regarding family histories of DPN among T2DM patients. Second, lifestyle personal data such as those related to cigarette, smoking habits, alcohol consumption, HbA1c, body mass index, physical activity, personal history and dietary habits could not be accessed, all of these factors were associated with DPN risk, which was not available.

Variables	Incident peripheral neuropathy									
	Two-year follow-up					Five-year follow-up				
	aOR[a]	95% CI			p-value	aOR[a]	95% CI			p-value
In female patients										
cDDD of metformin use										
DDD < 300	1.99	1.85	–	2.15	< 0.001	1.74	1.67	–	1.81	< 0.001
DDD 300–500	2.21	1.77	–	2.77	< 0.001	1.87	1.64	–	2.13	< 0.001
DDD > 500	1.00	0.25	–	4.03	0.996	1.28	0.68	–	2.40	0.439
Intensity of metformin use										
< 10	1.96	1.82	–	2.12	< 0.001	1.72	1.65	–	1.79	< 0.001
10–25	2.08	1.92	–	2.26	< 0.001	1.79	1.71	–	1.88	< 0.001
> 25	2.16	1.73	–	2.71	< 0.001	1.84	1.62	–	2.10	< 0.001
In male patients										
cDDD of metformin use										
DDD < 300	1.57	1.48	–	1.67	< 0.001	1.51	1.46	–	1.56	< 0.001
DDD 300–500	1.86	1.55	–	2.23	< 0.001	1.64	1.48	–	1.83	< 0.001
DDD > 500	2.84	1.50	–	5.38	< 0.001	2.36	1.58	–	3.52	< 0.001
Intensity of metformin use										
< 10	1.01	0.53	–	1.92	0.976	1.04	0.70	–	1.56	0.834
10–25	1.07	0.56	–	2.03	0.842	1.06	0.71	–	1.59	0.762
> 25	0.79	0.21	–	2.99	0.733	0.80	0.35	–	1.83	0.591
In patients aged 20–49										
cDDD of metformin use										
DDD < 300	1.85	1.68	–	2.03	< 0.001	1.71	1.62	–	1.80	< 0.001
DDD 300–500	2.45	1.92	–	3.14	< 0.001	1.89	1.64	–	2.19	< 0.001
DDD > 500	3.29	1.34	–	8.11	0.010	2.05	1.12	–	3.75	0.020
Intensity of metformin use										
< 10	1.83	1.66	–	2.01	< 0.001	1.71	1.62	–	1.80	< 0.001
10–25	1.88	1.69	–	2.09	< 0.001	1.72	1.62	–	1.82	< 0.001
> 25	2.47	1.94	–	3.15	< 0.001	1.90	1.64	–	2.20	< 0.001
In patients aged 50–54										
cDDD of metformin use										
DDD < 300	1.99	1.79	–	2.22	< 0.001	1.67	1.57	–	1.77	< 0.001
DDD 300–500	1.76	1.20	–	2.58	0.004	1.74	1.42	–	2.13	< 0.001
DDD > 500	2.97	0.72	–	12.15	0.131	3.05	1.50	–	6.19	0.002
Intensity of metformin use										
< 10	1.17	0.28	–	4.88	0.832	0.95	0.46	–	1.94	0.882
10–25	1.21	0.29	–	5.05	0.797	0.95	0.46	–	1.95	0.890
> 25	0.62	0.03	–	11.84	0.747	0.56	0.13	–	2.48	0.447
In patients aged 55–59										
cDDD of metformin use	1.76	1.58	–	1.96	< 0.001	1.66	1.56	–	1.76	< 0.001
DDD < 300	2.58	1.84	–	3.61	< 0.001	2.11	1.72	–	2.59	< 0.001
DDD 300–500	1.00	0.14	–	7.33	1.000	1.77	0.72	–	4.34	0.213
DDD > 500										
Intensity of metformin use										
< 10	4.41	0.61	–	32.05	0.143	1.96	0.80	–	4.81	0.142
10–25	4.94	0.68	–	35.95	0.115	2.03	0.83	–	4.98	0.123
> 25	17.19	0.31	–	951.55	0.165	3.01	0.48	–	18.89	0.240
In patients aged ≥ 65										
cDDD of metformin use										
DDD < 300	1.62	1.50	–	1.76	< 0.001	1.47	1.40	–	1.53	< 0.001
DDD 300–500	1.82	1.29	–	2.58	< 0.001	1.72	1.41	–	2.09	< 0.001
DDD > 500	1.40	0.34	–	5.74	0.637	1.73	0.88	–	3.37	0.110
Intensity of metformin use										
< 10	2.05	0.49	–	8.54	0.322	1.43	0.72	–	2.82	0.304
10–25	2.27	0.55	–	9.46	0.259	1.56	0.79	–	3.09	0.197
> 25	3.07	0.16	–	57.84	0.454	1.70	0.42	–	7.01	0.460

Continued

Variables	Incident peripheral neuropathy									
	Two-year follow-up					Five-year follow-up				
	aOR[a]	95% CI			p-value	aOR[a]	95% CI			p-value
In patients with DCSI score 0										
cDDD of metformin use										
DDD < 300	1.83	1.71	–	1.96	< 0.001	1.67	1.60	–	1.73	< 0.001
DDD 300–500	2.39	1.98	–	2.88	< 0.001	1.92	1.71	–	2.14	< 0.001
DDD > 500	3.96	1.95	–	8.05	< 0.001	2.97	1.92	–	4.59	< 0.001
Intensity of metformin use										
< 10	1.81	1.69	–	1.94	< 0.001	1.66	1.60	–	1.72	< 0.001
10–25	1.86	1.73	–	2.00	< 0.001	1.67	1.60	–	1.74	< 0.001
> 25	2.42	2.01	–	2.92	< 0.001	1.94	1.74	–	2.17	< 0.001
In patients with DCSI score 1										
cDDD of metformin use										
DDD < 300	2.01	1.76	–	2.28	< 0.001	1.98	1.84	–	2.13	< 0.001
DDD 300–500	2.65	1.87	–	3.74	< 0.001	2.59	2.11	–	3.16	< 0.001
DDD > 500	3.13	0.97	–	10.08	0.056	2.19	0.97	–	4.95	0.060
Intensity of metformin use										
< 10	1.68	0.52	–	5.38	0.386	2.31	1.02	–	5.23	0.045
10–25	1.74	0.54	–	5.60	0.353	2.42	1.07	–	5.49	0.034
> 25	1.89	0.17	–	21.00	0.606	3.61	0.68	–	19.26	0.133
In patients with DCSI score ≥ 2										
cDDD of metformin use										
DDD < 300	2.22	1.90	–	2.60	< 0.001	1.88	1.72	–	2.05	< 0.001
DDD 300–500	2.58	1.73	–	3.87	< 0.001	2.30	1.83	–	2.90	< 0.001
DDD > 500	1.16	0.16	–	8.57	0.885	2.25	1.08	–	4.66	0.030
Intensity of metformin use										
< 10	4.72	0.65	–	34.44	0.126	1.89	0.92	–	3.86	0.083
10–25	5.76	0.79	–	42.07	0.084	2.08	1.01	–	4.27	0.046
> 25	13.09	0.23	–	740.24	0.212	2.45	0.55	–	10.84	0.238

Table 4. Stratified analysis of incident peripheral neuropathy in new-onset diabetes mellitus patients with metformin. [a]aOR, adjusted odds ratio. Extraneous factors adjusted in the model were patients' characteristics, DCSI, and comorbidities.

The prediction of DPN occurrence in T2DM patients undergoing metformin treatment is influenced by factors such as vitamin B12 or homocysteine levels. However, the absence of this data limits the ability to interpret the underlying mechanisms. Third, the diagnoses of DPN and other comorbidities are completely dependent on ICD- 9-CM codes and ICD- 10-CM code. Nonetheless, the NHI Bureau of Taiwan randomly reviews the charts and interviews patients to verify the accuracy of the diagnoses. These processes improve the accuracy and validity of NHIRD. We agree that it is possible there are some uncontrolled confounding variables not solved. However, we proved that the results were approximately similar to prospective cohort trials. Last, this study was conducted using data from the Taiwanese population. However, ethnic and genetic factors may also play a role in the development of DPN, which could limit the generalizability of our findings to other populations.

Conclusions

To sum up, this study provides large-scale population-based longitudinal evidence that metformin use was associated with DPN odds among T2DM patients in a dose-dependent manner. The results suggest that T2DM patients received < 300, 300–500, ≥ 300 cDDD of metformin or use intensity of < 10, 10 ~ 25, > 25 DDD/month experienced higher risk of DPN at 2 and 5 years. The higher cDDD of metformin or use intensity, the higher DPN risk was found in this study. T2DM patients receiving metformin, being elderly and having a higher DCSI score were associated with an increased risk of DPN. In addition, T2DM patients comorbid with cerebrovascular disease also had a higher risk for the development of DPN. However, the lack of vitamin B12 data hinders a clear understanding of the underlying mechanisms. Comprehensive, large-scale studies are needed to assess the potential risks of metformin therapy for DPN in T2DM.patients.

Methods

Data source

This study used secondary data from the Longitudinal Health Insurance Database (LHID), a subset of the NHIRD, from 2001 to 2018 provided by the Health and Welfare Data Science Center (HWDC) of the Ministry of Health and Welfare in Taiwan. The LHID contains information on all beneficiaries enrolled in Taiwan's National

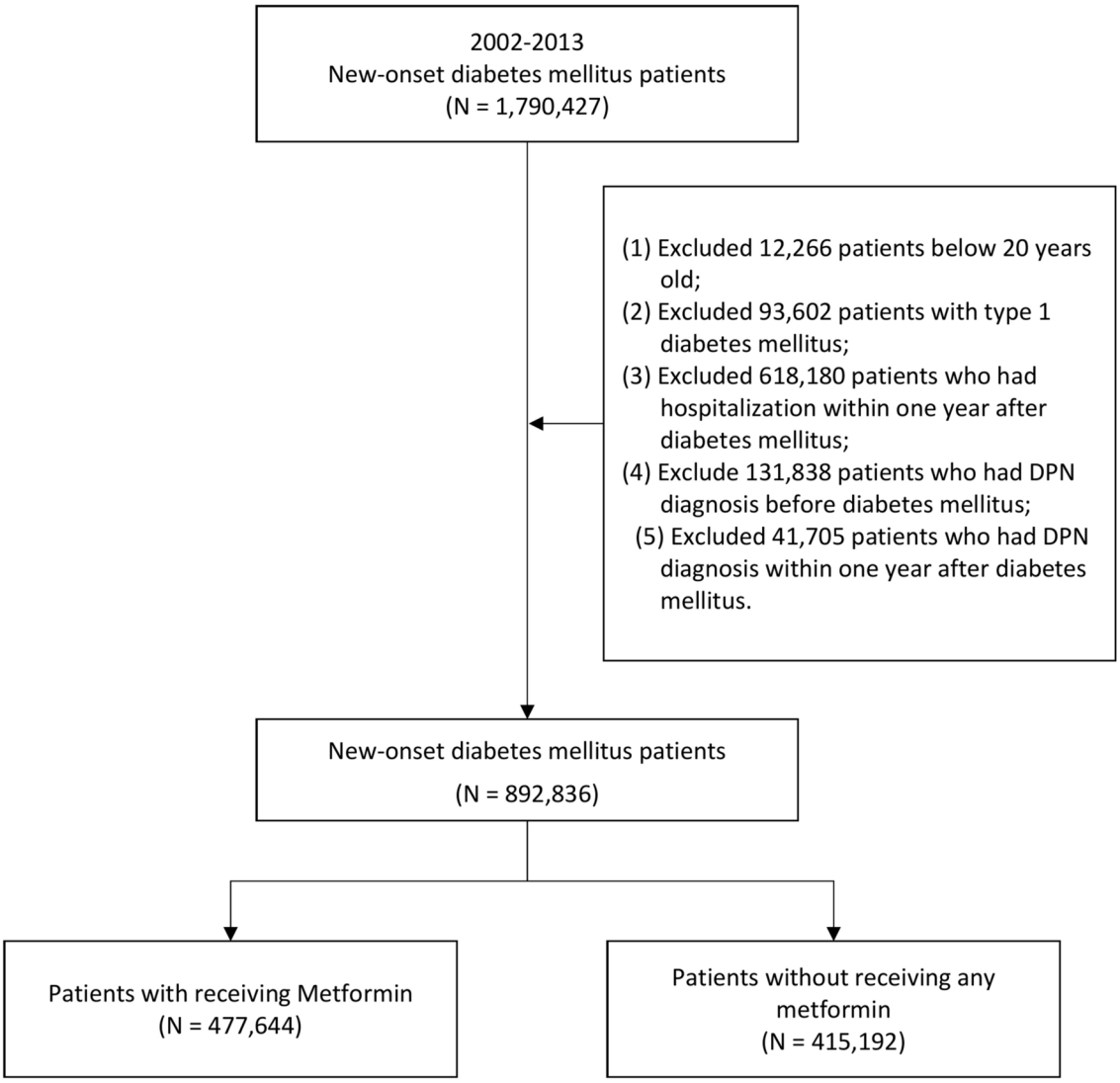

Fig. 1. Patient selection process. *DPN* diabetic peripheral neuropathy.

Health Insurance (NHI) program, which is a government-run, single-payer national social insurance program that has operated since 1995. The NHI contains health insurance claims data for 99% of Taiwan's 23 million residents. Disease diagnoses were coded according to the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) and ICD, 10th Revision, Clinical Modification (ICD-10-CM). The NHIRD can be used to obtain real-world evidence to support clinical decisions and health-care policy making[38,39]. This study was conducted in accordance with the Declaration of Helsinki. The patient data were extracted from the LHID released by the Health and Welfare Data Science Center, in which NHI beneficiaries are represented using scrambled random identification numbers to protect their privacy. Our study protocol was approved by the Central Regional Research Ethics Committee of China Medical University, Taiwan (No. CRREC-109–011). Because we used only de-identified data in the current study, the requirement for informed consent was waived.

Study subjects

We enrolled ≥ 20-year-old patients given a diagnosis of new-onset DM (ICD-9-CM: 250) over 2002–2013. A DM diagnosis was considered to be the presence of three outpatient DM diagnoses. Metformin use was coded using the Anatomical Therapeutic Chemical code A10BA02. To reduce bias, we excluded (1) patients with type 1 DM, (2) patients given a diagnosis of DPN before or within the first year of their DM diagnosis, and (3) patients hospitalized within 1 year of their DM diagnosis.

Figure 1 presents a flowchart of the patient selection process. We included 892,836 patients given a diagnosis of new-onset DM over 2002–2013. The case group comprised 477,644 patients treated with metformin within the first year of their DM diagnosis, whereas the comparison group comprised 415,192 patients not treated with metformin.

Study designs

We measured metformin intake in terms of the defined daily dose (DDD) of metformin—which is a standard method for measuring drug use and exposure. According to the World Health Organization, the DDD is defined as the assumed average maintenance dose per day in adults. However, the DDD does not necessarily reflect the recommended or prescribed daily dose[40]. One unit of DDD of metformin is equal to 2 g, which was used to measure the medication unit[41]. Several predictors were used to examine the correlation between metformin use and the risk of DPN. The first predictor was years of metformin use compared with metformin no use. The second predictor was the cumulative dose of metformin use, which was measured using the cumulative defined daily dose (cDDD).

In the current study, the observation period before metformin treatment was 1 year after initial DM diagnosis. A metformin DDD of 2 g was considered the baseline dose[41]. Based on the study design from several researches, we used 2 criteria for analyzing the dose-dependency of the metformin treatment–DPN risk association: The first criterion was defined using 3 ranges of cumulative DDD (cDDD) of metformin in the first year: < 300, 300–500, and > 500. The second criterion was defined using 3 ranges of the average monthly intensity of metformin use: < 10, 10–25, and > 25[11,42]. All patients were observed at their 2- and 5-year follow-ups after initial DM diagnosis.

The presence of DPN (ICD-9-CM: 250.6, 250.7, and 250.9; ICD-10-CM: E10.4, E11.4, E12.4, E13.4, E14.4, G63.2, and G62.9) was defined as a patient receiving ≥ 3 DPN diagnoses within 1 year. Sex, age, income level, urbanization level, diabetes severity, and comorbidities were considered control variables. We used the Diabetes Complications Severity Index (DCSI) to evaluate diabetes severity and assess adverse outcome risk; the index was calculated using the information from seven diabetes complication categories (retinopathy, nephropathy, neuropathy, cerebrovascular, cardiovascular, peripheral vascular disease, and metabolic)[31,43].

We also considered the following comorbidities: hyperuricemia (ICD-9-CM: 790.6), cerebrovascular disease (CVD; ICD-9-CM: 430–438), alcoholism (ICD-9-CM: 303), rheumatoid arthritis (RA; ICD-9-CM: 714), sleep disturbance (ICD-9-CM: 780), systematic lupus erythematosus (SLE; ICD-9-CM: 710.0), migraine (ICD-9-CM: 346.90), coronary artery disease (CAD; ICD-9-CM: 410–414), peripheral arterial disease (PAD; ICD-9-CM: 443.9), depression (ICD-9-CM: 296.2 and 296.3), sarcopenia (ICD-9-CM: 724.8, 728.3, 728.8, and 728.9), obesity (ICD-9-CM: 278.0), diabetic retinopathy (ICD-9-CM: 362.0), and chronic kidney disease (CKD; ICD-9-CM: 585).

Statistical analysis

All analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC, USA). The chi-square test was used to evaluate differences in the baseline characteristics between the patients treated and not treated with metformin. The difference in DPN risk among the patients treated with metformin was estimated using multiple logistic regression with adjustments for sex, age, income level, urbanization level, diabetes severity, and comorbidities. Odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated. Two adjusted models were developed to estimate metformin cDDD and metformin treatment intensities (expressed as DDDs/month). Furthermore, we conducted a stratified analysis to investigate the association between metformin use and DPN, containing sex, age, and DCSI. A P value of < 0.05 was considered to indicate statistical significance.

Data availability

The data that support the findings of this study are available from Health and Welfare Data Science Center, Ministry of Health and Welfare (HWDC, MOHW) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the corresponding authors upon reasonable request and with permission of Health and Welfare Data Science Center, Ministry of Health and Welfare (HWDC, MOHW, https://dep.mohw.gov.tw/dos/np-2497–113.html).

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Author contributions

All authors have participated in this study and have reviewed and agree with the final manuscript. Kuang-Hua Huang: Conceptualization, Funding acquisition, Methodology, Validation, Writing – original draft, and Writing – review & editing. Shiang-Wen Huang: Formal analysis and Writing – review & editing. Yih Yang: Formal

analysis, and Writing – review & editing. Shuo-Yan Gau: Formal analysis and Writing – review & editing. Tung-Han Tsai: Formal analysis, and Writing – review & editing. Ya-Lan Chang: Data curation, Formal analysis, and Writing – review & editing. Chien-Ying Lee: Conceptualization, Methodology, Validation, Writing – original draft, and Writing – review & editing.

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Declarations

Competing interests
The authors declare no competing interests.

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