CYP2C19 Loss-of-function Polymorphisms are Associated with Reduced Risk of Sulfonylurea Treatment Failure in Chinese Patients with Type 2 Diabetes

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Sulfonylureas (SUs) are predominantly metabolized by cytochrome p450 2C9 (CYP2C9) and cytochrome p450 2C19 (CYP2C19) enzymes. *CYP2C9* polymorphisms are associated with greater treatment response and hypoglycemic risk in SU users. However, there are no large scale pharmacogenetic studies investigating the effect of loss-of-function alleles *CYP2C19*2* and *CYP2C19*3*, which occur frequently in East Asians. Retrospective pharmacogenetic analysis was performed in 11,495 genotyped patients who were enrolled in the Hong Kong Diabetes Register between 1995 and 2017, with follow-up to December 31, 2019. The associations of *CYP2C19* polymorphisms with SU treatment failure, early HbA1c response, and severe hypoglycemia were analyzed by Cox regression or logistic regression assuming an additive genetic model. There were 2341 incident SU users that were identified (mean age 59 years, median diabetes duration 9 years), of which 324 were *CYP2C19* poor metabolizers (*CYP2C19 *2/*2* or **2/*3* or **3/*3*). *CYP2C19* poor metabolizers had lower risk of SU treatment failure (hazard ratio 0.83, 95% confidence interval (CI) 0.72–0.97, *P* = 0.018) and were more likely to reach the HbA1c treatment target < 7% (odds ratio 1.52, 95% CI 1.02–2.27, *P* = 0.039) than wild-type carriers (*CYP2C19 *1/*1*) following adjustment for multiple covariates. There were no significant differences in severe hypoglycemia rates among different *CYP2C19* genotype groups. *CYP2C19* polymorphisms should be considered during personalization of SU therapy.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Sulfonylureas (SUs) are predominantly metabolized by *CYP2C9* and *CYP2C19* enzymes. *CYP2C19* loss-of-function polymorphisms are common in East Asians. Pharmacokinetic studies showed *CYP2C19* loss-of-function polymorphisms were associated with higher plasma level of SU, mainly gliclazide.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Association of *CYP2C19* loss-of-function polymorphisms with sulfonylurea treatment failure, HbA1c response and severe hypoglycemia in Chinese patients with type 2 diabetes.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ *CYP2C19* loss-of-function polymorphisms are associated with reduced risk of SU treatment failure and better HbA1c response in Chinese patients with type 2 diabetes.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ *CYP2C19* poor metabolizers might particularly benefit from SUs used alone or in combination with other glucose lowering drugs without apparent increased risk of hypoglycemia. Genotyping for *CYP2C19* polymorphisms may facilitate the precision use of SU therapy particularly in Asians.

The growing epidemic of type 2 diabetes (T2D) is a major public health challenge in many Asian countries. Sulfonylureas (SUs) have been the cornerstone of glucose lowering drug (GLD) therapy for over 60 years and listed as one of the essential medicines by the World Health Organization. SUs are widely used as the second add-on GLD to metformin therapy in T2D due to its confirmed

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efficacy and low costs, albeit with increased risk of hypoglycemia.[1] In the territory-wide Hong Kong Diabetes Surveillance Database enrolling 0.7 million people in 2001–2016, 50% of patients with T2D were exposed to SUs.[2] Among the second generation SUs, gliclazide is widely prescribed due to its short half-life, low risk of hypoglycemia, and apparent safety in chronic kidney disease.[3,4]

Many patients treated with SUs have deterioration in glycemic control over time[5] with significant interindividual differences in SU treatment responses. SUs are metabolized predominantly in the liver by cytochrome p450 2C9 (*CYP2C9*) and cytochrome p450 2C19 (*CYP2C19*) enzymes.[6–9] Pharmacokinetic studies suggest that *CYP2C19* polymorphisms have a greater effect on gliclazide metabolism[10,11] than *CYP2C9*, whereas the reverse is true for glyburide and glipizide.[12,13] In a large retrospective pharmacogenetic study of incident SU users in Scotland, carriers with 2 copies of loss-of-function *CYP2C9*2* (rs1799853) or *CYP2C9*3* (rs1057910) alleles were 3.4-fold more likely to achieve HbA1c target and had lower risk of monotherapy failure than carriers of the wild type.[14] Whereas *CYP2C9*2/*2* was associated with increased risk of hypoglycemia, this was less certain for *CYP2C9*3*.[15,16]

*CYP2C9*2* and *CYP2C9*3* are common in White patients with respective minor allele frequency (MAF) of 11.7% and 5.6%, but these polymorphisms are rare in East Asians (MAF < 0.1% and 3.4%, respectively).[17] Instead, loss-of-function alleles *CYP2C19*2* (rs4244285) and *CYP2C19*3* (rs4986893) are common in East Asians with respective MAF of 31% and 6.7% as compared with 18% and < 0.1% in White patients.[17] Carriers of wild type *CYP2C19*1/*1* are regarded as extensive metabolizers (EMs), whereas heterozygote carriers for **2* or **3* loss-of-function alleles (**1/*2* and **1/*3*) are intermediate metabolizers (IMs) and homozygote carriers (**2/*2*, **2/*3*, and **3/*3*) are poor metabolizers (PMs). It is estimated that 15–30% of Asians are *CYP2C19* PMs.[18] In a pharmacokinetic study of healthy Chinese subjects, we showed that *CYP2C19*2/*2* was associated with 2-fold increase in total plasma gliclazide area-under-the-curve and plasma half-life with 50% reduction in oral drug clearance compared with *CYP2C19* EMs.[19]

Despite the common use of SUs in Asians, there are no large-scale pharmacogenetic studies examining the effect of *CYP2C19*2* or *CYP2C19*3* polymorphisms on SU treatment response or risk of hypoglycemia in this population. We therefore investigated the associations of *CYP2C19* loss-of-function polymorphisms with SU treatment failure, SU efficacy, and risk of hypoglycemia among incident SU users. Given potential differences in metabolism, we further compared the effect of *CYP2C19* polymorphisms in gliclazide versus other non-gliclazide users.

STUDY DESIGN AND METHODS

This was a retrospective pharmacogenetic analysis in 11,495 Chinese patients with valid genotype data who were enrolled in the Hong Kong Diabetes Register (HKDR) between 1995 and 2017 followed up to December 31, 2019. The HKDR was established by a doctor-nurse team at a university-affiliated, publicly funded, hospital-based diabetes center using a structured protocol for gathering data during regular comprehensive structured assessment of complications and metabolic control aimed at stratifying risk and individualizing treatment. The register consecutively enrolled patients referred to the Diabetes Mellitus and Endocrine Centre and details of the HKDR have been previously described.[20] Ethical approval was obtained from the Joint New Territories East Cluster and Chinese University of Hong Kong Clinical Research Ethics Committee. Written informed consent was obtained from all patients at the time of enrollment for collection of clinical information and bio-samples for research and publication purposes.

Ascertainment of sample

Among 11,495 genotyped patients in the HKDR, we selected patients with T2D who were incident SU monotherapy users (monotherapy group) or incident SU users added to metformin monotherapy throughout the study period (dual therapy group). The index date was defined as the date of SU initiation. All patients had been treated with SU therapy for at least 6 months after the index date. Patients who were started on or discontinued from a second (monotherapy group) or third (dual therapy group) glucose-lowering drug including insulin within 6 months before or after the index date, were considered to have unstable therapy and thus were excluded. The detailed ascertainment procedures are shown in **Figure S1**.

Data collection

Baseline characteristics of patients were captured by structured assessments at enrollment in the HKDR. These include age at diagnosis, diabetes duration, gender, body mass index (BMI), estimated glomerular filtration rate (eGFR; estimated by the Chronic Kidney Disease Epidemiology Collaboration equation),[21] lipid and glycemic profiles, self-reported use of antihypertensive drugs, and lipid-lowering drugs. The HKDR was linked to the territory-wide electronic health record system operated by the Hospital Authority, which captures all dispensing, laboratory, and hospitalization data. Using a unique identifier, the enrollment data were linked to the laboratory data, including on-treatment HbA1c measurements and dispensing data, including drug name and formulation, start and end date of drug, route, and dosage. All in-patient and out-patient medications were dispensed from the hospital or clinic on-site pharmacies. The International Classification of Diseases 9th Edition codes (ICD-9) were used to define hospitalization due to severe hypoglycemia.

Genotyping

Genotype data were obtained from stored DNA samples and performed as part of a diabetes genetic discovery program with reported methodology.[22] Genotyping was performed using Illumina Omni2.5 + exome array, Illumina Infinium Global Screening Array or Illumina Infinium Asian Screening Array among participants in the HKDR with available DNA samples. Quality control (QC) was performed on single nucleotide polymorphisms (SNPs) in chromosome 1 to 22. SNPs were excluded if they had (i) MAF < 0.05 with overall call rate < 99%; (ii) MAF ≥ 0.05 with overall call rate < 95%; (iii) overall

MAF < 1%; and (4) Hardy-Weinberg equilibrium test *P* value < 1×10^{-4}. Genotype data that had passed QC were then subject to imputation using Minimac3 with the 1000 Genomes Project Phase 3 data (version 5) as the reference panel. The imputed SNPs with R^2 < 0.5 and MAF < 1% were discarded. *CYP2C19*2* (rs4244285) and *CYP2C19*3* (rs4986893) were directly genotyped whereas *CYP2C9*3* (rs1057910) genotype data were imputed.

Definition of outcomes

SU treatment failure model. Time to SU treatment failure was defined as the time period from SU index date to a clinical end point defined as one of the following two events, whichever occurred first: (i) switching to other GLD therapy or addition of a second (monotherapy group) or third (dual therapy group) GLD, including insulin for more than 6 months; or (ii) 2 consecutive measurements of HbA1c ≥ 8.5% (3–12 months apart during treatment).[23,24]

HbA1c-based treatment response model. This HbA1c-based model was designed to reflect early response to SU therapy. In this dichotomous model, the success of SU treatment response was defined as attaining a treatment target of HbA1c < 7% within 18 months of the index date without experiencing SU failure. Patients with missing baseline HbA1c or baseline HbA1c < 7% were excluded in this model.[14]

Severe hypoglycemia model. A severe hypoglycemic event was defined as hospital admission for hypoglycemia as coded by any one of the top 15 discharge diagnosis with ICD 9 codes 250.3, 250.8, 250.81, 250.82, 250.83, and 251.2 during the study period.

Covariates

Covariates were selected based on prior knowledge of factors reported to influence treatment responses, including baseline age, sex, diabetes duration, baseline HbA1c, BMI, eGFR, average SU daily dose, therapy group (monotherapy or dual therapy), as well as use of antihypertensive drug and lipid-lowering drug at baseline. Baseline HbA1c and eGFR were defined as the last available measurement within 1 year prior to the SU index date. Diabetes duration was calculated from the year of diagnosis to the index date. Gender, age of diagnosis, and BMI were determined at enrollment. Use of antihypertensive drugs and lipid-lowering drugs were self-reported by patients at enrollment. The average daily treatment dose was calculated as the mean dose of prescriptions filled during the observation period. Different SUs were converted into equivalent doses by expressing the dosage as the percentage of maximum daily recommended treatment dose as defined in MIMS Drug Reference (Hong Kong), Issue 2, June 2020.

Statistical analysis

The differences in baseline characteristics among *CYP2C19* genotype groups were compared using one-way analysis of variance or Kruskal-Wallis test for continuous variables, and chi-squared test for categorical variables. Data are presented as mean ± standard deviation (SD) or median (interquartile range (IQR)) as appropriate. Hardy-Weinberg equilibrium was examined using the chi-squared test with two degrees of freedom to compare differences in allele frequencies. We assessed crude survival (for time to SU treatment failure) with the Kaplan-Meier survival plot, stratified by *CYP2C19* genotype groups. The Cox proportional-hazards model was performed to estimate the associations of combined *CYP2C19* genotype group with SU treatment failure expressed as hazard ratio (HR) with 95% confidence interval (CI). The logistic regression model was used to estimate the associations of combined *CYP2C19* genotype group with the success of SU treatment response and severe hypoglycemia expressed as odds ratio (OR) with 95% CI. We fitted multivariable adjusted models controlling for therapy group, baseline age, sex, diabetes duration, baseline HbA1c, BMI, eGFR, average SU daily dose, use of antihypertensive drugs, and use of lipid-lowering drugs. All analyses were carried out using IBM Statistical Package for Social Sciences version 26. A *P* value of < 0.05 (2-tailed) was deemed statistically significant.

Subanalysis of *CYP2C19* polymorphisms effect on gliclazide and non-gliclazide users

As we hypothesized differential effects of *CYP2C19* on metabolism of SU subgroups, we compared the baseline characteristics between gliclazide vs. non-gliclazide users and the associations of *CYP2C19* genotypes with treatment outcomes. We considered the interactive effects of *CYP2C19* genotype by SU drug subgroup (gliclazide vs. non-gliclazide) by including an interactive term in the adjusted models in the whole group. Outcome analyses were conducted based on the initial SU prescribed.

Interactive effects of *CYP2C9*3 and CYP2C19* loss-of-function polymorphisms

*CYP2C9*2* is rare in East Asians (MAF < 0.1%), whereas the MAF of *CYP2C9*3* polymorphism is 3.4%.[17] To clarify potential interactive effects of *CYP2C9*3 and CYP2C19* loss-of-function polymorphisms on SU responses, we added an interaction term to the model and repeated the analyses.

Sample size justification

Assuming 15% of Chinese are *CYP2C19* PMs and 40% are *CYP2C19* EMs and that 50% of patients experience SU treatment failure at 5 years, 1500 patients are needed for 90% power to detect a 20% risk reduction among PMs[14] with a 2-sided alpha level of 0.05.

Sensitivity analysis

In our prescription database, there were small time gaps between drug prescriptions due to patients' default or hospitalization or other reasons which could not be ascertained using this register. To investigate the impact of treatment discontinuation, we excluded users who have discontinued medications and repeated analyses on our outcome models. Discontinued SU users were defined by < 0.8 of total exposure to SU during observation period for all patients. Discontinued metformin users were defined by < 0.8 of total exposure to metformin among the dual therapy users.

RESULTS

Baseline characteristics

A total of 2341 incident SU users were included in our analyses with a mean (SD) baseline age of 58.8 (12.3) years and a median (IQR) diabetes duration of 9.0 (5.0–13.0) years. Of these, 435 patients received SU monotherapy and 1906 received SU and metformin dual therapy. In the latter group, 816 had been treated with metformin before the index date and 1090 were incident metformin users with add-on SU. In the whole cohort, the MAF of *CYP2C19*2* and *CYP2C19*3* were 31.7% and 5.5%, respectively. There was no deviation from Hardy-Weinberg equilibrium with either variant (*CYP2C19*2* $\chi^2 = 0.24$, $P = 0.885$; *CYP2C19*3* $\chi^2 = 0.37$, $P = 0.832$). Among these patients, 39% ($n = 922$) were *CYP2C19* EMs, 47% ($n = 1095$) were IMs, and 14% ($n = 324$) were PMs. The baseline characteristics were similar among these three genotype groups (**Table 1**). **Table S1** shows the patients' baseline characteristics stratified by the three outcomes of this study.

SU treatment failure

During a median (IQR) follow-up period of 4.5 (2.0–8.2) years, 71% ($n = 1660$) of patients experienced SU treatment failure. **Figure 1a** shows the Kaplan-Meier survival plot of overall SU users. The median survival time to SU treatment failure was 5.4 years in EMs, 5.7 years in IMs, and 5.9 years in PMs. Among the SU monotherapy users (**Figure 1b**), the median time to SU monotherapy failure was 3.2 years in EMs, 3.6 years in IMs, and 4.6 years in PMs.

In the Cox proportional-hazards model (**Figure 2**), *CYP2C19* PMs with genotypes of **2/*2* or **2/*3* or **3/*3* had an HR of 0.86 (95% CI 0.74–1.00, $P = 0.050$) of SU treatment failure compared with EMs (*CYP2C19*1/*1*) in the univariate analysis. In the multivariate analysis, after adjustment for other covariates, the HR was further reduced to 0.83 (95% CI 0.72–0.97, $P = 0.018$). *CYP2C19* IMs with only one copy of either **2* or **3* had intermediate risk between EMs and PMs (HR 0.91, 95% CI 0.82–1.01, $P = 0.066$).

In our study cohort, 12.2% ($n = 285$) discontinued SU and 10.6% ($n = 202$) of the dual therapy group discontinued metformin, defined as less than 0.8 of total time exposure to either treatment. In the remaining 1955 patients, after excluding discontinued SU or metformin users and adjusting for covariates, *CYP2C19* IMs (HR 0.88, 95% CI 0.78–0.98, $P = 0.021$) and PMs (HR 0.79, 95% CI 0.67–0.92, $P = 0.004$) had lower risk of SU treatment failure than the *CYP2C19* EMs (**Figure S2**).

The allelic risk association of *CYP2C19* polymorphisms on SU treatment failure is shown in **Table 2**. When analyzed by each single risk variant, only the *CYP2C19*2* loss-of-function allele was associated with lower risk of treatment failure (*CYP2C19*1/*2*, HR 0.86, 95% CI 0.76–0.97, $P = 0.012$; *CYP2C19*2/*2*, HR 0.78, 95% CI 0.65–0.94, $P = 0.009$) but not for the *CYP2C19*3* loss-of-function allele (*CYP2C19*1/*3*, HR 0.96, 95% CI 0.78–1.18, $P = 0.708$; *CYP2C19*3/*3*, HR 0.40, 95% CI 0.13–1.23, $P = 0.110$). The association of *CYP2C19* polymorphisms with SU treatment failure was most evident in the SU monotherapy group

Table 1 Baseline characteristics of overall SU users by *CYP2C19* combined genotype group and therapy group

	CYP2C19 genotype group				Therapy group		
	Extensive metabolizers	Intermediate metabolizers	Poor metabolizers				
Characteristics	**1/*1* (*n* = 922)	**1/*2 *1/*3* (*n* = 924) (*n* = 171)	**2/*2 *2/*3 *3/*3* (*n* = 243) (*n* = 76) (*n* = 5)	*P* value	SU monotherapy	SU + metformin dual therapy	*P* value
Patients (*n*)	922 (39.4%)	1095 (46.8%)	324 (13.8%)		435 (18.6%)	1906 (81.4%)	
Baseline age, years	58.9 ± 12.4	58.6 ± 12.1	59.0 ± 12.4	0.756	60.4 ± 13.3	58.4 ± 12.0	0.002
Male (*n*)	419 (45.4%)	509 (46.5%)	131 (40.4%)	0.156	208 (47.8%)	851 (44.6%)	0.231
Age of diagnosis, years	49.6 ± 12.4	48.7 ± 11.3	49.1 ± 11.8	0.299	51.4 ± 13.0	48.6 ± 11.5	< 0.001
Diabetes duration, years	8.0 (4.0–13.0)	9.0 (5.0–14.0)	9.0 (6.0–13.0)	0.143	8.0 (5.0–13.0)	9.0 (5.0–14.0)	0.027
Baseline HbA1c, %	7.7 ± 1.3	7.8 ± 1.5	7.6 ± 1.3	0.060	7.2 ± 1.4	7.8 ± 1.3	< 0.001
BMI, kg/m^2	25.6 ± 4.1	25.8 ± 4.3	25.6 ± 4.0	0.408	24.0 ± 3.4	26.1 ± 4.2	< 0.001
eGFR, mL/min/1.73m^2	84.2 (64.1–99.2)	84.3 (67.2–100.0)	79.6 (62.9–99.1)	0.319	68.3 (48.0–88.7)	85.9 (69.1–100.7)	< 0.001
Average SU daily dose, %	49.8 ± 28.5	49.5 ± 29.0	49.5 ± 28.2	0.952	36.2 ± 23.4	52.7 ± 29.0	< 0.001
Antihypertensive drug use, *n*	324 (35.1%)	371 (33.9%)	103 (31.2%)	0.539	140 (32.2%)	658 (34.5%)	0.383
Lipid-lowering drug use, *n*	144 (15.6%)	167 (15.3%)	39 (12.0%)	0.278	40 (9.2%)	310 (16.3%)	< 0.001

Means ± SD, medians (interquartile range), or number (percentage) of baseline characteristics are presented. One-way analysis of variance /Kruskal-Wallis test or two sample *t*-test /Mann-Whitney test were used for continuous variables, and chi-squared test was used for categorical variables. Average SU daily dose was expressed as a percentage of the maximum recommended daily dose.
BMI, body mass index; eGFR, estimated glomerular filtration rate; SU, sulfonylurea.

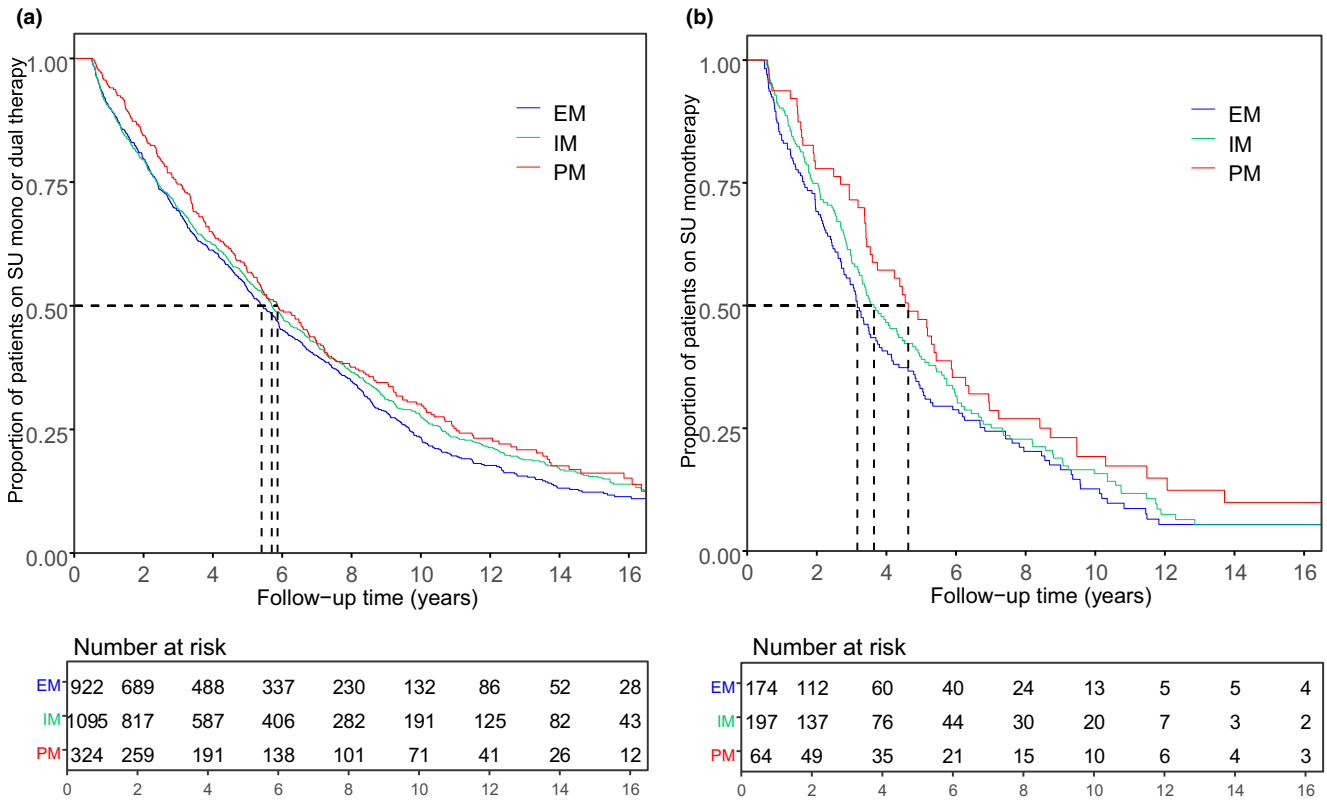

Figure 1 Kaplan-Meier survival plots of time to SU treatment failure by *CYP2C19* genotype groups. (**a**) Plot for overall SU users. (**b**) Plot for SU monotherapy users. SU, sulfonylurea; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

Outcome	Event/total number	Crude model		†Adjusted model	
SU treatment failure	1660/2341	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
CYP2C19 genotype group					
**1/*1*	665/922	1.00 (reference)		1.00 (reference)	
**1/*2 or *1/*3*	761/1095	0.93 (0.84-1.03)	0.157	0.91 (0.82-1.01)	0.066
**2/*2 or *2/*3 or *3/*3*	234/324	0.86 (0.74-1.00)	0.050	0.83 (0.72-0.97)	0.018
Reaching treatment target	592/1042	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
CYP2C19 genotype group					
**1/*1*	213/395	1.00 (reference)		1.00 (reference)	
**1/*2 or *1/*3*	290/508	1.14 (0.87-1.48)	0.343	1.19 (0.89-1.59)	0.238
**2/*2 or *2/*3 or *3/*3*	89/139	1.52 (1.02-2.27)	0.039	1.49 (0.97-2.29)	0.071
Severe hypoglycaemia	156/2341	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
CYP2C19 genotype group					
**1/*1*	64/922	1.00 (reference)		1.00 (reference)	
**1/*2 or *1/*3*	66/1095	0.86 (0.60-1.23)	0.405	0.87 (0.60-1.26)	0.466
**2/*2 or *2/*3 or *3/*3*	26/324	1.17 (0.73-1.88)	0.517	1.13 (0.69-1.87)	0.619

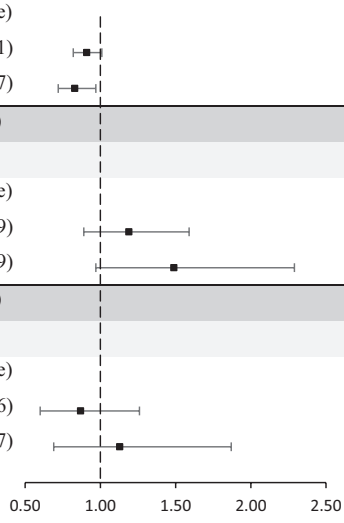

Figure 2 Associations of *CYP2C19* polymorphisms with (i) SU treatment failure, (ii) reaching treatment target (HbA1c < 7%), and (iii) severe hypoglycemia among overall SU users. †Adjusted models were adjusted for covariates including therapy group (monotherapy or dual therapy), baseline age, sex, diabetes duration, baseline HbA1c, body mass index, estimated glomerular filtration rate, average SU daily dose, baseline use of antihypertensive drug and baseline use of lipid-lowering drug. CI, confidence interval; HR, hazard ratio; OR, odds ratio; SU, sulfonylurea.

Table 2 Genotypic effect of *CYP2C19*2* and *CYP2C19*3* on SU treatment failure among patients after excluding discontinued drug users

		Crude model		Adjusted model[a]	
CYP2C19 genotype	Event/total number	HR (95% CI)	*P* value	HR (95% CI)	*P* value
**1/*1* (*n* = 747)	575/759	1.00 (reference)		1.00 (reference)	
**1/*2* (*n* = 766)	555/783	0.91 (0.81–1.02)	0.103	0.86 (0.76–0.97)	**0.012**
**1/*3* (*n* = 145)	115/148	0.94 (0.77–1.15)	0.523	0.96 (0.78–1.18)	0.708
**2/*2* (*n* = 197)	145/198	0.83 (0.69–1.00)	**0.044**	0.78 (0.65–0.94)	**0.009**
**2/*3* (*n* = 63)	49/63	0.83 (0.62–1.11)	0.217	0.84 (0.63–1.13)	0.249
**3/*3* (*n* = 4)	3/4	0.56 (0.18–1.73)	0.311	0.40 (0.13–1.23)	0.110

Significant *P* values < 0.05 are shown in bold.
CI, confidence interval; HR, hazard ratio; SU, sulfonylurea.
[a]Adjusted model was adjusted by covariates including therapy group (monotherapy or dual therapy), baseline age, sex, diabetes duration, baseline HbA1c, body mass index, estimated glomerular filtration rate, average SU daily dose, baseline use of antihypertensive drug and lipid-lowering drug.

(**Table S2**), where PMs had an HR of 0.67 (95% CI 0.48–0.92, $P = 0.014$) compared with EMs.

HbA1c-based treatment response

After excluding patients with missing baseline HbA1c or baseline HbA1c < 7%, we identified 1042 subjects with a baseline HbA1c ≥ 7%, of whom 592 patients had attained HbA1c < 7% within 18 months. **Figure 2** shows the logistic regression for reaching treatment target in an additive genetic model. Patients with 2 copies of loss-of-function *CYP2C19* alleles were 1.5 times more likely to attain treatment target compared with EMs (OR 1.52, 95% CI 1.02–2.27, $P = 0.039$) which was attenuated after adjustment for multiple covariates (OR 1.49 95% CI 0.97–2.29, $P = 0.071$; **Figure 2**).

Severe hypoglycemia

Among 2341 SU users, 3 patients in the monotherapy group and 153 in the dual therapy group experienced at least one severe hypoglycemic event requiring hospitalization during the observation period. In logistic regression analysis, no association was observed between *CYP2C19* loss-of-function polymorphisms with severe hypoglycemia rate (**Figure 2**). *CYP2C19* PMs tended to have increased odds, albeit not significant (OR 1.46, 95% CI 0.81–2.61, $P = 0.209$) of severe hypoglycemia vs. the EMs after excluding discontinued drug users and adjustment for other covariates (**Figure S2**).

Gliclazide vs. non-gliclazide subgroups

There were 1455 gliclazide users (including gliclazide and gliclazide modified release) and 886 non-gliclazide users (including glibenclamide, glipizide, glimepiride, tolbutamide, and chlorpropamide) based on the initial SU prescribed. **Table S3** shows the patients' clinical profiles in gliclazide and non-gliclazide subgroups. The association of greater HbA1c response among PMs expressed as OR in the gliclazide subgroup was 1.75 (95% CI 1.05–2.93) vs. 1.10

Table 3 Associations of *CYP2C19* polymorphisms with SU treatment failure and reaching treatment target (HbA1c < 7%) among gliclazide and non-gliclazide users

Outcome	Event/total number	Gliclazide users		Event/total number	Non-gliclazide users		*P* value for interaction of *CYP2C19* genotype group and SU drug subgroup
SU treatment failure	**1020/1455**	**HR (95% CI)**	***P* value**	**640/886**	**HR (95% CI)**	***P* value**	
CYP2C19 genotype group							
**1/*1*	418/578	1.00 (reference)		247/344	1.00 (reference)		0.940
**1/*2 or *1/*3*	453/669	0.90 (0.79–1.03)	0.122	308/426	0.88 (0.74–1.04)	0.128	
**2/*2 or *2/*3 or *3/*3*	149/208	0.82 (0.68–1.00)	**0.046**	85/116	0.84 (0.65–1.07)	0.160	
Reaching treatment target	**427/731**	**OR (95% CI)**	***P* value**	**165/311**	**OR (95% CI)**	***P* value**	
CYP2C19 genotype group							
**1/*1*	147/272	1.00 (reference)		66/123	1.00 (reference)		0.387
**1/*2 or *1/*3*	208/354	1.32 (0.93–1.87)	0.120	82/154	0.97 (0.57–1.65)	0.895	
**2/*2 or *2/*3 or *3/*3*	72/105	1.75 (1.05–2.93)	**0.033**	17/34	1.10 (0.47–2.59)	0.822	

All analyses were adjusted for therapy group (monotherapy or dual therapy), baseline age, sex, diabetes duration, baseline HbA1c, body mass index, estimated glomerular filtration rate, average SU daily dose, baseline use of antihypertensive drug and lipid-lowering drug.
Significant *P* values < 0.05 are shown in bold.
CI, confidence interval; HR, hazard ratio; OR, odds ratio; SU, sulfonylurea.

(95% CI 0.47–2.59) in the non-gliclazide subgroup. In the overall model, interactive effect between SU subgroup and *CYP2C19* genotypes were not interactive with respect to SU treatment failure or odds of attaining HbA1c target (**Table 3**).

Interactive effects of *CYP2C9*3* polymorphisms on risk of SU treatment failure and HbA1c response

Among 2341 SU users, 137 patients were heterozygous *CYP2C9*3* carriers and 1 patient was a homozygous *CYP2C9*3* carrier. None of the *CYP2C9*3* carriers were *CYP2C19* PMs in our cohort. We added an interaction term to the model and did not detect any interactive effect between combined *CYP2C19* and *CYP2C9* genotypes on the risk of SU treatment failure and HbA1c response ($P = 0.444$ and $P = 0.290$, respectively; **Table S4**). After adjusting for *CYP2C9*3* effects, the association of *CYP2C19* PMs with reduced risk of SU treatment failure (HR 0.85, 95% CI 0.73–0.99, $P = 0.032$) and higher odds of attaining HbA1c treatment target (OR 1.60, 95% CI 1.04–2.48, $P = 0.034$) remained significant (**Table S4**).

DISCUSSION

In this pharmacogenetic study involving 2341 Chinese incident SU users with T2D, we demonstrated that the loss-of-function allele *CYP2C19*2* was associated with greater response to SU. Patients with the *CYP2C19*2* polymorphism had lower risk of SU treatment failure and were more likely to attain the American Diabetes Association recommended treatment target HbA1c of 7%.[25] When analyzed by different SU drugs, *CYP2C19* PMs exhibited better HbA1c response than EMs following gliclazide initiation. These findings were particularly robust in homozygous carriers of *CYP2C19*2*.

The results of this analysis in real-world settings support our previous pharmacokinetic studies regarding the higher plasma level of gliclazide in *CYP2C19* PMs.[19] Here, *CYP2C19*2* also had pharmacodynamic effects on short-term HbA1c response and long-term treatment failure in patients receiving SU monotherapy or dual therapy of SU and metformin. Patients who were *CYP2C19* PMs were 1.5 times more likely to achieve HbA1c target within 18 months of SU initiation. We observed consistent associations of improved SU response with one copy of *CYP2C19*2* loss-of-function allele. Although *CYP2C19*3/*3* carriers had better response than non-carriers, this was not significant, which might in part be due to the lower allelic frequency and small sample size. In a small pharmacokinetic study of 18 Han Chinese subjects, individuals with 2 copies of *CYP2C19*3* had higher plasma gliclazide concentrations and prolonged half-life than *CYP2C19 *1/*1* or **1/*2* or **1/*3* carriers.[10]

In the present study, we did not observe differences in severe hypoglycemia rates among different *CYP2C19* genotype groups. In a 3-month clinical study involving 108 predominantly White or Han patients treated with SU, researchers reported that *CYP2C9* (60% vs. 39%) and *CYP2C8* (47% vs. 27%) variant alleles tended to be more frequent in patients with mild hypoglycemic events compared with non-carriers. This trend was not seen in *CYP2C19* loss-of-function allele carriers vs. *CYP2C19* wild type carriers (27% vs. 28%).[16] In our study population, 156 patients had severe hypoglycemia, 3 of whom received SU monotherapy with the majority treated with both SU and metformin. We did not find any genetic association with risk of severe hypoglycemia in this relatively small cohort.

Our subanalyses indicated that the association of better SU treatment response with *CYP2C19* loss-of-function alleles tended to be stronger among gliclazide users compared with other SU users. In a clinical trial conducted in healthy Chinese subjects, *CYP2C9*, but not *CYP2C19*, polymorphisms influenced glyburide pharmacokinetic and pharmacodynamic parameters.[12] Similarly, *CYP2C19* metabolizer status did not affect tolbutamide pharmacokinetics and glucose response.[26,27] Combining the evidence from pharmacokinetic studies in volunteers and the present pharmacogenetic study in patients with T2D, *CYP2C19* may play a more important role in gliclazide metabolism compared with other SUs. In our study, *CYP2C9*3* occurred at a low frequency and we did not observe any significant interactive effects between *CYP2C9* and *CYP2C19* loss-of-function polymorphisms on efficacy or safety. We did not observe significant interaction between gliclazide and *CYP2C19* genotypes in treatment responses, although a larger sample size will be needed to evaluate different pharmacogenetic effects among different SU subgroups.

To the best of our knowledge, this is the first large-scale pharmacogenetic study which explored the association of *CYP2C19* polymorphisms with SU response in the Asian population with T2D. The strengths of the study include the long median follow-up period of 4.5 years and detailed SU prescription data with complete information on dosage, agent, and duration of treatment. Despite the rarity of *CYP2C9*3* polymorphisms present in less than 5% of our population, we adjusted for their effects and found that the association of *CYP2C19* loss-of-function polymorphisms with efficacy of SU remained significant.

Our study also has limitations. Due to the relatively small sample size of the SU monotherapy group, we included dual SU and metformin users, and cannot exclude the potential influence of metformin use on the outcomes. Apart from genetic variants in drug-metabolizing enzymes of *CYP2C19* and *CYP2C9*, we did not examine polymorphisms of SU drug transporter (*TCF7L2*) and SU drug target genes (*ABCC8* and *KCNJ11*), which will be further explored in the future. We adjusted for self-reported lipid-lowering drugs including statins at baseline, which has been associated with modest increases in HbA1c among patients with diabetes.[28] We did not have information on non-diabetic drug prescription data, such as corticosteroids, which could also influence glycemic control. Drug-drug interactions with *CYP2C19* inhibitors, such as omeprazole, were not adjusted in this study, which may potentially influence individual variations in SU pharmacodynamics and pharmacokinetics.[29,30] Our findings require further confirmation in larger independent cohorts with adequate power to detect differences in severe hypoglycemia rates, which are rare events.

Our results have clinical implications. In our cohort, 60% of Chinese patients with T2D had at least one copy of *CYP2C19* loss-of-function allele with 14% being *CYP2C19* PMs. These accord with the reported frequency of 15–30% among Asians.[18]

When considering common factors that influence SU treatment response, *CYP2C19* polymorphisms may not be neglected with larger effect sizes compared with other variants, such as *KCNJ11*. *CYP2C19* polymorphisms are potentially actionable in the setting of diabetes therapy with genotype data available at the point of prescribing pre-emptively.[31] As the costs of genotyping continue to fall, genotyping for such common drug metabolizing enzyme polymorphisms may become embedded in electronic health records. *CYP2C19* genotype guided *P2Y12* antiplatelet therapy with point-of-care testing has been shown to improve outcomes following percutaneous coronary interventions, as compared with an unselected approach.[32] Our data suggested that *CYP2C19* PMs might particularly benefit from gliclazide used alone or in combination with other GLDs without increased risk of hypoglycemia. Although sodium glucose co-transporter 2 inhibitors and glucagon-like peptide 1 receptor agonists (GLP1-ra) may be preferred in patients at high cardiovascular-renal risk,[33,34] we must not forget that SU is still useful with high affordability. This drug class also has the highest glucose lowering efficacy among oral glucose lowering agents, reducing HbA1c by 1–1.5%, which is comparable to that of injectable therapies, such as insulin and GLP1-ra. In young patients who face long disease duration, optimal glycemic control is critically important to avoid glycemic deterioration and treatment escalation and long-term development of complications. This efficacy may outweigh the risk of hypoglycemia, which may be relatively low in younger adults. Our results suggested that SU remain an important low-cost and efficacious glucose lowering drug, especially in some patients with genetic predisposition with increased responsiveness.

In conclusion, *CYP2C19* PM genotype were associated with reduced risk of SU treatment failure and better HbA1c response in Chinese patients with T2D without apparent increased risk in severe hypoglycemia rates. Future independent replication studies are needed to validate our results. Genotyping for *CYP2C19* polymorphisms may facilitate the precision use of SU therapy particularly in Asians.

AUTHOR CONTRIBUTIONS

E.C., J.C.N.C., A.Y., and K.W., wrote the manuscript. E.C., J.C.N.C., and B.T. designed the research. K.W., A.Y., S.M., C.T., C.K.P.L., B.F., and H.M.L. performed the research. K.W., A.Y., S.M., and E.S.H.L. analyzed the data. R.C.W.M., A.O.Y.L., A.P.S.K., and J.C.N.C. contributed to reagents and analytical tools.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

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