Original Article

SLC22A2 gene 808 G/T variant is related to plasma lactate concentration in Chinese type 2 diabetics treated with metformin

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Aim: To investigate the potential relationship between the SLC22A2 gene polymorphism and blood lactate concentration in Shanghai Hans suffering from type 2 diabetes mellitus (T2DM).

Methods: The SLC22A2 single nucleotide polymorphism (SNP) 808G/T was genotyped in 400 T2DM patients, including a metformin-treated group ($n=200$) and a non-metformin-treated group ($n=200$). Fasting plasma lactic acid levels were measured with an enzyme-electrode assay. Biochemical indexes, including plasma alanine aminotransferase (ALT), creatinine (Cr), and glycolated hemoglobin (HbA1c), were also measured.

Results: The fasting plasma lactate concentration in the metformin-treated group was significantly higher than that in the non-metformin-treated group (1.29 ± 0.45 mmol/L vs 1.18 ± 0.44 mmol/L, $P=0.015$). Additionally, the ratio of patients with hyperlactacidemia was 8% (16/200) for the metformin-treated group and 5.5% (11/200) for the non-metformin-treated group, with no lactic acidosis found in either group. The frequency of the SLC22A2 808G/T T allele was 12.9%. Patients with the mutant genotype (TT) had a higher blood lactate concentration in the metformin-treated group than those in the non-metformin-treated group ($t=2.492$, $P=0.013$). This trend was not observed in the GG and GT genotypes when compared with metformin-treated and non-metformin-treated groups. Patients with the mutant genotype (TT) in the metformin-treated group also had a higher incidence of hyperlactacidemia compared with the GG genotype (40.0% vs 6.9%, $P=0.050$) in the metformin-treated group and the GG (6.0%, $P=0.042$) or GT (4.3%, $P=0.043$) genotypes in the non-metformin-treated group. In the metformin-treated group, there were significant gender differences in lactate concentrations in the TT (2.18 ± 0.15 vs 1.04 ± 0.27 mmol/L, $P=0.008$) and GG genotypes (1.40 ± 0.51 vs 1.19 ± 0.35 mmol/L, $P=0.004$). The lactate levels of women with the TT genotype were the highest in the metformin-treated group, but differences in lactate levels among the genotypes were not observed in the non-metformin-treated group.

Conclusion: There is an 808G/T polymorphism in the SLC22A2 gene in Chinese Hans with T2DM. The 808G>T variance in the SLC22A2 gene can affect the plasma lactate level and the incidence of hyperlactacidemia in T2DM patients undergoing metformin therapy. Additionally, the female patients carrying the TT genotype are prone to lactatemia.

Keywords: ASP-PCR; metformin; SLC22A2 gene; organic cation transporter 2; lactate; single nucleotide polymorphism; type 2 diabetes mellitus

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Introduction

Metformin is one of the most commonly used drugs for the treatment of type 2 diabetes mellitus (T2DM). It also has beneficial effects in cardiovascular disease, impaired glucose tolerance and polycystic ovary syndrome through an insulin-sensitizing effect^[1]. However, metformin therapy is characterized by considerable inter-individual variability in clinical efficacy^[2]. Some studies have shown that metformin is not

metabolized but is transported by at least two organic cation transporters (OCTs), OCT1 and OCT2^[3, 4]. The human OCT1 transporter (SLC22A1) is primarily expressed in the liver and is not detected in the kidney. Furthermore, OCT2 (SLC22A2) has been found to be the most abundant organic cation transporter in the basolateral membranes of human kidney^[5, 6]. A kinetic analysis of metformin transport and its distribution in rats suggested that metformin is a superior substrate for renal OCT2 rather than hepatic OCT1, and renal OCT2 plays a dominant role in metformin pharmacokinetics^[4]. In addition, OCT2 is responsible for the observed gender differences in

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renal basolateral membrane organic cation transporting activity^[7-9]; however, to date no studies related to human OCT2 and its relevance to metformin-treatment in diabetic populations have been reported.

Genetic factors have been estimated to account for 64% to 94% of individual variations in renal clearance of various drugs, including metformin, amoxicillin, and ampicillin^[10]. In Chinese people in particular, genetic factors have been shown to account for up to 75% of the inter-individual variability in drug disposition and its effects^[11]. The existence of genetic polymorphisms in the human OCT2 gene has been previously reported in some populations. A total of 28 genetic variations in the OCT2 gene have been identified in non-Asian American populations, as reported by Leabman. Among those variants, three non-synonymous SNPs (T199I, T201M, and A270S) in the SLC22A2 gene decreased the renal clearance of metformin compared to the reference genotype^[12]. The non-synonymous SNP, 808G>T (Ala270Ser), was found to be present in 13.3% of individuals from a healthy population of Chinese from Hong Kong. Subjects who are homozygous for 808G>T tend to have higher plasma exposure to metformin, and this can be further enhanced in elderly patients or those with renal dysfunction^[13]; however, other variations in the OCT2 gene were rare (less than 3%) in the Chinese population. Thus, the variant that is the most likely to be related to metformin sensitivity and plasma lactate in Chinese Hans with T2DM may be the 808G/T transversion in the SLC22A2 gene.

Serum lactate level is a balance between its synthesis and elimination. Lactate, which is produced in the gut, liver, and peripheral tissues, such as erythrocytes and the skin, is utilized to form glucose in the liver^[14]. The portion of lactate that is not metabolized is excreted almost completely in the urine by the kidney. Two possible mechanisms for raised lactate levels due to metformin have been proposed: 1) increases in glycolytic lactate production in peripheral tissues^[15] and 2) inhibition of lactate metabolism/transport in the liver and other tissues such as heart and muscle. Wang DS reported that the plasma lactate concentrations of metformin-treated wild-type mice were 2.5-fold greater than that in metformin-treated OCT (-/-) mice^[16]. However, prospective large-scale studies in patients with diabetes still lack the necessary evaluation of the clinical relevance of the SLC22A2 genetic polymorphisms and their impact on the beneficial and adverse effects of metformin.

The allele-specific primer polymerase chain reaction (ASP-PCR) assay is a method used to determine SNPs based on DNA amplification, and the SNPs can be determined by whether the primers anneal to the target DNA^[17, 18]. The present study was designed to determine SNPs at position 808 of the SLC22A2 gene using ASP-PCR and to investigate the potential relationship between SLC22A2 gene polymorphisms and blood lactate concentrations in Shanghai Hans with T2DM.

Materials and methods

Subjects

A total of 400 patients with T2DM [diagnosed according to

1999 World Health Organization (WHO) and American Diabetes Association standards] were enrolled in the study between April 2007 and October 2008 from the Shanghai Diabetes Center, including a group receiving metformin treatment ($n=200$) and another group with no metformin treatment ($n=200$). The patients had a male: female ratio of 200:200 and a median age of 60 years (range 35–77 years). All participants with pre-disposing conditions, such as congestive heart failure, renal insufficiency, chronic lung disease with hypoxia, history of alcohol use, or aged older than 80 years, were excluded from the study. These conditions were determined from their medical history, physical examination, electrocardiographic evaluation, and routine laboratory tests (blood chemistry evaluation, hematologic testing, and urinalysis). The patients did not take any medications other than hypoglycemic agents (sulfonylureas and glitazones) starting three days before being admitted to the hospital.

Measurement of plasma lactate concentrations and biochemical indexes

After taking metformin [500 mg three times daily (tid)] at least three days from the first day of admission to the hospital, blood samples from metformin-treated and non-metformin-treated groups were collected in the morning of the fourth day after an overnight fast and without any physical activity. The fasting venous plasma glucose (FPG), creatinine (Cr), urea nitrogen (BUN), alanine aminotransferase (ALT), and HbA1c were determined on the same morning with lactate. FPG was determined with the glucose oxidase method. Kidney and liver function was measured on an automatic analyzer (Hitachi 7180 biochemistry automatic analyzer, Japan). HbA1c was measured with a high-performance liquid chromatography (HPLC). Body mass index (BMI) was calculated by dividing body weight in kg divided by the square of the height in m. The glomerular filtration rate (GFR) was detected by a Technetium-99 isotope scan. The total GFR was calculated as the sum of the left and right kidneys. If GPT>60 U/L or Cr>110 $\mu\text{mol/L}$ or GFR<60 mL/min, the patient was excluded from the study. A total of 400 patients satisfied the above criteria and were recruited into this study. Written informed consent was obtained from all subjects.

SNP determination (808G>T) in the SLC22A2 gene using the ASP-PCR method

The genomic sequence of SLC22A2 exon4 was obtained using the BLAST program (accession number: rs316019) (<http://www.ncbi.nlm.nih.gov/SNP>). The genotyping of the SNP (808G>T) of SLC22A2 was performed using the ASP-PCR method according to previous reports^[18]. The primer set (forward primer: 5'-AAAGGTTCTACCGTCCAA-3'; reverse primer for 808G: 5'-GTGGTTGCAGTTCACAGTAGC-3'; reverse primer for 808T: 5'-GGTGGTTCAGTTCACAGTATC-3') was designed to amplify a 359-bp and 360-bp portion of the SLC22A2 gene encoding the SNP. ASP-PCR was carried out in 20 μL reaction volumes consisting of 2*reaction buffer, 4 mmol/L dNTPs, 15 pmol primer mixture, 2U *Taq*

DNA polymerase (5 U/ μ L), and 25 ng genomic DNA (20 ng/ μ L) (Sai Bai Sheng (sbs) gene company, Shanghai, China). The reaction mixtures were placed in an ABI 9700 thermal cycler (Applied Biosystems) that was programmed for touch-down PCR to improve the specificity of PCR amplification^[19]. For this procedure, the annealing temperature was 57 °C for two PCR cycles and decreased by 1 °C per two cycles for the next 4 cycles until an optimal annealing temperature of 55 °C was reached. Then, there were 35 PCR cycles at the annealing temperature of 55 °C with a final extension at 72 °C for 10 min. PCR products were analyzed by electrophoresis on 3% agarose gels followed by ethidium bromide staining and inspection under UV light.

Direct sequencing of the *SLC22A2* gene in randomly selected individuals with and without the 808G/T mutation

To confirm the results of the ASP-PCR-based determination of the 808G>T SNP in the *SLC22A2* gene, exon4 of the *SLC22A2* gene, including site 808, was sequenced using DNA samples from randomly selected individuals diagnosed to be wt (wild-type), heterozygote or mutant homozygote.

Statistical analyses

All numerical values are expressed as the mean $\pm$ SD, and the count data are expressed as percentages. The Hardy-Weinberg equilibrium was tested by SHEsis. The plasma lactate concentration between different groups and different genders of the three genotypes was compared by factorial design variance analysis. The blood lactate concentration of the three genotypes was compared by one-way analysis of variance (ANOVA) followed by Fisher's exact test to find statistically significant differences in the frequency of genotypes of *SLC22A2* (808G>T) between the two groups. Whether Cr, ALT, or GFR values and plasma lactate concentration differed among genders and groups was tested using Student's *t* test or Chi-square test. Finally, the incidence of hyperlactacidemia in different genotypes was compared using Fisher's exact test. All analyses were performed with SPSS software (version 16.0; SPSS, Chicago, Illinois, USA). All *P*-values were two-sided and a *P* value of <0.05 was considered significant.

Results

Identification of organic cation transporter 2 genetic polymorphisms

The results of ASP-PCR showed that there were three geno-

types of the *SLC22A2* gene at position 808: GG (*n*=308), GT (*n*=81) and TT (*n*=11) in 400 T2DM patients of Chinese Hans (Table 1 and Figure 1), and this was confirmed by direct sequencing (Figure 2A–2C). The gene distributions were consistent with Hardy-Weinberg equilibrium in all of the groups. In short, the frequencies of the GT genotype and the TT genotype in all patients were 20.2% and 2.8%, respectively, and the T allele frequency was 12.9%.

Figure 1. Representative patterns of electrophoresis of allele specific primer-polymerase chain reaction (ASP-PCR) products with different primers for determination of 808G/T genotypes of the *SLC22A2* gene. When the PCR products were observed in the lanes of 808G primers and no PCR products were observed in lanes for 808T primers (359 bp and 360 bp, respectively), the strain was diagnosed as wild-type (wt) (A). When the PCR products were observed in the lanes of 808G and 808T primers (359 bp and 360 bp, respectively), the strain was considered as heterozygote (A). When the PCR products were observed in the lanes of 808T and no PCR products were observed in lanes for 808G primers, the strain was diagnosed as mutant homozygotes. (B) Applying the clear genotype of 8 samples as control for each test.

The general biochemical characteristics in different genotypes of the two groups

Table 2 shows the clinical characteristics of patients (*n*=400) in this study. The patients with the TT genotype in the metformin-treated group (*n*=200) had significantly lower HbA1c compared to the GG or GT genotypes in the metformin-treated group (*P*=0.022 and *P*=0.033, respectively). There were no differences in age, BMI, duration of diabetes, FPG and BUN,

Table 1. The numbers and frequencies of three genotypes and alleles in groups with and without metformin therapy.

Exon	Subjects	Metformin (<i>n</i> =200)		Non-metformin (<i>n</i> =200)		Total (<i>n</i> =400)	
		Genotypes <i>n</i> (%)	Allele <i>n</i> (%)	Genotypes <i>n</i> (%)	Allele <i>n</i> (%)	Genotypes <i>n</i> (%)	Allele <i>n</i> (%)
Exon4	808G/T	GG 160 (80)	G 355 (88.7)	GG 148 (74)	G 342 (85.5)	GG 308 (77)	G 697 (87.1)
		GT 35 (17.5)	T 45 (11.3)	GT 46 (23)	T 58 (14.5)	GT 81 (20.2)	T 103 (12.9)
		TT 5 (2.5)		TT 6 (3)		TT 11 (2.8)	

Hardy-Weinberg balance test; Fisher's exact test, *P*=0.383 vs non-metformin group.

Figure 2. Direct sequence results of sense strands of *SLC22A2* genotypes of (A) wildtype (wt), (B) 808T, (C) 808G/T strains. In the strains with wt, the sense base at position 808 was G (A). In the strain with 808T mutation, the sense base at position 808 was T (B). In the strain with 808G/T, the sense bases at position 808 were G and T, respectively.

Cr, ALT, or GFR between different genotypes both in the metformin-treated and the non-metformin-treated groups ($P>0.05$; Table 2).

Plasma lactate concentration in different genotypes of the two groups

Of these 400 cases, the fasting plasma lactate concentration in the metformin-treated group (1.29 ± 0.45 mmol/L) was higher than in the non-metformin-treated group (1.18 ± 0.44 mmol/L, $P=0.015$), and the ratio of patients with hyperlactacidemia in the metformin-treated group was slightly higher than in the non-metformin-treated group (8% vs 5.5%, $P=0.58$), but no lactic acidosis was found in any of the patients. Figure 3 indicates that the patients in the metformin-treated group that carried the mutant homozygote genotype (TT) had higher lactate concentrations than patients in the non-metformin-treated group with the TT genotype ($P=0.013$); however, the difference in blood lactate concentration between the two groups with GG and GT genotypes was not significant ($P=0.053$, $P=0.344$,

Figure 3. The plasma lactate levels of three genotypes in two groups with and without metformin treatment. By the method of analysis of variance of factorial design, there was no significant difference in the blood lactate levels of GG and GT genotypes in two groups ($P=0.053$, $P=0.344$). The plasma lactate level of metformin group carried TT genotype was higher obviously than that of non-metformin group with TT ($t=2.492$, $P=0.013$). Each column represents as mean $\pm$ SEM. ^b $P=0.013$, compared with non-metformin group with TT genotype.

Table 2. Comparison of the clinical characteristics and biochemical indexes between subjects with and without metformin treatment.

	Metformin group			Non-metformin group		
	GG	GT	TT	GG	GT	TT
N	160	35	5	148	46	6
Age	57.24 $\pm$ 11.22	59.91 $\pm$ 12.07	59.20 $\pm$ 12.23	63.21 $\pm$ 11.83	63.32 $\pm$ 11.31	64.5 $\pm$ 9.79
BMI	25.99 $\pm$ 3.22	26.76 $\pm$ 3.89	25.79 $\pm$ 4.40	24.04 $\pm$ 3.12	23.11 $\pm$ 3.17	24.73 $\pm$ 2.88
DUR	7.11 $\pm$ 6.37	6.93 $\pm$ 6.46	8.2 $\pm$ 5.4	8.06 $\pm$ 7.53	8.07 $\pm$ 7.05	10.66 $\pm$ 11.41
FPG	8.76 $\pm$ 2.81	8.48 $\pm$ 2.10	8.11 $\pm$ 1.92	7.99 $\pm$ 2.59	7.61 $\pm$ 2.29	6.80 $\pm$ 1.11
HbA1c	8.97 $\pm$ 2.03	8.88 $\pm$ 1.75	7.70 $\pm$ 0.84 ^{be}	9.01 $\pm$ 2.23	8.93 $\pm$ 2.32	8.36 $\pm$ 0.76
BUN	5.50 $\pm$ 1.47	5.46 $\pm$ 1.54	6.06 $\pm$ 2.69	6.24 $\pm$ 2.01	6.22 $\pm$ 1.76	5.72 $\pm$ 0.81
Cr	70.78 $\pm$ 16.30	68.03 $\pm$ 17.83	74.70 $\pm$ 22.56	79.57 $\pm$ 17.97	75.28 $\pm$ 15.34	74.00 $\pm$ 12.14
ALT	29.60 $\pm$ 17.16	25.57 $\pm$ 14.83	28.00 $\pm$ 15.60	26.17 $\pm$ 16.27	20.86 $\pm$ 11.68	22.83 $\pm$ 13.22
GFR	97.93 $\pm$ 21.03	93.43 $\pm$ 17.96	99.28 $\pm$ 25.60	84.00 $\pm$ 21.91	85.25 $\pm$ 20.25	86.65 $\pm$ 16.19

Data were expressed as mean $\pm$ SD (range). Student's *t* test and Chi-square test were used. BMI: body mass index; DUR: duration after diabetes onset; FPG: fasting plasma glucose; HbA1c: glycosylated hemoglobin; BUN: urea nitrogen; Cr: creatinine; ALT: alanine transferase. GFR: glomerular filtration rate; ^b $P=0.022$, ^e $P=0.033$, compared to GG or GT genotype in metformin group.

respectively). Furthermore, the incidence of hyperlactacidemia among the three genotypes in both groups is shown in Table 3. The results reveal that patients carrying the TT genotype in the metformin-treated group had a higher incidence of hyperlactacidemia compared to patients with the GG genotype (40.0% vs 6.9%, $P=0.050$) in the metformin-treated group and with the GG (6.0%, $P=0.042$) or GT (4.3%, $P=0.043$) genotype in the non-metformin-treated group. In contrast, the difference in lactatemia in patients with the TT genotype between the two groups was not significant.

Gender differences in lactate concentrations

The ANOVA of factorial design indicated that the blood lactate concentration in women who carried the GG and TT genotype in the *SLC22A2* gene was significantly higher than that of men ($P=0.001$, $P=0.025$, respectively), but the difference in lactate concentrations in the GT genotype was not significant ($P=0.529$, Figure 4). In the metformin-treated group, the blood lactate concentration in women who carried the GG or TT genotypes in the *SLC22A2* gene was significantly higher than that of men ($P=0.004$, $P=0.008$, respectively) and that of women who carried the TT genotype was significantly higher than that of women with the GG or GT genotypes ($P=0.045$, $P=0.041$, respectively, Figure 5). However, the levels of plasma Cr in women were significantly lower than those in men ($P=0.000$). In the non-metformin-treated group, there

Figure 4. The plasma lactate levels of three genotypes in different genders. In 400 subjects, the blood lactate concentration in women carried GG and TT genotype of *SLC22A2* gene was higher significantly than that of men, and the difference of lactate concentration in GT genotype was no significance by the analysis of variance of factorial design. ^b $P=0.025$, ^c $P=0.001$, compared to lactate levels of males.

Figure 5. The plasma lactate levels of three genotypes between different genders in metformin group. The blood lactate concentration in women carried GG and TT genotype of *SLC22A2* gene was higher significantly than that of men. The blood lactate concentration in women carried TT genotype of *SLC22A2* gene was higher significantly than those with GG or GT genotypes. ^c $P=0.004$, ^h $P=0.008$, compared to lactate levels of males; ^f $P<0.05$ ($P=0.045$ for GG genotype, $P=0.041$ for GT genotype) compared to lactate levels of females with TT genotype.

was no significant relationship between lactate concentrations and any of the three genotypes.

Discussion

In the present study, the ASP-PCR method was performed to detect the 808G>T SNP in the *SLC22A2* gene, which encodes OCT2. By applying touchdown PCR conditions, better specificity was achieved, and the results were reliable. This analysis showed that there were three genotypes (GG, GT, and TT) at position 808 in the *SLC22A2* gene in Chinese Hans with T2DM. The genotype in the two groups and the total group were in accordance with Hardy-Weinberg equilibrium, and the T allele frequency was 12.9%, which was similar to the data in healthy populations from Hong Kong (13.3%), Japan (16.8%), and Korea (11%)^[13, 20, 21].

This research further investigated the relationship between the *SLC22A2* 808G>T polymorphism and plasma lactate levels in these T2DM patients treated with or without metformin. The results reveal that the mean lactate level of patients with the mutant homozygote genotype (TT) was the highest in all groups, especially in patients treated with metformin; however, the HbA1c level was the lowest. In other words, the *SLC22A2* gene (808G>T) variation can affect the plasma lactic acid and HbA1c levels of T2DM patients receiving metformin

Table 3. The number of cases and the incidence of hyperlactacidemia of three genotypes in groups treated with or without metformin.

	Metformin (n=200)			Non-metformin (n=200)		
	GG n (%)	GT n (%)	TT n (%)	GG n (%)	GT n (%)	TT n (%)
hLA	11 (6.9%)	3 (8.6%)	2 (40% ^{bch})	9 (6.0%)	2 (4.3%)	0 (0%)
nLA	149 (93.1%)	32 (91.4%)	3 (60%)	139 (94.0%)	44 (95.7%)	6 (100%)

hLA: hyperlactacidemia, nLA: normal lactate concentration. The lactate level higher than 1.96 mmol/L was defined as hyperlactacidemia. Fisher's exact test, ^b $P=0.042$, ^c $P=0.043$ compared to GG or GT genotype in non-metformin group; ^h $P=0.05$, compared with GG genotype in metformin group.

therapy. To date, a few studies examining the relevance of the 808G/T gene polymorphism and metformin pharmacokinetics have been reported, but studies about these polymorphisms and their association with blood lactate concentration are rare. The above-mentioned Hong Kong study in the general population demonstrated that the *SLC22A2* (808G>T) polymorphism influenced metformin metabolism *in vivo* and that the discharge of metformin was reduced in subjects with the TT genotype^[14]. Similarly, the present study indicates that the incidence of lactatemia in T2DM patients carrying the TT genotype in the metformin-treated group increased and their HbA1c decreased significantly compared to the non-metformin-treated group. However, no marked difference was found in patients with the GG or GT genotypes. Thus, it is likely that a metformin concentration in the plasma clears more glucose, decreasing the level of HbA1c and increasing the plasma lactate level by inhibiting lactate metabolism in the liver. That is to say, *SLC22A2* gene (808G>T) polymorphisms increase the plasma lactate level by elevating the circulating concentration of metformin.

Earlier studies have demonstrated that OCT1-mediated hepatic uptake of guanides play an important role in lactic acidosis^[16, 22]. However, an animal study revealed that metformin is a superior substrate for renal OCT2 rather than hepatic OCT1, and renal OCT2 plays the dominant role in metformin pharmacokinetics^[4]. From the results of this study, we can infer that OCT2 also takes part in the occurrence of hyperlactacidemia. After administration of metformin, the OCT2 transporter of T2DM patients carrying allele T at position 808 of the *SLC22A2* gene could not bind with the drug and subsequently excrete the drug into the urine. This escalated the blood concentration of metformin, which caused the production of lactate. Therefore, this study proved the relevance of the *SLC22A2* 808 G/T polymorphism and the raised blood lactate concentration/hyperlactacidemia in the metformin-treated group and supplied a possible explanation for the decreased excretion of metformin. However, in this study, there were no obvious differences between patients with the GG and TT genotypes, which may be the result of the small sample size. Thus, it is necessary to expand the sample size to further prove these findings in the future.

The study additionally showed that compared to the non-metformin-treated group, there was a gender difference in plasma lactate levels. The plasma lactate concentrations in females were higher than in males with the same *SLC22A2* 808G/T genotype in the metformin-treated group, and women carrying the TT genotype had significantly higher plasma lactate levels than those with the GG or GT genotypes. Diabetic patients recruited in this study had normal liver and renal function. In addition, Cr, BUN, and ALT levels of women in the metformin-treated group were not higher, and their Cr levels were lower than in men. Thus, it is unlikely that abnormal liver production or renal excretion of lactate caused this result. Therefore, the gender differences in lactate levels are more likely related to the fundamental difference in sex hormone levels in males and females. Urakami demonstrated in rats

that OCT2 is responsible for the gender differences in renal basolateral membrane OCT activity, and OCT2, but not OCT1, is sexually up-regulated by testosterone and down-regulated by estradiol in the kidney^[8-10]. In our clinical study of 1021 patients with T2DM, we observed that blood lactate levels were due to gender differences, with females having significantly higher blood lactate levels than males treated with metformin (unpublished data). Until now, no related study has reported the relationship between OCT2 gene polymorphisms and gender. From the results of this study and previous animal experiments, we speculate that the gender differences in blood lactate concentration might be due to differential OCT2 expression in males and females. Specifically, the low expression of OCT2 in females may reduce the renal excretion of metformin, cause its accumulation, and induce a higher level of lactic acid. The in-depth mechanisms of metformin metabolism and excretion need to be confirmed by further studies. Even so, this study may have important clinical meaning for metformin therapy. Because of the relatively high frequency of G808T in the diabetic population, we can use ASP-PCR to rapidly detect the highest allele frequency (A270S) and predict the risk of lactatemia in patients carrying the T allele, especially in females with the TT genotype, which may prevent the occurrence of lactic acidosis.

Limitations of this study include the indirect measurement of blood metformin concentrations and the lack of control of other hypoglycemic agents in these patients. These may limit the significance of this study, and we cannot certify the speculation directly. Another limitation of the present research was small sample size, and therefore more studies with larger sample sizes should be done to further prove these findings.

In summary, our results demonstrate that there is an 808G/T polymorphism in the *SLC22A2* gene in Chinese Hans with T2DM. The 808G>T variance in the *SLC22A2* gene can increase plasma lactate levels and the incidence of hyperlactacidemia in T2DM patients undergoing metformin therapy. Female patients carrying the TT genotype are prone to lactatemia.

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Author contribution

Fang LIU and Wei-ping JIA designed the research; Qing LI and Tai-shan ZHENG performed the research; Jun-ling TANG and Hui-juan LU contributed new analytical reagents and tools; Qing LI analyzed the data; Qing LI and Fang LIU wrote the paper.

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