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Original Article

Genetic variants of *OCT1* influence glycemic response to metformin in Han Chinese patients with type-2 diabetes mellitus in Shanghai

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Abstract: Aims/hypothesis: Genetic variation in *OCT1* can influence the glycemic response to metformin. We evaluated the effects of the *OCT1* single-nucleotide polymorphisms (SNPs), rs1867351, rs4709400, rs628031, and rs2297374, on metformin efficacy in type-2 diabetes mellitus (DM) patients. Methods: We performed a single-center prospective analysis of the distributions of these SNPs in a cohort of Han Chinese subjects in Shanghai, China (HCS), and evaluated the effects of each SNP on glycemic control in HCS DM patients following 3 months of incident metformin treatment. Results: The allele frequencies of rs4709400 and rs628031 in our HCS control group differed from those previously reported for Han Chinese subjects in Beijing (HCB), as well as those previously reported for Caucasians and Africans, whereas the allele frequencies of rs1867351 and rs2297374 were more similar to those in HCB subjects. The DM patients with the rs1867351 T/T or rs4709400 G/G genotype exhibited greater reductions in postprandial plasma glucose (PPG), compared to those with different genotypes of these SNPs. The DM patients with the rs2297374 C/T, rs4709400 G/G, or rs628031 G/G genotype exhibited greater reductions in fasting plasma glucose (FPG), and those with the rs1867351 T/T, rs628031 A/A, or rs2297374 C/T genotype exhibited greater reductions in HbA_{1c}, compared to those with different genotypes of these SNPs. Conclusions /interpretation: The rs1867351, rs4709400, rs628031, and rs2297374 SNPs of *OCT1* have selective effects on FPG, PPG, and HbA_{1c} in HCS DM patients in response to metformin treatment. Future studies of these SNPs in larger samples of HCS DM patients are warranted.

Keywords: Type-2 diabetes mellitus, *OCT1*, metformin, polymorphism, glycemic control

Introduction

Type 2 diabetes mellitus (DM) is a major public health problem worldwide, especially in developed regions. China has one of the world's largest populations of DM patients [1, 2], and the prevalence of DM in China is increasing more rapidly than that in North America or Europe [3, 4]. Multiple pharmacological interventions are available for the treatment of DM, among which metformin is recommended as the first-line treatment, according to national and international guidelines [5, 6]. However, the pharmacological mechanism underlying the metformin-mediated inhibition of hepatic gluconeogenesis has not been fully elucidated, and substantial interpatient variation in glycemic response to metformin treatment for DM has been widely reported [7, 8], highlighting the need for more patient-centered care for DM [6].

With recent advancements in genome-wide association studies, multiple genetic variants have been identified that contribute to the variation in clinical response to metformin treatment for DM [9-15]. The ataxia telangiectasia mutated (*ATM*) gene, which functions in DNA repair and cell cycle control, also plays a role in the activation of adenosine monophosphate-activated protein kinase in rat hepatocytes in response to metformin treatment [16], and genetic variation in *ATM* can alter the glycemic response to metformin therapy. A previous study in the UK reported a frequency of 44% for the C allele of rs11212617 A>C, a single-nucleotide polymorphism (SNP) in the *ATM* gene, and showed that the C allele was associated with a glycated hemoglobin (HbA_{1c}) level of less than 7% [16]. Zhou et al [17] also found a significant association between the C allele of rs11212617 and HbA_{1c} level in a cohort of Han Chinese DM patients in Shanghai.

Variants of other genes can also affect the glycemic response to metformin. The rs622342 A>C variant of the *OCT1* gene (SLC22A1) and the rs2289669 G>A variant of the *MATE1* gene (SLC47A1) have each been shown to influence the reduction of HbA_{1c} in response to metformin treatment [18, 19]. These SNPs exhibit an additive effect on the blood glucose-lowering property of metformin based on the number of C and G alleles of rs622342 (genotypes C/C or A/C) and rs2289669 (genotypes G/G or G/A), respectively, with a greater number of these alleles associated with a lower level of HbA_{1c} [20]. Various other genetic variants of *OCT1* have also been reported in the literature, some which have been shown to influence the efficacy of metformin treatment for DM [21, 22].

The genotype distributions and allele frequencies of SNPs implicated in the glycemic response to metformin vary between Caucasian, African, and Asian DM patients [Becker 2010]. The genotype and allele distributions of rs11212617 has even been shown to vary between different populations of Han Chinese DM patients within China [17]. Given the large geographic expanse of China and its large population, it is possible that variation exists in other *OCT1* polymorphisms within China's Han Chinese population, which comprises >90% of the total population of China. Furthermore, the individual and combined effects of these SNPs on the glycemic response to metformin treatment for DM in Asian patients have not been thoroughly investigated. The aim of our current study was to determine the contributions of genetic variants of *OCT1* and *MATE1* to the clinical effects of metformin treatment in Han Chinese DM patients. We evaluated the genotype and allele frequencies of seven genetic variants of *OCT1* and one genetic variant of *MATE1* in a cohort of Han Chinese subjects in Shanghai, China, and compared the clinical effects of each variant on the glycemic response to metformin treatment.

Patients and methods

Patients

We examined genetic variants in *OCT1* and *MATE1* in 153 Han Chinese patients who received incident metformin treatment for DM at the Shanghai Xuhui District Dahua Hospital between July 2012 and July 2014. All of the patients with DM were diagnosed based on the 1999 World Health Organization criteria for DM, and were treated with 500 to 2000 mg/day metformin orally. Patients who were also prescribed insulin, acarbose, rosiglitazone, or pioglitazone during the study period were excluded from our study. Patients with stage-3 to -5 chronic kidney disease, liver cirrhosis, endocrine disorders, malignancies, or systemic inflammatory diseases were also excluded from our study. We enrolled 124 healthy volunteers as the control group. The metformin-resistant (MR) group consisted of 10 DM patients whose HbA_{1c} had declined by <1% after 3 months of incident metformin treatment during the study period. The study protocol was approved by the ethics review boards of the Dahua Hospital and written informed consent was obtained from the patients.

Study design

All of the participant's sex, age, systolic and diastolic blood pressure (BP), body-mass index (BMI), HbA_{1c} level, fasting plasma glucose (FPG) level, and postprandial plasma glucose (PPG) level were recorded at baseline. A second set of HbA_{1c}, FPG, and PPG measurements were recorded at 3 months after enrollment, which corresponded to at least 3 months of metformin monotherapy in the DM and MR groups. Each patient's genomic DNA was analyzed to determine the genotypes of the following genetic variants in the genes indicated: rs1867351 (SLC22A1), rs12208357 (SLC22A1), rs4709400 (SLC22A1), rs628031 (SLC22A1), rs2297374 (SLC22A1), rs72552763 (SLC22A1), rs113569197 (SLC22A1), and rs36056065 (SLC47A1).

Genotyping analysis

Peripheral blood samples were collected from each patient, and the genomic DNA of polymorphonuclear leukocytes was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Genotyping of the selected SNPs was performed using PCR reactions with primer that were designed to specifically span DNA fragments containing variations at rs1867351, rs12208357, rs4709400, rs628031, rs2297374, rs72552763, rs113569197 or rs36056065. Each PCR sample contained 12.5 μL of 2 × AmpliTaq Gold 360 Master Mix (Applied Biosystems, Life Technologies, Carlsbad, CA,

Table 1. Comparison of demographic and baseline clinical variables based on *OCT1* genetic variant

Variable	Genotypes			
rs1867351	C/C	C/T	T/T	*P*-value
Men	21 (53.8)	28 (50.0)	20 (47.6)	0.86
Women	18 (46.2)	28 (50.0)	22 (52.4)	
Age (y)	55.6 ± 8.90	56.9 ± 12.7	55.2 ± 12.3	0.76
BMI (kg/m^2)	25.6 ± 3.02	25.9 ± 4.05	25.7 ± 4.28	0.92
Systolic BP (mmHg)	130.6 ± 11.62	127.9 ± 10.54	132.6 ± 11.82	0.12
Diastolic BP (mmHg)	80.8 ± 7.44	78.4 ± 6.43	80.6 ± 7.34	0.57
rs4709400	C/C	C/G	G/G	*P*-value
Men	13 (44.8)	30 (52.6)	26 (51.0)	0.79
Women	16 (55.2)	27 (47.4)	25 (49.0)	
Age (y)	55.2 ± 13.2	56.5 ± 12.5	55.9 ± 9.37	0.89
BMI (kg/m^2)	25.8 ± 4.81	25.9 ± 3.92	25.6 ± 3.14	0.91
Systolic BP (mmHg)	131.7 ± 12.52	129.0 ± 11.91	130.4 ± 10.05	0.58
Diastolic BP (mmHg)	79.9 ± 6.64	79.7 ± 7.42	79.8 ± 7.00	0.99
rs628031	A/A	A/G	G/G	*P*-value
Men	6 (66.7)	26 (44.1)	37 (53.6)	0.34
Women	3 (33.3)	33 (55.9)	32 (46.4)	
Age (y)	56.0 ± 8.54	55.9 ± 13.3	56.1 ± 10.3	0.99
BMI (kg/m^2)	25.9 ± 3.46	26.1 ± 4.33	25.5 ± 3.45	0.66
Systolic BP (mmHg)	134.1 ± 12.13	130.3 ± 12.71	129.4 ± 9.99	0.50
Diastolic BP (mmHg)	81.89 ± 4.91	79.17 ± 7.41	79.99 ± 6.99	0.52
rs2297374	C/C	C/T	T/T	*P*-value
Men	40 (53.3)	22 (46.8)	7 (46.7)	0.80
Women	35 (46.7)	25 (53.2)	8 (53.3)	
Age (y)	55.6 ± 10.9	57.5 ± 12.6	53.5 ± 11.4	0.46
BMI (kg/m^2)	25.5 ± 3.27	26.4 ± 4.39	24.8 ± 4.59	0.32
Systolic BP (mmHg)	130.6 ± 11.33	130.1 ± 11.85	127.5 ± 10.13	0.62
Diastolic BP (mmHg)	79.81 ± 6.700	80.00 ± 8.280	78.73 ± 4.480	0.83

USA), 2 μL forward primer, 2 μL reverse primer, 2 μL DNA template and 6.5 μL ddH_2O with PCR conditions according to the manufacturer's instructions. After PCR amplification, the DNA fragments were isolated by agarose gel electrophoresis and AxyPrep DNA Gel Extraction Kit (Axygen, Shanghai, China). Finally after cloning into TOPO TA vectors (Invitrogen Life Technologies, Carlsbad, CA, USA), the DNA has been sequenced by a domestic company.

Statistical analysis

The statistical analysis was performed using the SPSS version 13.0 software (IBM, Armonk, NY, USA). Continuous variables are presented as the mean ± standard error of the mean. Intergroup differences in the continuous variables were evaluated using unpaired Student *t*-tests and an analysis of variance. Differences in genotype and allele frequencies were evaluated using a chi-squared analysis. Linear regression models adjusted for potential confounders were used for assessing changes in HbA_{1c}, FPG and PPG in response to metformin treatment based on the SNP genotypes. For the distributions of the genotypes, deviations from Hardy-Weinberg equilibrium were evaluated using chi-squared tests where appropriate.

Results

Patient characteristics

The mean age of the participants in the three study groups was 56.4 ± 11.6 years, and the mean BMI was 25.7 ± 3.7 kg/m^2. No significant intergroup difference in sex, age, BMI, or mean systolic or diastolic BP was observed between the DM, MR, and control groups at baseline.

Table 2. Genotype and allele frequencies of the *OCT1* and *MATE1* genetic variants

Polymorphism (gene)	Genotype-Allele	DM group *n* (frequency)[a]	MR group *n* (frequency)[b]	Control group *n* (frequency)[c]	*P*-value
rs1867351 (*OCT1*)	C/C	39 (28.5)	3 (30.0)	16 (12.9)	
	C/T	56 (40.9)	4 (40.0)	62 (50.0)	0.040
	T/T	42 (30.7)	3 (30.0)	46 (37.0)	
	C	49.0	50.0	38.0	0.165
	T	51.0	50.0	62.0	
Hardy-Weinberg equilibrium (*p*)		0.03	0.44	0.53	
rs4709400 (*OCT1*)	C/C	29 (21.2)	1 (10.0)	29 (23.4)	
	C/G	57 (41.6)	4 (40.0)	58 (46.8)	0.64
	G/G	51 (37.2)	5 (50.0)	37 (29.8)	
	C	42.0	30.0	46.8	0.044[d]
	G	58.0	70.0	53.2	
Hardy-Weinberg equilibrium (*p*)		0.09	0.45	0.88	
rs628031 (*OCT1*)	G/G	69 (50.4)	9 (90.0)	69 (55.6)	
	G/A	59 (43.1)	0 (0.0)	45 (36.3)	0.049
	A/A	9 (6.60)	1 (10.0)	10 (8.1)	
	G	72.0	90.0	73.8	0.003[de]
	A	28.0	10.0	26.2	
Hardy-Weinberg equilibrium (*p*)		0.44	0.49	<0.01	
rs2297374 (*OCT1*)	C/C	75 (54.7)	3 (30.0)	52 (41.9)	
	C/T	47 (34.3)	6 (60.0)	59 (47.6)	0.14
	T/T	15 (11.0)	1 (10.0)	13 (10.5)	
	C	71.9	60.0	65.7	0.207
	T	28.1	40.0	34.3	
Hardy-Weinberg equilibrium (*p*)		0.08	0.53	0.43	
rs12208357 (*OCT1*)	C/C	136 (99.3)	10 (100.0)	124 (100.0)	
	C/T	0 (0.0)	0 (0.0)	0 (0.0)	1.0
	T/T	1 (0.7)	0 (0.0)	0 (0.0)	
	C	93.0	100.0	100.0	---
	T	7.0	0.0	0.0	
Hardy-Weinberg equilibrium (*p*)		<0.01	---	---	
rs72552763 (*OCT1*)	Wt/Wt	0 (0.0)	0 (0.0)	0 (0.0)	
GAT deletion (3^-)	Wt/3^-	0 (0.0)	0 (0.0)	0 (0.0)	---
	$3^-/3^-$	136 (100.0)	10 (100.0)	124 (100.0)	
Hardy-Weinberg equilibrium (*p*)		---	---	---	
rs113569197 (*OCT1*)	Wt/Wt	128 (93.4)	9 (90.0)	114 (91.9)	
TGGTAAGT insertion (8^+)	Wt/8^+	0 (0.0)	0 (0.0)	0 (0.0)	0.63
	$8^+/8^+$	9 (6.6)	1 (10.0)	10 (8.1)	
Hardy-Weinberg equilibrium (*p*)		---	---	---	
rs36056065 (*MATE1*)	Wt/Wt	0 (0.0)	0 (0.0)	0 (0.0)	
GTAAGTTG insertion (8^+)	Wt/8^+	58(42.3)	0 (0.0)	46 (37.1)	0.020[f]
	$8^+/8^+$	79 (57.7)	10 (100.0)	78 (62.9)	
Hardy-Weinberg equilibrium (*p*)		---	---	---	

[a]*N* = 137, [b]*N* = 124, [c]*N* = 10, [d]MR group compared to control group, [e]DM group compared to MR group, [f]DM group compared to control group; Wt: wild type (no deletion or insertion).

Sixteen patients were lost to follow-up. Among these, 12 DM patients failed to provide blood samples after completing 3 months of metformin treatment, and four patients elected to ter-

Table 3. Comparison of study variables based on rs1867351 genotypes

Glycemic index	C/C	C/T	T/T	*P*-value
FPG baseline (mM)	8.9 ± 2.3	8.9 ± 2.5	9.9 ± 2.9	0.97
FPG 3 mos (mM)	7.0 ± 1.2	7.4 ± 1.3	8.2 ± 3.3	0.040
ΔFPG (mM)	-1.9 ± 2.3	-1.5 ± 2.1	-1.6 ± 1.1	0.77
PPG baseline (mM)	11.9 ± 2.70	11.6 ± 3.10	13.6 ± 5.20	0.86
PPG 3 mos (mM)	10.4 ± 2.70	10.2 ± 3.00	10.9 ± 3.60	0.37
ΔPPG (mM)	-1.5 ± 2.9	-1.4 ± 3.1	-2.7 ± 2.3	0.060
HbA_{1c} baseline (%)	8.0 ± 1.5	8.3 ± 2.0	7.9 ± 2.1	0.21
HbA_{1c} 3 mos (%)	7.2 ± 0.90	7.1 ± 0.90	6.9 ± 0.60	0.24
ΔHbA_{1c} (%)	-1.0 ± 0.90	-0.90 ± 1.3	-2.1 ± 4.8	0.020

Table 4. Comparison of study variables based on rs4709400 genotypes

Glycemic index	C/C	C/G	G/G	*P*-value
FPG baseline (mM)	9.5 ± 3.7	8.5 ± 3.5	9.2 ± 2.2	0.43
FPG 3 mos (mM)	8.5 ± 3.5	7.2 ± 1.0	7.1 ± 1.6	0.0060
ΔFPG (mM)	-0.90 ± 1.6	-1.9 ± 2.0	-2.0 ± 2.2	0.046
PPG baseline (mM)	13.7 ± 5.90	11 ± 4.0	12 ± 2.5	0.68
PPG 3 mos (mM)	11 ± 4.0	10.4 ± 2.70	10.2 ± 2.80	0.52
ΔPPG (mM)	-2.7 ± 2.6	-1.2 ± 3.0	-1.9 ± 2.9	0.070
HbA_{1c} baseline (%)	8.3 ± 2.2	7.0 ± 0.70	8.1 ± 1.4	0.12
HbA_{1c} 3 mos (%)	7 ± 0.7	7 ± 0.8	7.2 ± 0.9	0.39
ΔHbA_{1c} (%)	0.5 ± 0.5	0.7 ± 1.2	1 ± 0.8	0.68

minate metformin treatment during the study period. Thus, the DM group contained 137 patients in our final analyses.

No significant intragroup difference in sex, age, BMI, or mean systolic or diastolic BP was observed at baseline based on the presence of the rs1867351, rs4709400, rs628031, or rs2297374 SNP of *OCT1* (**Table 1**). In addition, no significant intragroup difference in the demographic or baseline clinical variables was observed based on the presence of the *OCT1* polymorphism rs72552763, rs113569197, or rs12208357 or the *MATE1* polymorphism rs36056065 (data not shown). The patients' baseline HbA_{1c} levels in the DM group ranged from 7% to 12% following 3 months of metformin treatment.

Allele frequencies of the genetic variants

The genotype distributions, allelic frequencies, and Hardy-Weinberg equilibrium status for the eight variants in our Han Chinese cohort are summarized in **Table 2**. The rs1867351 allele frequencies were 49.0% (C) and 51.0% (T) in the DM group, 50.0% (C) and 50.0% (T) in the MR group, and 38.0% (C) and 62.0% (T) in the control group. No significant difference in the rs1867351 allele frequencies were observed between the DM, MR, and control groups (P = 0.165).

The rs4709400 allele frequencies were 42.0% (C) and 58.0% (G) in the DM group, 30% (C) and 70% (G) in the MR group, and 46.8% (C) and 53.2% (G) in the control group. The rs4709400 allele frequencies in the DM group were significantly different from those in the MR group (P = 0.044). The rs628031 allele frequencies were 72.0% (G) and 28.0% (A) in the DM group, 90% (G) and 10% (A) in the MR group, and 73.8% (G) and 26.2% (A) in the control group. The rs628031 allele frequencies in the MR group were significantly different than those in the DM (P = 0.003) and control groups (P = 0.003). The rs2297374 allele frequencies were 71.9% (C) and 28.1% (T) in the DM group, 60% (C) and 40% (T) in the MR group, and 65.7% (C) and 34.3% (T) in the control group. No significant difference in the rs2297374 allele frequencies were observed between the DM, MR, and control groups (P<0.207). The frequencies of the C/C genotype and the C allele of rs12208357 were >99% in all three study groups, with only one patient in the DM group having a different genotype (T/T).

The allele frequency of the 3-bp deletion (GAT) mutation at rs72552763 was 100% in all of the study groups, with no subjects in any of the groups having the wild-type (Wt) allele. The allele frequencies for the 8-bp insertion mutation (8^+) at rs113569197 were 93.4% (Wt) and 6.6% (8^+) in the DM group, 90% (Wt) and 10% (8^+) in the MR group, and 91.9% (Wt) and 8.1% (8^+) in the control group, with no subjects in any of the three groups having the heterozygous genotype (Wt/8^+). The allele frequencies of the

Table 5. Comparison of study variables based on rs628031 genotype

Glycemic index	A/A	A/G	G/G	*P*-value
FPG baseline (mM)	8.03 ± 2.42	7.98 ± 1.85	8.47 ± 2.63	0.48
FPG 3 mos (mM)	7.4 ± 2.6	7.6 ± 2.2	7.1 ± 1.5	0.34
ΔFPG (mM)	-0.63 ± 1.5	-0.38 ± 1.7	-1.37 ± 2.1	<0.010
PPG baseline (mM)	10.4 ± 2.65	10.7 ± 3.27	11.1 ± 3.29	0.72
PPG 3 mos (mM)	9.8 ± 1.7	10.6 ± 3.40	10.2 ± 2.60	0.63
ΔPPG (mM)	-0.58 ± 1.7	-0.10 ± 1.0	-0.91 ± 1.8	0.92
HbA_{1c} baseline (%)	7.71 ± 2.42	7.89 ± 1.83	7.51 ± 1.68	0.49
HbA_{1c} 3 mos (%)	6.1 ± 0.50	7.0 ± 0.60	7.2 ± 0.90	<0.010
ΔHbA_{1c} (%)	-1.1 ± 1.7	-0.89 ± 1.4	-0.31 ± 1.6	0.020

Table 6. Comparison of study variables based on rs2297374 genotype

Glycemic index	C/C	C/T	T/T	*P*-value
FPG baseline (mM)	7.93 ± 2.19	8.66 ± 2.15	8.42 ± 3.20	0.22
FPG 3 mos (mM)	7.1 ± 1.2	7.6 ± 2.6	8.4 ± 1.7	0.035
ΔFPG (mM)	-0.83 ± 2.3	-1.1 ± 1.5	-0.020 ± 1.5	0.0020
PPG baseline (mM)	10.9 ± 3.33	11.3 ± 3.16	9.79 ± 2.97	0.36
PPG 3 mos (mM)	10.2 ± 2.80	11.2 ± 3.30	8.8 ± 1.1	0.015
ΔPPG (mM)	-0.66 ± 3.3	-0.05 ± 2.4	-0.99 ± 1.9	0.92
HbA_{1c} baseline (%)	7.48 ± 1.58	7.91 ± 1.94	8.03 ± 2.26	0.32
HbA_{1c} 3 mos (%)	7.3 ± 0.90	6.8 ± 0.70	7.0 ± 0.10	0.0030
ΔHbA_{1c} (%)	-0.18 ± 3.5	-1.1 ± 3.7	-1.0 ± 4.5	0.039

8-bp insertion mutation (8^+) at rs36056065 were 21.1% (Wt) and 78.9% (8^+) in the DM group, 0.0% (Wt) and 100% (8^+) in the MR group, and 18.6% (Wt) and 81.5% (8^+) in the control group, with no subjects in any of the three study groups having the homozygous Wt genotype. We excluded rs12208357, rs7255-2763, rs113569197, and rs36056065 from our analysis of the clinical variables because the genotype distributions of each deviated substantially from Hardy-Weinberg equilibrium.

Clinical effects of the genetic variants

We compared changes in the levels of FPG, PPG, and HbA_{1c} between the different genotypes of the rs1867351, rs4709400, rs628-031, and rs2297374 genetic variants of *OCT1*. The DM patients with the T/T genotype of rs1867351 exhibited significantly greater reductions in their PPG (P = 0.06) and HbA_{1c} (P = 0.02) levels following metformin treatment than those with the other rs1867351 genotypes (**Table 3**). The DM patients with the G/G genotype of rs4709400 exhibited significantly greater reductions in their FPG (P = 0.046) and PPG (P = 0.07) levels following metformin treatment than those with the other rs4709400 genotypes (**Table 4**). Following metformin treatment, the DM patients with the G/G genotype of rs6280-31 exhibited significantly greater reductions in their FPG level (P<0.01), and those with the A/A genotype of rs628031 exhibited significantly greater reductions in their HbA_{1c} level (P<0.02), compared to those with the heterozygous genotype (G/A) (**Table 5**). The DM patients with the C/T genotype of rs2297374 exhibited significantly greater reductions in their FPG (P = 0.002) and HbA_{1c} (P = 0.039) levels following metformin treatment than those with the homozygous rs2297374 genotypes (C/C and T/T) (**Table 6**).

Discussion

We determined the genotype and allele frequencies of eight genetic variants of *OCT1* and *MATE1* in a cohort of Han Chinese subjects in Shanghai, China (HCS), and evaluated the effects of the rs1867351, rs4709400, rs628031, and rs2297374 SNPs of *OCT1* on the glycemic response to metformin treatment in HCS DM patients. We found that, although none of these *OCT1* genetic variants were associated with differences in the baseline demographic or clinical variables, these SNPs exhibited selective effects on FPG, PPG, and HbA_{1c}. The HCS DM patients with the rs1867-351 T/T or rs4709400 G/G genotype exhibited greater reductions in their PPG level following metformin treatment than those with the other rs1867351 and rs4709400 genotypes. The HCS DM patients with the rs2297374 C/T genotype or the G/G genotype of rs4709400 or rs628031 exhibited greater reductions in their FPG level following metformin treatment than those with the other genotypes of these SNPs. The HCS DM patients with the rs186-7351 T/T, rs628031 A/A, or rs2297374 C/T genotype exhibited greater reductions in their

HbA_{1c} level following metformin treatment than those with the other genotypes of these SNPs.

The OCT1-mediated uptake of metformin into hepatocytes is essential for the inhibitory effect of the drug on hepatic gluconeogenesis. Genetic variation in *OCT1* can therefore affect the glycemic effects of metformin. Shikata et al [23] were the first to investigate the effects of *OCT1* polymorphisms, including rs628031, on the efficacy of metformin for DM, and reported that *OCT1* genetic variants were not associated with changes in HbA_{1c}. In that same year, Shu *et al [24]* reported that patients with at least one *OCT1* variant exhibited higher plasma glucose levels in the oral glucose tolerance test. Furthermore, although certain *OCT1* genetic variants, such as rs11212617, have been shown to be associated with improved metformin efficacy in some populations of DM patients [14, 17, 25], similar results have not been observed in other populations [10, 25]. These findings demonstrate the need for the use of multiple markers of glycemic response in studies of the contribution of *OCT1* polymorphisms to the glucose-lowering effects of metformin, and indicate that the role of *OCT1* polymorphisms in modulating responsiveness to metformin treatment for DM is highly dependent on the genetic background of the patient.

Differences in *OCT1* genotype and allele frequencies have been shown to occur between different races. In a recent study, Zhou et al [17] showed that a significant relationship existed between the C allele of rs11212617 and greater reductions in FPG, PPG, and Hb1Ac in HCS DM patients, with the C/C genotype exhibiting the greatest reductions in these glycemic parameters. Although the frequencies of rs11212617 alleles in their healthy HCS control group (A, 32.5%; C, 67.5%) differed substantially from those previously reported by the International HapMap Project (IHP) [26] for two American populations of African and European descent (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=11212617), they were more similar to those previously reported by the IHP for a population of Han Chinese subjects in Beijing (HCB) (A: 39%, C: 61%). In our current study, although the allele frequencies of rs4709400 and rs628031 in our HCS control group (**Table 2**) differed from those in the HCB sample (C: 38.9%, G: 61.1% and G: 83.7%, A: 16.3%, respectively), the allele frequencies of rs1867351 and rs2297374 in our HCS control group (**Table 2**) were more similar to those reported for HCB subjects (C: 35%, T: 65% and C: 68.3%, G 31.7%, respectively). These data show that differences in the allele frequencies of *OCT1* polymorphisms can exist within a single ethnic group, highlighting the importance of understanding the contributions of *OCT1* polymorphisms to metformin responsiveness in the development of patient-centered treatment strategies for DM.

To evaluate the contributions of rs1867351, rs4709400, rs628031, and rs2297374 to metformin response in HCS DM patients, we compared changes in the levels of FPG, PPG, and HbA_{1c} between the different genotypes of these genetic variants following 3 months of incident metformin treatment. We found that the HCS DM patients with the T/T genotype of rs1867351 exhibited greater reductions in the PPG ($P = 0.06$) and HbA_{1c} ($P = 0.02$) levels, compared with those of the HCS DM patients with the other rs1867351 genotypes (**Table 3**) The rs1867351 locus (http://www.ncbi.nlm.nih.gov/snp/?term = rs1867351) contains a promoter-linked SNP in OCT1, and a previous study has shown that rs1867351 was associated with reduced renal clearance of metformin [27]. Therefore, it is likely that the T allele of rs1867351 in our HCS DM patients contributed to reductions in the FPG and Hb1Ac levels by maintaining a higher relative concentration of metformin in their blood.

The A>G polymorphism at rs628031 causes a missense mutation in exon 7 of *OCT1*, consisting of an amino acid substitution at position 408 in the OCT1 protein (Met408Val). One of the most common *OCT1* polymorphism in Caucasian populations [19], the rs628031 polymorphism does not affect OCT1 expression or function [24, 28], and Shikata et al [23] reported that rs628031 had no significant effect on the clinical efficacy of metformin based on the level of HbA_{1c} in a cohort of Japanese DM patients. In our current study, DM patients with the G/G genotype of rs628031 exhibited a significantly greater reduction in their FPG level following metformin treatment, and those with the A/A genotype exhibited a significantly greater reduction in HbA_{1c}, compared to those with the other rs628031 genotypes (**Table 5**). These data show that substantial variation in the contribution of

rs628031 to metformin efficacy exists between different Asian populations.

We found that the intron variants rs2297374 and rs4709400 also affected metformin efficacy in HCS DM patients. Following 3 months of incident metformin treatment, the HCS DM patients with the C/T genotype of rs2297374 exhibited significantly greater reductions in their FPG and HbA_{1c} levels than those with the other rs2297374 genotypes (**Table 6**). The HCS DM patients with the G/G genotype of rs4709400 exhibited significantly greater reductions in their FPG and PPG levels than those with the other rs4709400 genotypes (**Table 4**). Although it is unlikely that such SNPs alter OCT1 function, it is possible that variation at these loci might affect OCT1 mRNA processing. Future studies of the effects of rs628031, rs2297374, and rs4709400 on metformin responsiveness in Han Chinese DM patients are warranted to determine whether our results are representative of the contributions of these SNPs in other Chinese populations.

In our current study, we also observed significant differences in the allele frequencies of rs4709400, rs628031, and rs2297374 between the DM and MR groups in our HCS cohort. These data suggest that these polymorphisms might play a role in the development of metformin resistance. Future studies are warranted to determine whether these polymorphisms may represent prognostic indicators of poor response to metformin treatment. Future studies of rs4709400, rs628031, and rs2297374 might also provide insight into the molecular interaction between OCT1 and metformin, which could facilitate the development of new metformin analogs that are refractory to these metformin-resistant mutations.

Our findings are, however, subject to certain limitations. Our single-center study design and our relatively small sample of HCS subjects, especially the MR group (n = 10), may have contributed to shortcomings in our data sets, particularly concerning the criteria for establishing Hardy-Weinberg equilibrium. Our preliminary investigation included the *OCT1* polymorphisms, rs72552763, rs113569197, and rs12208357, and the *MATE1* polymorphism, rs36056065, but the genotype distributions of these variants demonstrated substantial deviation from Hardy-Weinberg equilibrium (**Table 2**). Although the genotype frequencies of these polymorphisms in our HCS sample were similar to those reported for Asian populations in the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/snp), the database samples were also relatively small. Therefore, we excluded these genetic variants from our analysis of metformin efficacy. Future studies of the distribution of these polymorphisms in larger cohorts of Han Chinese subjects are warranted to provide a foundation for studies of their effects on glycemic response to metformin therapy in Han Chinese DM patients.

In conclusion, we examined the genotype distributions of rs1867351, rs4709400, rs628031, and rs2297374 in HCS DM, MR, and control subjects. We found that the allele frequencies of rs4709400 and rs628031 in our HCS control group differed from those in HCB subjects, whereas the allele frequencies of rs1867351 and rs2297374 in our control group were more similar to those reported for HCB subjects. We also found that these SNPs exhibited selective effects on FPG, PPG, and HbA_{1c} levels in HCS DM patients in response to 3 months of incident metformin monotherapy. The DM patients with the rs1867351 T/T or rs4709400 G/G genotype exhibited greater reductions in their PPG level following metformin treatment, compared to those with the other rs1867351 and rs4709400 genotypes. The HCS DM patients with the rs2297374 C/T, rs4709400 G/G, or rs628031 G/G genotype exhibited greater reductions in their FPG level following metformin treatment, compared to those with the other genotypes of these SNPs, and the HCS DM patients with the rs1867351 T/T, rs628031 A/A, or rs2297374 C/T genotype exhibited greater reductions in their HbA_{1c} level in response to metformin treatment, compared to those with the other genotypes of rs1867351, rs628031, and rs2297374. Future studies of these *OCT1* polymorphisms in larger samples of CHS DM patients are warranted to confirm our findings and identify the molecular mechanisms through which these SNPs affect the metformin-mediated inhibition of gluconeogenesis in hepatocytes.

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Disclosure of conflict of interest

None.

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