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Clinical Research Article

Renal Protective Effect of Metformin in Type 2 Diabetes Patients

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Abstract

Background: Inhibiting the development and progression of diabetic kidney disease (DKD) is an important issue, but the renoprotective effect of metformin is still controversial.

Objective: To assess the renoprotective effect of metformin in patients with type 2 diabetes.

Methods: This retrospective observational multicenter cohort study included 316 693 patients with type 2 diabetes from 7 hospitals. After matching for age, gender, medical year, baseline estimated glomerular filtration rate (eGFR), urine protein (dipstick), glycated hemoglobin (HbA1c) and propensity score; a total of 13 096 metformin and 13 096 non-metformin patients were included. The main results were doubling of serum creatinine, eGFR $\leq$ 15 mL/min/1.73 m^2 and end-stage kidney disease (ESKD).

Results: After conducting a multivariable logistic regression analysis on the variables, the metformin group was revealed to have better renal outcomes than the non-metformin group, including a lower incidence of doubling of serum creatinine (hazard ratio [HR], 0.71; 95% CI, 0.65-0.77), eGFR $\leq$ 15 mL/min/1.73 m^2 (HR 0.61; 95% CI, 0.53-0.71), and ESKD (HR 0.55; 95% CI, 0.47-0.66). The subgroup analyses revealed a consistent renoprotective effect across patients with various renal functions. Furthermore, when considering factors such as age, sex, comorbidities, and medications in subgroup analyses, it consistently showed that the metformin group experienced a slower deterioration in renal function across nearly all patient subgroups.

Conclusion: Metformin decreased the risk of renal function deterioration.

Key Words: chronic kidney disease, diabetes mellitus, diabetic kidney disease, end-stage kidney disease, kidney, metformin, renal function

Abbreviations: AMPK, AMP-activated protein kinase; CKD, chronic kidney disease; DKD, diabetic kidney disease; DM, diabetes mellitus; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GLP-1, glucagon-like peptide 1; HbA1c, glycated hemoglobin; HR, hazard ratio; ICD, International Classification of Diseases; NF-κB, nuclear factor-kappa B; NNT, number needed to treat; SGLT2, sodium-glucose cotransporter type 2.

Diabetic kidney disease (DKD) is the main cause of end-stage kidney disease (ESKD). Inhibiting the development and progression of DKD is an important issue. However, effective therapy for DKD is still limited.

Metformin is an oral antidiabetic agent that belongs to the biguanide class. Metformin reduces intestinal glucose absorption, improves peripheral glucose uptake, reduces fasting insulin concentration, and increases insulin sensitivity of peripheral tissues (1). In addition, metformin inhibits gluconeogenesis by activating AMP-activated protein kinase (AMPK) (2). AMPK is an important participant in regulating energy metabolism and plays a key role in diabetes and related diseases. Studies have shown that AMPK is necessary to maintain glucose stability (3). Because metformin has the advantages of low cost, safety, and cardioprotective effect, it is now the first-line recommended drug for patients with type 2 diabetes mellitus (DM) according to the guidelines of American Diabetes Association/European Association for Study of Diabetes.

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Previous animal experiments have shown that metformin can exert renal protection through a variety of mechanisms (4-17). However, it is worth noting that previous studies among diabetic patients have yielded conflicting results regarding the efficacy of metformin in renal protection. While certain investigations suggest a beneficial impact, others fail to demonstrate significant effect (18-24). Therefore, the purpose of this study was to explore whether metformin has a renoprotective effect in patients with type 2 diabetes in clinical settings.

Patients and Methods

Study Design

This is a multicenter, retrospective observational cohort study. Patients who have received diabetes treatment in the institution within a specified period of time were grouped according to their use or not of metformin. The changes in kidney function in different groups over time were monitored.

Database and Study Sample

A retrospective cohort study was conducted. All included diabetes patients were treated at Chang Gung Memorial Hospital, which is composed of 7 medical institutions from the northeast to the south of Taiwan from January 2006 to December 2016 ($n = 316\,693$). Unidentified patient data were retrieved from electronic medical records, including birth date, sex, diagnostic codes according to International Classification of Diseases (ICD)-9 and ICD-10-Clinical Modification, drug prescriptions, and laboratory test results.

In this study (Fig. 1), patients with type 2 DM between January 2006 to December 2016 were included for analysis ($n = 316\,693$). Patients who did not receive any diabetes medications during identification period ($n = 104\,765$) were excluded (refusing medicine, not requiring drug treatment, or receiving treatment in an external hospital). Subsequently, only patients newly diagnosed with type 2 DM and who began treatment between January 2007 and December 2016 were included in the study ($n = 137\,514$). Patients who were already undergoing treatment for diabetes before January 2007 were excluded from the study. These patients were then divided into 2 groups based on whether they used metformin in their initial prescription for diabetes. If a patient in the non-metformin group received metformin after the index date, they were also excluded. After that, we excluded patients aged < 18 years, without baseline estimated glomerular filtration rate (eGFR) data, baseline eGFR < 30 mL/min/1.73 m^2, without urine dipstick, or glycated hemoglobin (HbA1c) data during identification period. The number of patients in the group who received metformin was 56 451 and the number in the non-metformin group was 16 327. Then the metformin group was randomly selected from the remaining patients at a ratio of 1:1 with the non-metformin group according to age, gender, medical year (which corresponds to the index date), baseline eGFR, urine protein (dipstick), HbA1c, and propensity score matching. Finally, a metformin group ($n = 13\,096$) and a non-metformin group ($n = 13\,096$) were included.

Matching

To ensure comparability between the groups, individual matching for age, gender, medical year, baseline eGFR, urine protein (dipstick), HbA1c, and propensity score (caliper = 0.1) matching was used in this study. The propensity scores were estimated using logistic regression analyses and were adjusted for baseline eGFR, age, sex, comorbidity (hypertension, hyperlipidemia, coronary artery disease, cerebrovascular disease, congestive heart failure, gout, nephrolithiasis, chronic hepatitis and liver cirrhosis, autoimmune disorders, chronic lung disease, malignancy) and initial medication in the index data (sulfonylurea, meglitinide, alpha-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase-4 [DPP4] inhibitors, insulin, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, renin inhibitors, statin, anti-gout, calcium channel blockers, beta blockers, diuretics, aspirin, and nonsteroidal anti-inflammatory drugs). Propensity scores were not adjusted for glucagon-like peptide-1 (GLP-1) inhibitors and sodium-glucose cotransporter type 2 (SGLT2) inhibitors due to the small percentage of patients initially using them, as indicated in the index data.

Data Collection

The eGFR is calculated from serum creatinine using the Chronic Kidney Disease Epidemiology Collaboration equation (25).

Outcome

The main results were doubling of serum creatinine sustained for at least 3 months, eGFR ≤ 15 mL/min/1.73 m^2 sustained for at least 3 months, and ESKD. The ESKD was defined as eGFR ≤ 15 mL/min/1.73 m^2 sustained for at least 3 months combined with an ESKD code (ICD-9: 585.6 or ICD-10: N18.6) or kidney transplant status code (ICD-9: V42.0 or ICD-10: Z94.0).

Duration of Follow-Up/Censor Date

The duration of follow-up or censor date varied across participant groups in the study. For instances in which participants remained under continuous medical care until the end date of the 2016 database, follow-up extended until the conclusion of the database, serving as the censor date. Participants experiencing outcomes prior to the end of the database were followed until the occurrence of the outcome. Individuals who passed away during follow-up were tracked until their date of death, while those lost to follow-up were monitored until their last recorded medical visit date.

Statistical Analysis

To compare the baseline characteristics, the χ^2 test was used for categorical variables and the unpaired Student *t* test was performed for continuous variables. The Cox proportional hazards model was used to calculate hazard ratios (HRs) and 95% CIs. Multivariable Cox proportional hazard models were then used to investigate the effect of metformin on renal outcome. Follow-up duration was defined as the time from initiation of therapy to death, or to the last follow-up date. We use several different models. In Model 1, we adjusted for age, sex, and comorbidity (where absolute standardized mean difference is more than 0.1). In Model 2, we adjusted for age, sex, comorbidity, and laboratory data (systolic blood pressure, diastolic blood pressure, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, hemoglobin, and HbA1c). In Model 3, we adjusted

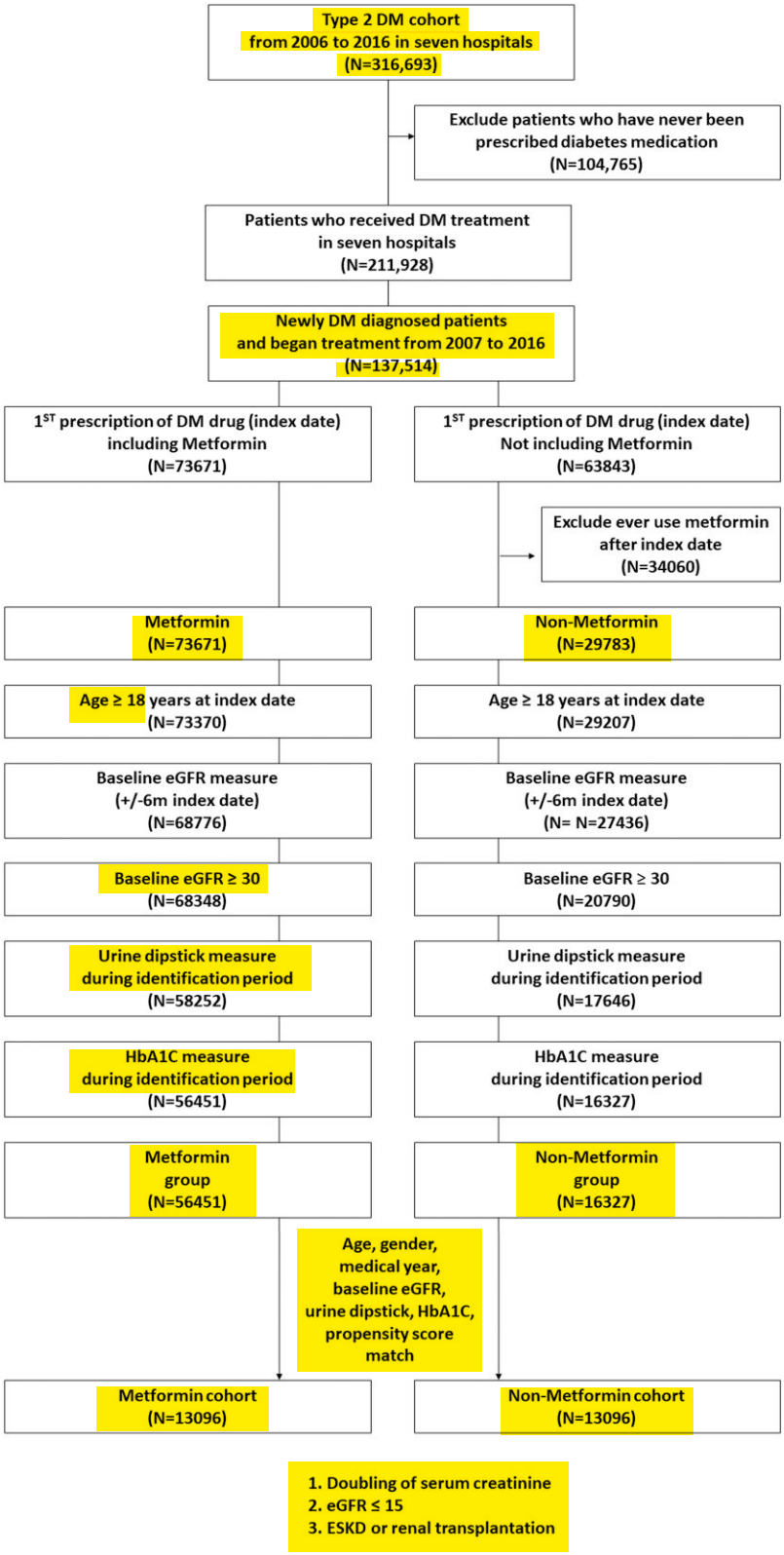

Figure 1. Study flow chart of metformin effect on kidney function.

for age, sex, comorbidity, laboratory data, and medication ever used throughout the entire follow-up period, including sulfonylurea, meglitinide, alpha-glucosidase inhibitors, thiazolidinediones, DPP4 inhibitors, GLP-1 inhibitors, SGLT2 inhibitors, insulin, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, renin inhibitor, statin, anti-gout, calcium channel blockers, beta blockers, diuretics, aspirin, and nonsteroidal anti-inflammatory drugs. In Model 4, a competing risk model was utilized. In Model 5, a time and dose-dependent Cox model was employed. All statistical analyses were

conducted using SAS 9.4 statistical software (SAS Institute Inc., Cary, NC, USA) and R statistical software, version 3.0.3 (R Foundation for Statistical Computing).

Sensitivity Analyses

To test the impact of another potential residual confounder on the observed result, we used the R-package "obsSens." (26) In this analysis, we added another hypothetical unmeasured confounder with a similar risk effect as our result. Subsequently, we tested how this added factor confounded our observation with different prevalence in the metformin and non-metformin groups (Supplementary Figs. S1-S3) (27-29).

Results

Baseline Characteristics

Table 1 presents the demographic characteristics of the 2 groups stratified by metformin use. There were 13 096 and 13 096 patients in the metformin and non-metformin groups, respectively. The median eGFR was 79.5 mL/min/1.73 m^2 (58.3-104.6 mL/min/1.73 m^2) in the metformin group and 78.5 mL/min/1.73 m^2 (57.5-104.7 mL/min/1.73 m^2) in the

Table 1. Demographic characteristics of the metformin and non-metformin cohorts before and after matching

Variables	Before matching			After matching		
	Metformin (N = 56451)	Non-Metformin (N = 16327)	aSMD	Metformin (N = 13096)	Non-Metformin (N = 13096)	aSMD
	n (%)/median (IQR)	*n* (%)/median (IQR)		*n* (%)/median (IQR)	*n* (%)/median (IQR)	
Baseline eGFR, mL/min/1.73^2			0.716			0
≥90	29789 (52.77)	5503 (33.70)		4986 (38.07)	4986 (38.07)	
60 ≤ 90	21036 (37.26)	4678 (28.65)		4465 (34.09)	4465 (34.09)	
45 ≤ 60	4267 (7.56)	2931 (17.95)		2422 (18.49)	2422 (18.49)	
30 ≤ 45	1359 (2.41)	3215 (19.69)		1223 (9.34)	1223 (9.34)	
Median (IQR)	91.77 [74.14,113.22]	72.37 [48.92,101.44]	0.605	79.51 [58.34,104.65]	78.58[57.53,104.71]	0.028
Urine dipstick			0.370			0
Negative	38962 (69.02)	8746 (53.57)		7787 (59.46)	7787 (59.46)	
Trace	6698 (11.87)	2031 (12.44)		1639 (12.52)	1639 (12.52)	
1+	5276 (9.35)	2101 (12.87)		1555 (11.87)	1555 (11.87)	
2+	3969 (7.03)	2082 (12.75)		1428 (10.90)	1428 (10.90)	
3+ & 4+	1546 (2.74)	1367 (8.37)		687 (5.25)	687 (5.25)	
Age			0.353			0
<45	7243 (12.83)	1497 (9.17)		1019 (7.78)	1019 (7.78)	
45 ≤ 55	12624 (22.36)	2386 (14.61)		1962 (14.98)	1962 (14.98)	
55 ≤ 65	16977 (30.07)	3863 (23.66)		3203 (24.46)	3203 (24.46)	
≥65	19607 (34.73)	8581 (52.56)		6912 (52.78)	6912 (52.78)	
Median (IQR)	59.71 [51.30,69.20]	66.07 [55.69,75.74]	0.372	66.06 [56.09,74.23]	66.11 [56.14,75.73]	0.051
DM duration (months)	0.36 [0.00,3.35]	0.79 [0.00,10.84]	0.141	0.46 [0.00,5.52]	0.76 [0.00,10.63]	0.072
Follow-up duration (years)	3.72 [1.69,6.25]	2.53 [0.93,4.76]	0.368	3.55 [1.59,5.98]	2.59 [0.95,4.85]	0.292
Male	30441 (53.92)	9611 (58.87)	0.100	7399 (56.50)	7399 (56.50)	0
Comorbidity						
Hypertension	25203 (44.65)	8613 (52.75)	0.163	6578 (50.23)	6741 (51.47)	0.025
Hyperlipidemia	16489 (29.21)	3791 (23.22)	0.137	3486 (26.62)	3147 (24.03)	0.060
Coronary artery disease	6243 (11.06)	2174 (13.32)	0.069	1684 (12.86)	1638 (12.51)	0.011
Cerebrovascular disease	7366 (13.05)	2722 (16.67)	0.102	2116 (16.16)	2212 (16.89)	0.020
Congestive heart failure	2031 (3.60)	1123 (6.88)	0.148	646 (4.93)	812 (6.20)	0.055
Gout	3012 (5.34)	1300 (7.96)	0.106	856 (6.54)	940 (7.18)	0.025
Nephrolithiasis	1619 (2.87)	498 (3.05)	0.011	357 (2.73)	385 (2.94)	0.013
Chronic hepatitis and liver cirrhosis	7828 (13.87)	2541 (15.56)	0.048	1703 (13.00)	2088 (15.94)	0.084
Autoimmune disorders	554 (0.98)	166 (1.02)	0.004	107 (0.82)	142 (1.08)	0.028
Chronic lung disease	3597 (6.37)	1381 (8.46)	0.080	999 (7.63)	1130 (8.63)	0.037
Malignant	7326 (12.98)	2815 (17.24)	0.119	1778 (13.58)	2281 (17.42)	0.106
Laboratory data						
SBP, ≥ 140 mmHg	24698 (45.21)	6803 (44.64)	0.011	5905 (46.64)	5384 (44.16)	0.050
DBP, ≥ 90 mmHg	9211 (16.86)	2101 (13.79)	0.085	1956 (15.45)	1652 (13.55)	0.054

(*continued*)

Table 1. Continued

Variables	Before matching			After matching		
	Metformin (N = 56451)	Non-Metformin (N = 16327)	aSMD	Metformin (N = 13096)	Non-Metformin (N = 13096)	aSMD
	n (%)/median (IQR)	*n* (%)/median (IQR)		*n* (%)/median (IQR)	*n* (%)/median (IQR)	
TC, ≥ 200 mg/dL	20661 (37.77)	4554 (30.29)	0.158	4481 (35.54)	3614 (30.01)	0.118
LDL, ≥ 100 mg/dL	33643 (62.61)	7874 (54.59)	0.163	7494 (60.68)	6328 (54.91)	0.117
HDL, ≥40 mg/dL	34641 (64.77)	8175 (57.16)	0.157	7679 (62.51)	6718 (58.74)	0.077
TG, ≥ 150 mg/DL	23991 (44.15)	5937 (40.11)	0.082	5296 (42.35)	4672 (39.43)	0.059
Hb, ≥ 12 g/dL	39926 (78.85)	8785 (57.45)	0.472	8700 (72.19)	7458 (61.17)	0.236
HbA1c			0.114			0
≥ 9%	17449 (30.91)	4768 (29.20)		3729 (28.47)	3729 (28.47)	
8%≤ 9%	7796 (13.81)	2272 (13.92)		1770 (13.52)	1770 (13.52)	
7%≤ 8%	14657 (25.96)	3766 (23.07)		3045 (23.25)	3045 (23.25)	
< 7%	16549 (29.32)	5521 (33.82)		4552 (34.76)	4552 (34.76)	
Initial medications in the index data						
Sulfonylurea, yes	579 (1.03)	219 (1.34)	0.029	136 (1.04)	147 (1.12)	0.008
Meglitinide, yes	17 (0.03)	46 (0.28)	0.064	2 (0.02)	15 (0.11)	0.039
AGIs, yes	19 (0.03)	56 (0.34)	0.071	3 (0.02)	13 (0.10)	0.031
TZD, yes	16 (0.03)	10 (0.06)	0.016	2 (0.02)	6 (0.05)	0.017
DPP4 inhibitor, yes	16 (0.03)	10 (0.06)	0.027	57 (0.44)	49 (0.37)	0.010
GLP-1, yes	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0
SGLT2 inhibitor, yes	0 (0.00)	3 (0.02)	0.019	0 (0.00)	3 (0.02)	0.021
Insulin, yes	99 (0.18)	983 (6.02)	0.342	74 (0.57)	75 (0.57)	0.001
ACEI, yes	1826 (3.23)	463 (2.84)	0.023	380 (2.90)	376 (2.87)	0.002
ARB, yes	8223 (14.57)	2384 (14.60)	0.001	1971 (15.05)	1904 (14.54)	0.014
Renin inhibitor, yes	143 (0.25)	51 (0.31)	0.011	32 (0.24)	35 (0.27)	0.005
Statin, yes	7575 (13.42)	1821 (11.15)	0.069	1663 (12.70)	1505 (11.49)	0.037
Anti-gout, yes	1724 (3.05)	769 (4.71)	0.086	551 (4.21)	576 (4.40)	0.009
CCBs, yes	8955 (15.86)	2444 (14.97)	0.025	2157 (16.47)	1940 (14.81)	0.046
Beta blockers, yes	7529 (13.34)	1931 (11.83)	0.046	1708 (13.04)	1551 (11.84)	0.036
Diuretics, yes	7054 (12.50)	2895 (17.73)	0.147	1948 (14.87)	2219 (16.94)	0.057
Aspirin, yes	6223 (11.02)	1879 (11.51)	0.015	1625 (12.41)	1530 (11.68)	0.022
NSAID, yes	4180 (7.40)	1147 (7.03)	0.015	960 (7.33)	978 (7.47)	0.005

Match were made with age (< 45, 45-54, 55-64, ≥ 65 years), gender, medical year (which corresponds to the index date), baseline eGFR (30 ≤ 45, 45 ≤ 60, 60 ≤ 90, 90 mL/min/1.73^2), urine dipstick, HbA1c, propensity score (caliper = 0.1).
Propensity score matching treatment model include baseline eGFR, age, sex, comorbidity, and initial medication.
Abbreviations: ACEI, angiotensin-converting enzyme inhibitors; AGI, alpha-glucosidase inhibitor; ARB, angiotensin-receptor blocker; aSMD, absolute standardized mean difference; CCB, calcium channel blocker; DBP, diastolic blood pressure; DPP4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide-1; Hb, hemoglobin; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; NSAID, nonsteroidal anti-inflammatory drug; SBP, systolic blood pressure; SGLT2, sodium-glucose cotransporter type 2; TC, total cholesterol; TG, triglyceride; TZD, thiazolidinedione.

non-metformin group. The median age was 66.0 (56.0-74.2) years in the metformin group and 66.1 (56.1-75.7) years in the non-metformin group. Table 1 also displays all the initial medications used for diabetes treatment in both the metformin and non-metformin groups at the beginning.

Incidence of Doubling of Serum Creatinine

Supplementary Table S1 shows the number of events of doubling of serum creatinine during the follow-up period (30). Among all patients, 2224 patients developed doubling of serum creatinine during the study period (1038 patients in the metformin group and 1186 patients in the non-metformin group).

In Table 2, after multivariable Cox proportional hazard regression analysis of variables, the results showed that the metformin group had a significantly lower incidence of doubling of serum creatinine (HR 0.71; 95% CI, 0.65-0.77; adjusted model 3). A subgroup analysis stratified according to the baseline renal function was then performed and patients were divided into 4 groups: group 1, eGFR ≥ 90 mL/min/1.73 m^2; group 2, 60 ≤ eGFR < 90 mL/min/1.73 m^2, group 3, 45 ≤ eGFR < 60 mL/min/1.73 m^2; and group 4, 30 ≤ eGFR < 45 mL/min/1.73 m^2. The results showed a lower incidence of serum creatinine doubling in the metformin group with different renal functions. Furthermore, we performed competing risk analysis using adjusted model 4, which involved subdistribution hazard function, with death considered as a

Table 2. Adjusted hazard ratios of renal function deterioration among the cohort of sampled patients during the follow-up years

	All			eGFR ≥ 90 mL/min/1.73 m^2			60 mL/min/1.73 m^2 ≤ eGFR, < 90 mL/min/1.73 m^2			45 mL/min/1.73 m^2 ≤ eGFR, < 60 mL/min/1.73 m^2			30 mL/min/1.73 m^2 ≤ eGFR, < 45 mL/min/1.73 m^2		
	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value	HR	95% CI	*P* value
Doubling of serum creatinine															
Model 1[a]	0.67	(0.62-0.73)	<.0001	0.69	(0.60-0.80)	<.0001	0.63	(0.54-0.74)	<.0001	0.66	(0.56-0.79)	<.0001	0.71	(0.58-0.86)	.0007
Model 2[b]	0.75	(0.69-0.82)	<.0001	0.79	(0.68-0.93)	.0036	0.72	(0.61-0.86)	.0002	0.70	(0.58-0.84)	.0001	0.78	(0.64-0.96)	.0204
Model 3[c]	0.71	(0.65-0.77)	<.0001	0.73	(0.62-0.85)	<.0001	0.68	(0.57-0.81)	<.0001	0.68	(0.56-0.81)	<.0001	0.74	(0.60-0.91)	.0042
Model 4[d]	0.72	(0.66-0.79)	<.0001	0.74	(0.63-0.87)	.0003	0.70	(0.59-0.83)	<.0001	0.70	(0.58-0.85)	.0002	0.75	(0.61-0.92)	.0071
Model 5[e]	0.69	(0.64-0.75)	<.0001	0.76	(0.67-0.86)	<.0001	0.61	(0.52-0.71)	<.0001	0.70	(0.60-0.83)	<.0001	0.69	(0.57-0.84)	.0002
Estimated GFR ≤ 15															
Model 1[a]	0.63	(0.55-0.72)	<.0001	0.66	(0.40-1.09)	.1074	0.56	(0.41-0.77)	.0004	0.60	(0.47-0.76)	<.0001	0.68	(0.55-0.84)	.0005
Model 2[b]	0.68	(0.59-0.79)	<.0001	0.80	(0.47-1.36)	.4181	0.62	(0.44-0.87)	.0058	0.61	(0.48-0.79)	.0002	0.75	(0.60-0.94)	.0120
Model 3[c]	0.61	(0.53-0.71)	<.0001	0.68	(0.40-1.16)	.1591	0.57	(0.40-0.80)	.0013	0.58	(0.45-0.75)	<.0001	0.71	(0.57-0.89)	.0028
Model 4[d]	0.63	(0.54-0.73)	<.0001	0.69	(0.40-1.21)	.1979	0.59	(0.42-0.83)	.0024	0.60	(0.46-0.79)	.0002	0.72	(0.57-0.91)	.0055
Model 5[e]	0.50	(0.43-0.57)	<.0001	0.47	(0.29-0.75)	.0015	0.39	(0.28-0.56)	<.0001	0.61	(0.47-0.78)	<.0001	0.67	(0.54-0.83)	.0002
ESKD															
Model 1[a]	0.56	(0.47-0.66)	<.0001	0.55	(0.29-1.02)	.0591	0.44	(0.30-0.66)	<.0001	0.56	(0.41-0.75)	.0001	0.64	(0.50-0.83)	.0007
Model 2[b]	0.62	(0.52-0.74)	<.0001	0.68	(0.35-1.30)	.2413	0.60	(0.40-0.92)	.0187	0.56	(0.41-0.77)	.0003	0.69	(0.53-0.90)	.0065
Model 3[c]	0.55	(0.47-0.66)	<.0001	0.58	(0.29-1.13)	.1064	0.56	(0.37-0.85)	.0065	0.53	(0.39-0.72)	<.0001	0.66	(0.51-0.87)	.0027
Model 4[d]	0.57	(0.47-0.68)	<.0001	0.59	(0.29-1.19)	.1386	0.58	(0.38-0.88)	.0106	0.55	(0.40-0.76)	.0003	0.67	(0.51-0.88)	.0037
Model 5[e]	0.38	(0.31-0.47)	<.0001	0.48	(0.27-0.87)	.0162	0.27	(0.15-0.46)	<.0001	0.53	(0.38-0.75)	.0004	0.54	(0.39-0.74)	.0001

Abbreviations: eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GFR, glomerular filtration rate; HR, hazard ratio.
[a]Adjusted for age, sex, and comorbidity.
[b]Adjusted for age, sex, comorbidity, and laboratory data.
[c]Adjusted for age, sex, comorbidity, laboratory data, and medication ever used throughout the entire follow-up period, including sulfonylurea, meglitinide, alpha-glucosidase inhibitors, thiazolidinedione, dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 inhibitors, sodium-glucose cotransporter type 2 inhibitors, insulin, angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, renin inhibitor, statin, anti-gout, calcium channel blockers, beta blockers, diuretics, aspirin, and NSAIDs.
[d]Sub-distribution hazard function adjusted for age, sex, comorbidity, laboratory data, and medication, with death considered as a competing risk.
[e]Time and dose-dependent Cox model treating the utilization of metformin as time and dose-dependent variables.

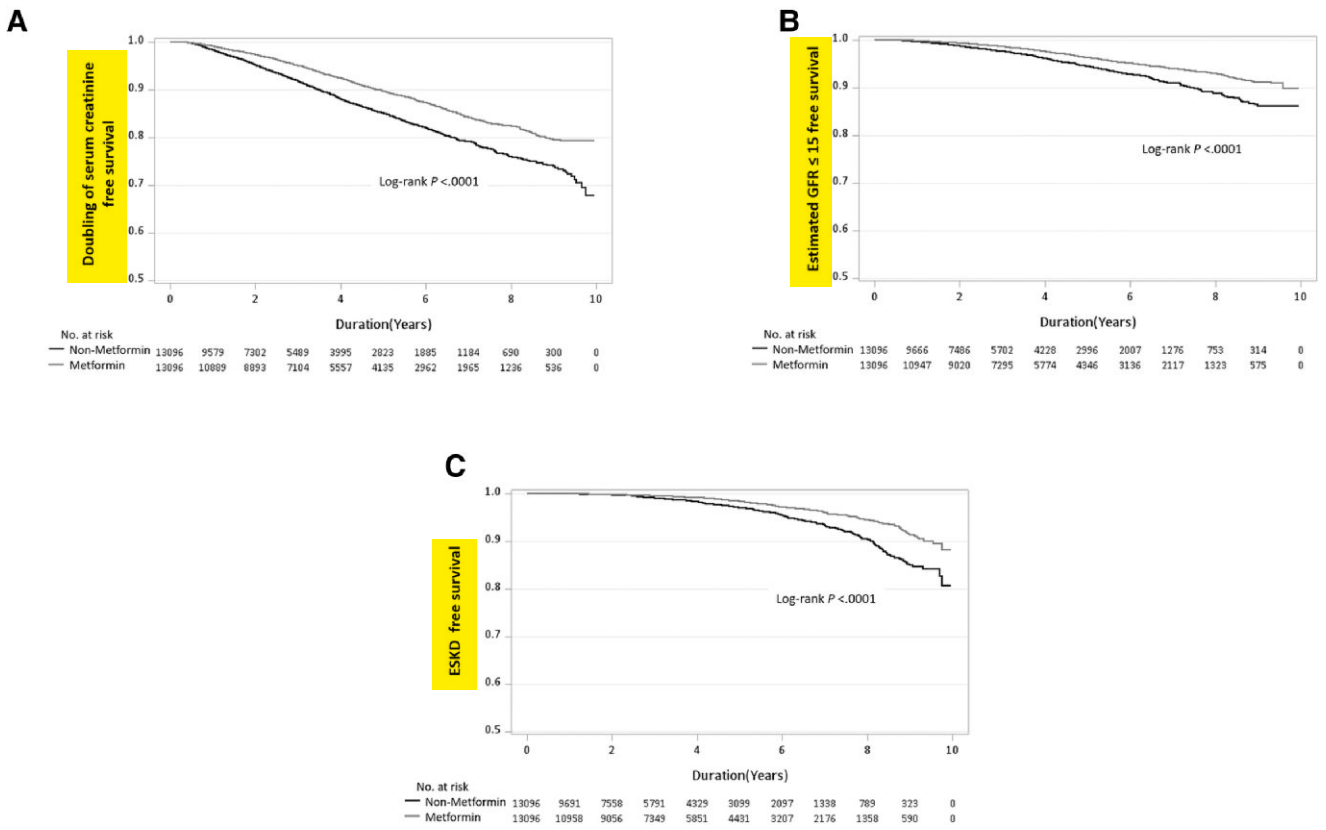

Figure 2. Kaplan-Meier curves for (A) doubling of serum creatinine, (B) eGFR ≤ 15 mL/min/1.73 m^2, and (C) ESKD in the total group of patients. After multivariable Cox proportional hazard regression analysis of variables, renoprotection effect was observed in the metformin group.

Table 3. Impact of metformin on renal outcome with number needed to treat analysis

Outcome	NNT
Doubling of serum creatinine	89
Estimated GFR ≤ 15	208
ESKD	239

Abbreviations: ESRD, end-stage renal disease; GFR, glomerular filtration rate; NNT, number needed to treat.

competing risk. Additionally, we conducted time and dose-dependent analyses using adjusted model 5, which employed a time and dose-dependent Cox model treating the utilization of metformin as time and dose-dependent variables. The results indicated that the lower incidence of renal endpoints in patients treated with metformin was similar after adjustment for other potential factors using models 1 to 5. In the Kaplan-Meier analysis, the metformin group had a lower incidence of doubling of serum creatinine ($P < .0001$) (Fig. 2A). We have also computed the number needed to treat (NNT), and the results are presented in Table 3 (31).

Incidence of eGFR ≤ 15 mL/min/1.73 m^2

Supplementary Table S1 shows the incidence of eGFR ≤ 15 mL/min/1.73 m^2 during the follow-up period (30). Of all patients, 815, including 376 in the metformin group and 439 in the non-metformin group, developed eGFR ≤ 15 mL/min/1.73 m^2 during the study period. In Table 2, after multivariable Cox proportional hazard regression analysis of variables, the results showed that the metformin group had a significantly lower incidence of eGFR ≤ 15 mL/min/1.73 m^2 during the follow-up period (HR 0.61; 95% CI, 0.53-0.71; adjusted model 3). Apart from the group with eGFR > 90 mL/min/1.73 m^2, the subgroup analyses revealed a consistent renoprotective effect in patients with varying levels of renal function. In competing risk analysis (adjusted model 4) and implemented time and dose-dependent analyses (adjusted model 5), the results remained largely consistent. In the Kaplan-Meier analysis, the metformin group had a lower incidence of eGFR ≤ 15 mL/min/1.73 m^2 ($P < .0001$) (Fig. 2B). We have also computed the NNT, and the results are presented in Table 3.

Incidence of ESKD

Supplementary Table S1 shows the incidence of ESKD during the follow-up period (30). Of all patients, 551, including 248 in the metformin group and 303 in the non-metformin group, developed ESKD during the study period. In Table 2, after multivariable Cox proportional hazard regression analysis of variables, the results showed that the metformin group had a significantly lower incidence of ESKD during the follow-up period (HR 0.55; 95% CI, 0.47-0.66; adjusted model 3). Apart from the group with eGFR > 90 mL/min/1.73 m^2, the subgroup analyses revealed a consistent renoprotective effect in patients with varying levels of renal function. In competing risk analysis (adjusted model 4) and implemented time and dose-dependent analyses (adjusted model 5), the results remained largely consistent. In the Kaplan-Meier analysis, the

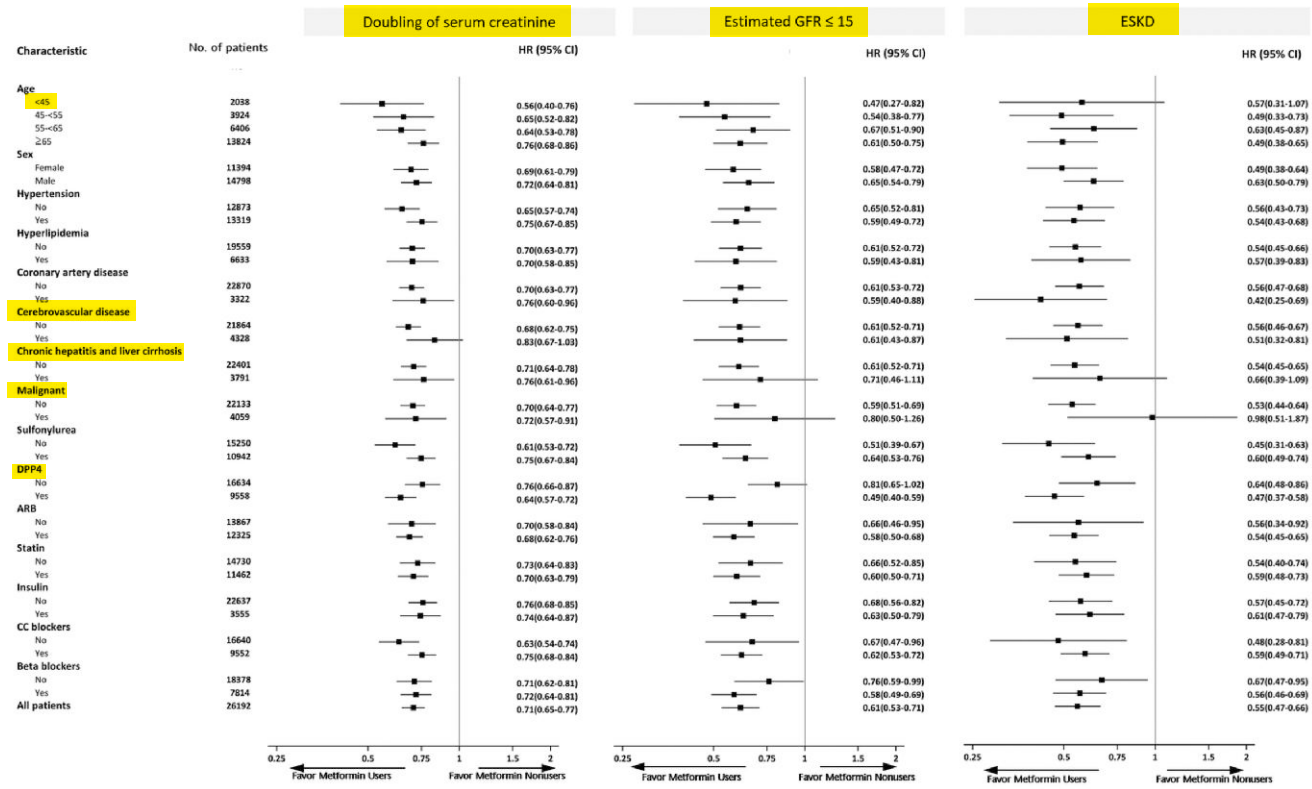

Figure 3. Subgroup analysis of doubling of serum creatinine, eGFR ≤ 15 mL/min/1.73 m^2, and ESKD among all patients. After a multivariable Cox proportional hazard regression analysis of variables, the metformin group showed slow renal function deterioration consistent across almost all patient subgroups.

metformin group had a lower incidence of ESKD ($P < .0001$) (Fig. 2C). We have calculated the NNT, and the findings are showcased in Table 3.

Subgroup Analysis

Subgroup analysis stratified according to comorbidity and medication was conducted. Following multivariable Cox proportional hazard regression analysis of variables, it was observed that the metformin group consistently exhibited slower renal function deterioration across most patient subgroups (Fig. 3). However, notable exceptions were observed in certain scenarios, where no significant difference was noted: among patients aged < 45 years, metformin did not demonstrate a significant effect on ESKD outcomes. Similarly, in patients with cerebrovascular disease, no significant difference was found in the doubling of serum creatinine outcome. In patients with chronic liver disease and malignancy, metformin did not significantly affect outcomes related to eGFR ≤ 15 mL/min/1.73 m^2 and ESKD. Additionally, in patients not using DPP4 inhibitors, no significant difference was observed in the outcome of eGFR ≤ 15 mL/min/1.73 m^2.

Sensitivity Analysis of Blood Glucose Control

As the prognosis of renal function is intertwined with patients' glycemic control, we performed a sensitivity analysis focusing on glycemic control (Supplementary Table S2) (32). We adjusted patient HbA1c levels as a time-varying covariate, and after a multivariable logistic regression analysis of variables, the metformin group exhibited a consistently slow renal function deterioration in nearly all patient subgroups.

Sensitivity Analysis of Potential Residual Confounder

To investigate the impact of another potential residual confounder on the observed result, we also conducted a sensitivity analysis to investigate the estimated trend of the metformin group, as well as the adjusted HR of the incidence of doubling of serum creatinine, eGFR ≤ 15 mL/min/1.73 m^2 and ESKD on a Cox proportional hazard regression model with the add-on of a residual confounding factor (Supplementary Figs. S1-S3) (27-29). For example, when all non-metformin subjects had the add-on residual confounder (prevalence of the unmeasured confounder is 1.0), and no subject in the metformin group had this residual confounder (prevalence of the unmeasured confounder is 0.0), the impact of metformin was protection for doubling of serum creatinine (HR, 0.42; the leftmost point on the bottom line in Supplementary Fig. S1) (27). Supplementary Fig. S1 shows that, in almost all situations, patients with metformin had a lower risk of doubling of serum creatinine occurrence relative to non-metformin subjects, even if an unmeasured confounder exists. Supplementary Figs. S2 and S3 also shows that, in most situations, metformin patients had a lower risk of eGFR ≤ 15 mL/min/1.73 m^2 and ESKD relative to non-metformin subjects (28, 29).

Discussion

In this study, 316 693 patients with diabetes were included to investigate whether metformin has a renoprotective effect. The results showed that patients with diabetes who took metformin were revealed to have better renal outcomes, including lower incidence of doubling of serum creatinine,

eGFR ≤ 15 mL/min/1.73 m^2, and ESKD. The subgroup analyses showed a consistent renoprotection effect in almost all groups.

Previous studies have investigated the renoprotective effect of metformin. However, the findings of these studies are controversial. In the United Kingdom Prospective Diabetes Study, Holman et al enrolled 5102 patients with type 2 DM, and 10 years of intensive metformin therapy resulted in a risk reduction of 16% in microvascular complications, defined as retinal photocoagulation, vitreous hemorrhaging, or renal failure (18). In a short-term 12-week study, Amador-Licona et al analyzed 51 type 2 DM patients, and the effect of switching from glibenclamide to metformin was examined (20). The results showed that metformin significantly decreased albuminuria by a mean of 24.2 mg/day. De Jager et al enrolled 390 type 2 DM patients with insulin treatment. Both metformin and placebo were added to the insulin therapy. After 4.3 years, there was no evidence to prove the superiority of metformin's effect on albuminuria, although metformin did improve endothelial function (22). In A Diabetes Outcomes Prevention Trial (ADOPT), Lachin et al studied 4351 patients with type 2 DM who were randomized to metformin or rosiglitazone or glyburide (23). After 5 years, the result showed metformin had no microvascular protection effect. Recently, Kwon et al studied 10 426 patients with type 2 DM and DKD, with a median follow-up period of 7.3 years. Findings indicated a significantly decreased risk of ESKD in the metformin group (21). The study by Yi et al examined the impact of metformin use on diabetic nephropathy and major adverse kidney events in patients with type 2 DM (24). They compared a control cohort of type 2 DM patients who were prescribed oral hypoglycemic agents other than metformin and never subsequently received metformin, with a cohort of type 2 DM patients prescribed metformin. After propensity score matching, 1994 individuals each from the metformin users and the control cohort were selected. The results showed that metformin use was consistently associated with a decreased risk of overt diabetic nephropathy and major adverse kidney events across various stages of renal function.

There are some limitations to previous studies. First, many of these studies included only a small number of subjects and did not include multiple center studies. In addition, the follow-up duration was relatively short. Second, the metformin and non-metformin groups usually had different baseline characteristics, which might have induced selection bias if only age- and sex-matched patients were used in the analysis. However, some previous studies did not use propensity score matching to correct for this bias. Third, albuminuria is a very important factor if the focus of the study was to investigate renal function deterioration in patients with diabetes, but these previous studies did not consider different degrees of albuminuria. Finally, it is important to mention that time-varying exposure models and competing risk analysis are valuable considerations; however, these aspects were not consistently incorporated into previous studies.

The mechanism by which metformin may have renoprotective effects is still not well known, and a multifactorial etiology is likely. There are several possible reasons for the protective effect of metformin on DKD (5-7, 10, 14, 15, 33-39). First, studies indicate that metformin may ameliorate glomerulosclerosis and fibrosis. Evidence suggests that in diabetic rats, metformin inhibits kidney transforming growth factor β expression and extracellular matrix production by mitigating oxidative stress and inflammation, thereby reducing proteinuria (6). Additionally, metformin has demonstrated the ability to reduce high glucose-induced activation of nuclear factor-kappa B (NF-κB) in vitro (35). Raphaëlle Corremans et al investigated the renoprotective effects of metformin in a rat model of DKD (33). Their study revealed that metformin reduced tubular injury and collagen accumulation. The dependent renal protective effect of metformin on AMPK has also been confirmed in a chronic kidney disease (CKD) animal model (5). Second, some studies have shown that metformin can ameliorate tubular injury (10, 36, 37). Metformin attenuates apoptosis by inhibiting NF-κB activation and reactive oxidant species (ROS) production in renal tubular cells (38, 39) In addition, Kim et al proved that lipotoxicity-induced apoptosis is mediated by the downregulation of GLP-1 receptor, and metformin can prevent it in vitro and in vivo (7). Third, previous studies have indicated that metformin can ameliorate autophagy. Metformin has been shown to reduce renal histological changes through autophagy by activating the AMPK/Sirt1/FoxO1 signaling pathway (14, 15). Furthermore, Raphaëlle Corremans et al discovered that metformin effectively slows the progression of nondiabetic CKD in rats, halting functional decline and reducing inflammation (34). Metformin's kidney protection is linked to Hippo signaling pathway activation, suggesting its potential in CKD treatment. Taken together, these results suggest that the renoprotective effect of metformin may be mediated by inducing autophagy and attenuating inflammation, oxidative stress, and fibrosis. Meanwhile, a pertinent issue that needs special mention is the role of metformin in inducing glycolysis in the kidney (8, 40-47). Although metformin has been shown to promote glycolysis in various tissues, including the liver and skeletal muscles, its specific effects on renal glycolysis and their implications for renal function and metabolism remain incompletely understood. A comprehensive understanding of these processes is essential for optimizing metformin therapy, particularly in individuals with diabetes and renal disease. Future research efforts should aim to unravel these mechanisms and clarify their relevance in the context of metformin therapy.

There were some limitations to this study. First, it is only an observational cohort study and not a randomized controlled trial. Second, the basic characteristics of the metformin and non-metformin groups were different. Although propensity score matching was used to correct for the difference, there were still some unmeasured confounding factors. Third, the diagnoses of ESKD and other comorbid medical conditions relied on administrative claims data and misclassification is possible. Another limitation of our study is the inability to further explore which patients may derive greater renal protective effects from metformin based on our research findings. For example, certain groups, such as individuals with high insulin resistance in type 2 DM (which has been shown to have higher rates of adverse cardiorenal outcomes) (48) could not undergo further analysis due to the lack of such data in our database.

In this extensive multicenter retrospective cohort study, metformin was identified to exhibit renoprotective effects.

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Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The data that support the findings of this study are available from Kaohsiung Chang Gung Memorial Hospital, but restrictions may apply to the availability of these data, which were approved by individual hospital institutional review board for the current study, and thus not publicly available. However, processed datasets can be requested and made available from the authors with the permission of Kaohsiung Chang Gung Memorial Hospital.

Ethics Approval

The study was approved by the ethics committee/institutional review board of the Chang Gung Memorial Hospital (IRB number: 202300420B0).

Informed Consent Statement

Informed consent is waived by the ethics committee. The administrative data is held by Kaohsiung Chang Gung Memorial Hospital. The access to the research data has been reviewed and approved by the Kaohsiung Chang Gung Memorial Hospital Institutes of Health Review Board.

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