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PHARMACOGENETICS

Association analysis of *SLC30A8* rs13266634 and rs16889462 polymorphisms with type 2 diabetes mellitus and repaglinide response in Chinese patients

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Abstract
Objective Genome-wide association studies (GWASs) identified that *SLC30A8* genetic polymorphism was a risk of type 2 diabetes mellitus (T2DM) in several populations. This study aimed to investigate whether the *SLC30A8* rs13266634 and rs16889462 polymorphisms were associated with T2DM susceptibility and repaglinide therapeutic efficacy in Chinese T2DM patients.
Methods We conducted a case–control study of 443 T2DM patients and 229 healthy volunteers to identify *SLC30A8* rs13266634 and rs16889462 genotypes by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay. Forty-eight patients were randomly selected and underwent an 8-week repaglinide treatment (3 mg/d). Fasting plasma glucose (FPG), postprandial plasma glucose (PPG), glycated hemoglobin (HbAlc), fasting serum insulin (FINS), postprandial serum insulin (PINS), homeostasis model assessment for insulin resistance (HOMA-IR), serum triglyceride, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c) were determined before and after repaglinide treatment.
Results *SLC30A8* rs13266634 risk C allele frequency was higher in T2DM patients than in healthy controls ($P<0.05$). There was a better repaglinide response on FINS ($P<0.05$) and PINS ($P<0.01$) in patients with rs13266634 CT+TT genotypes compared with CC genotype carriers. Patients with rs16889462 GA genotype showed an enhanced repaglinide efficacy on FPG ($P<0.01$), PPG ($P<0.01$) and HbAlc ($P<0.05$) compared with GG genotype individuals.
Conclusions *SLC30A8* rs13266634 and rs16889462 polymorphisms were associated with repaglinide therapeutic efficacy in Chinese T2DM patients.

Keywords *SLC30A8* rs13266634 · *SLC30A8* rs16889462 · Genetic polymorphisms · Type 2 diabetes mellitus · Repaglinide · Zinc transporter protein member 8

Abbreviations

GWASs	genome-wide association studies
SNPs	single nucleotide polymorphisms
T2DM	type 2 diabetes mellitus
PCR-RFLP	polymerase chain reaction–restriction fragment length polymorphism

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ZnT-8	zinc transporter protein member 8
SLC30A8	zinc transporter solute carrier family 30 member 8 gene
BMI	body mass index
WHR	waist to hip ratio
FPG	fasting plasma glucose
PPG	postprandial plasma glucose
HbAlc	glycated hemoglobin
FINS	fasting serum insulin
PINS	postprandial serum insulin
HOMA-IR	homeostasis model assessment for insulin resistance
TC	total cholesterol
LDL-c	low-density lipoprotein-cholesterol
HDL-c	high-density lipoprotein-cholesterol
DV	differential value (postadministration minus preadministration)
IFG	impaired fasting glycemia
$[Ca^{2+}]i$	intracellular free calcium
ABCC8	adenosine triphosphate (ATP)-binding cassette superfamily subfamily C (CFTR/MRP), member 8
KCNJ11	potassium inwardly rectifying channel subfamily J, member 11
VDCC	voltage-dependent calcium channels

Introduction

Nowadays, the rapidly increasing prevalence of type 2 diabetes mellitus (T2DM) is becoming a tremendous public health problem that affects more than 170 million patients worldwide [1]. T2DM is a complex metabolic disorder with two major pathophysiological features: insulin resistance and pancreatic β-cell dysfunction [2]. The mechanism of this disease remains unknown; however, environmental factors and genetic variations are considered two major contributors to onset and development of T2DM. Recent genome-wide association studies (GWASs), which are high-throughput assays covering 3 million to 5 million single nucleotide polymorphisms (SNPs), provide some novel susceptibility loci for T2DM, six (*SLC30A8*, *HHEX*, *CDKAL1*, *CDKN2A-2B*, *IGF2BP2*, and *FTO*) of which correlate with modest T2DM susceptibility [odds ratio (OR) 1.14–1.20]. This has been confirmed in Caucasians populations [3–9]. Another large-scale case–control study also confirms their association in Asian populations [10]. Among them, the zinc transporter solute carrier family 30 member 8 gene (*SLC30A8*) is an especially interesting candidate gene because of its exclusive expression in the pancreas and major in β cells [6, 11].

SLC30A8 encodes ion channel zinc transporter protein member 8 (ZnT-8), which is thought to be the β-cell zinc concentration regulator. ZnT-8 is a critical molecule during the insulin maturation and release process that carries zinc from the cytoplasm into insulin secretory vesicles [11]. Therefore, its polymorphisms may affect its activity, which in turns correlates with T2DM susceptibility and therapeutic efficacy. In fact, two SNPs have been found to be associated with T2DM onset and development. Rs13266634 polymorphism (973C>T) is a nonsynonymous SNP that causes an amino acid change from arginine (R) to tryptophan (W) at position 325 (Arg325Trp). This SNP is associated with T2DM onset and development in several populations [3–9]. Another missense mutation, rs16889462 (974G>A), which changes amino acid 325 from arginine (R) to glutamine (Q) (Arg325Gln), is related to T2DM susceptibility. The association is also verified in Chinese population [10, 12].

Repaglinide, an insulin secretagogue agent, is a safe and effective medication for T2DM [14–16]. Repaglinide can enhance insulin secretion from pancreatic β-cells and reduce the concentration of blood glucose by inhibiting adenosine triphosphatase (ATP)-sensitive potassium (K^+) channels (KATP) and activating calcium (Ca^{2+}) channels [14, 17]. In vitro study shows it can promote insulin secretion and biosynthesis in pancreatic isles by glucose stimulating [18]. Although individual variations of repaglinide therapeutic efficacy have been found, its mechanism of action remains unknown. Previous studies show SNPs in cytochrome P450 (CYP) 2C8 and organic anion-transporting polypeptide 1B1 (*OATP1B1*) genes can influence the concentration of repaglinide in plasma [19–21]. However, that cannot fully account for the individual variations of repaglinide therapeutic efficacy. As described above, ZnT-8 promotes large amounts of zinc to participate in insulin release. Furthermore, zinc hyperpolarizes pancreatic β-cell membranes by activating KATP [11, 22, 23]. Thus, we speculate that *SLC30A8* genetic polymorphisms may be involved in T2DM development and repaglinide therapeutic efficacy. In this study, we investigated the association of *SLC30A8* gene polymorphisms (rs13266634 and rs16889462) with T2DM and repaglinide efficacy in Chinese patients.

Patients and methods

Patients

A total of 443 unrelated T2DM patients (233 men, 210 women) aged between 25 and 70 (mean 49.13± 10.79) years, and 229 healthy controls (129 men, 100 women) aged between 25 and 70 (mean 47.55±10.93) years from Hunan province, China, were recruited for this study.

Table 1 Distribution of *SLC30A8* rs13266634 and rs16889462 polymorphisms in T2DM patients and health controls

Genotype	T2DM patients n=443 (frequency)	Health controls n=229 (frequency)	P value
rs13266634 genotypes			
CC	134 (30.25%)	64 (27.95%)	
CT	211 (47.63%)	93(40.61%)	
TT	98 (22.12%)	72 (31.44%)	0.036*
rs13266634 alleles			
C	479 (54.06%)	221 (48.25%)	
T	407 (45.94%)	237(51.75%)	0.043*
rs16889462 genotypes			
GG	386 (87.13%)	208(90.83%)	
GA	56 (12.64%)	20 (8.73%)	
AA	1 (0.23%)	1 (0.44%)	0.287
rs16889462 alleles			
G	828 (93.45%)	436 (95.20%)	
A	58 (6.55%)	22 (4.80%)	0.201

The allelic frequencies are indicated in absolute values (percentage). P values are determined by Pearson χ^2 test

*P<0.05

All patients were evaluated through medical histories, physical examinations, and routine clinical laboratory tests. T2DM was diagnosed according to World Health Organization criteria in 1999, which was: fasting plasma glucose test (FPG) ≥ 7.0 mmol/l or 2 h postprandial plasma glucose test (PPG) ≥ 11.1 mmol/l. The inclusion criteria of all patients were that patients had a body mass index (BMI) between 18.5 and 30 kg/m^2 and did not administer any insulin secretagogue agents, agonists, or inhibitors of CYP2C8, CYP3A4, and OATP1B1 (such as rifampin, glitazones, gemfibrozil, antiviral drugs, antimycotic agents, and glucocorticoids) in the past 3 months. Patients with T1DM, a history of ketoacidosis, ischemic heart disease, congestive heart failure, trauma, kidney, or liver diseases; patients receiving insulin treatment; and pregnant or lactating women were excluded. The study protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University. Written informed consent was obtained from each participant before the start of this study. We received a clinical admission from tje

Table 2 The baseline level of clinical and biochemical characteristics of different *SLC30A8* genotype patients

Parameters (see "Abbreviations")	rs13266634 genotypes		P value	rs16889462 genotypes		P value
	CC	CT+TT		GG	GA+AA	
No. (male/female)	135 (67/68)	308 (166/142)	0.048[b]	386 (203/183)	57 (29/28)	0.809[b]
Age (years)	48.66±10.25 (48.86, 50.45)	49.38±11.04 (48.10, 50.65)	0.530	49.67±10.78 (48.53, 50.81)	47.29±11.21 (44.14, 50.45)	0.145
BMI (kg/m^2)	25.26±3.29 (24.68, 25.84)	25.34±3.43 (24.94, 25.74)	0.829	25.34±3.35 (24.98, 25.70)	25.33±3.54 (24.33, 26.32)	0.979
WHR	0.91±0.06 (0.90, 0.92)	0.91±0.06 (0.89, 0.92)	0.999	0.91±0.06 (0.90, 0.91)	0.92±0.05 (0.90, 0.93)	0.261
FPG (mmol/l)	9.92±4.38 (9.18, 10.67)	8.42±2.78 (8.11, 8.73)	0.000***[a]	9.78±4.04 (9.35, 10.20)	8.22±2.71 (7.45, 8.98)	0.016*[a]
PPG (mmol/l)	16.56±6.94 (15.34, 17.79)	14.48±5.13 (13.86, 15.10)	0.003**	15.27±5.92 (14.63, 15.91)	15.04±5.52 (13.40, 16.68)	0.804
FINS (mU/l)	10.37±12.40 (8.15, 12.59)	13.02±16.10 (11.11, 14.93)	0.210[a]	10.50±7.01 (9.73, 11.27)	8.80±5.28 (7.21, 10.38)	0.123[a]
PINS (mU/l)	47.86±44.46 (39.79, 55.93)	49.95±43.32 (44.60, 55.30)	0.395[a]	50.81±44.83 (45.79, 55.83)	49.20±37.09 (38.06, 60.35)	0.819[a]
HOMA-IR	4.08±4.62 (3.25, 4.90)	4.54±4.66 (3.99, 5.10)	0.646[a]	4.03±2.72 (3.73, 4.33)	3.17±2.13 (2.52, 3.81)	0.027*[a]
HbA1c (%)	9.87±6.58 (8.64, 11.10)	8.36±2.19 (8.08, 8.64)	0.005**[a]	9.22±7.02 (8.39, 10.06)	8.49±2.64 (7.68, 9.30)	0.500[a]
Triglyceride(mmol/l)	3.03±3.27 (2.43, 3.63)	2.64±2.87 (2.27, 3.01)	0.471[a]	2.79±3.12 (2.42, 3.15)	2.03±1.26 (1.64, 2.43)	0.170[a]
TC (mmol/l)	5.11±1.62 (4.80, 5.42)	4.91±1.70 (4.69, 5.13)	0.431	4.99±1.69 (4.79, 5.18)	4.74±1.40 (4.29, 5.20)	0.252
HDL-c (mmol/l)	1.48±0.95 (1.29, 1.66)	1.33±0.87 (1.22, 1.45)	0.106[a]	1.41±0.96 (1.29, 1.52)	1.24±0.34 (1.13, 1.36)	0.291[a]
LDL-c (mmol/l)	2.71±1.11 (2.49, 2.93)	2.82±1.01 (2.68, 2.95)	0.400	2.77±1.02 (2.65, 2.89)	2.84±1.17 (2.45, 3.22)	0.717

Data are given as mean ± standard deviation (95% confidence interval). P values represent the statistical difference between CC and CT+TT (GG and GA+AA) groups assessed by two-sample t test

[a] P values are determined by Mann–Whitney test. [b] P values are determined by Pearson χ 2 test.

*P < 0.05, **P < 0.01, ***P < 0.001

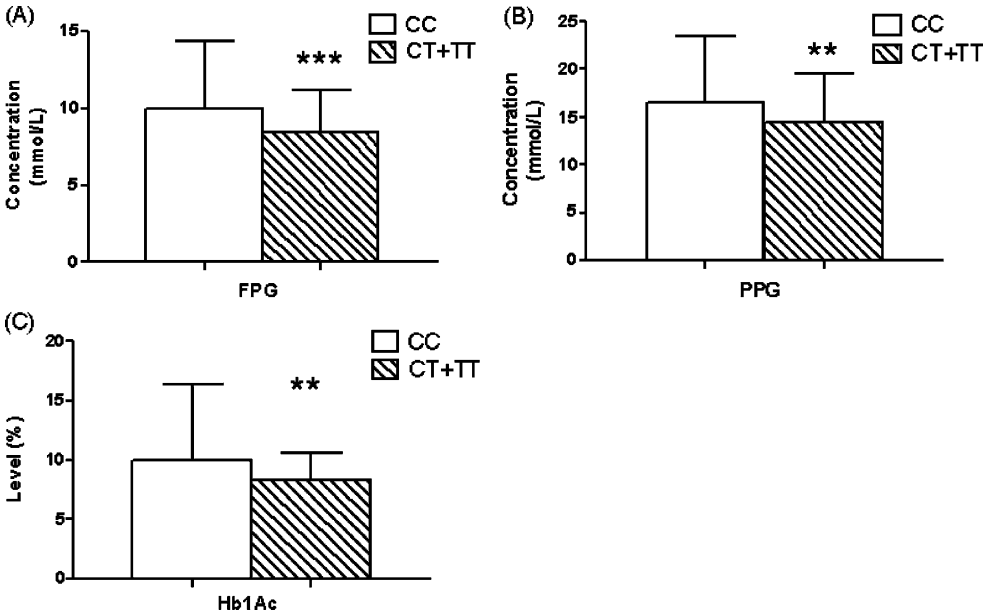

Fig. 1 Baseline levels of FPG (**a**), PPG (**b**), and HbA1c (**c**) in T2DM patients with different rs13266634 genotypes. Data are expressed with mean ± standard deviation (SD). $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with CC genotype group (n=443). (See "Abbreviations")

Chinese Clinical Trial Register (registration number ChiCTR-CCC00000406). A total of 48 T2DM patients (25 men, 23 women) with different *SLC30A8* rs13266634 and rs16889462 genotypes took monodrug repaglinide (1 mg×3/d day preprandial treatment) for 8 consecutive weeks.

Clinical measurements

On the 0 and 8th week after administration, venous blood samples were collected after an overnight fast and 2 h after a standardized breakfast. Concentrations of FPG, total cholesterol (TC), and triglycerides were determined by enzymatic colorimetric assay. High-density lipoprotein-cholesterol (HDL-c) concentration was measured by lipoprotein electrophoresis. Low-density lipoprotein-cholesterol (LDL-c) concentration was calculated according to the Friedewald formula [24]. Plasma insulin and glycated hemoglobin (HbAlc) levels were measured using radioimmunoassay kit (BNIBT, Beijing, China) and by high-performance liquid chromatography (HPLC) assay, respectively. Homeostasis model assessment for insulin resistance (HOMA-IR) value was calculated to estimate the level of insulin sensitivity using of the following formula: fasting serum insulin (mU/l) × fasting blood glucose (mmol/l) / 22.5 [25].

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using SQ Blood DNA Kit (Omega, CO, USA). Genotypes of the *SLC30A8* rs13266634 and rs16889462 were analyzed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) assay. The following primers were used: forward primer: 5′- GGACAGAAAGAGTTCCCATAGCG-3′, reverse primer: 5′- ATAGCAGCATGTTTGAAGGTGGC-3′. The PCR products were then digested by HpaII (Fermentas, MD, USA), BstNI (NEB, MA, USA), and MaeI (Fermentas) for rs13266634 and rs16889462 genotype. *OATP1B1* genotypes were also detected by PCR-RFLP. We used the following primers: forward primer: 5′-AAAGGAATCTGGGTCATACATGTGGAT-3′, reverse primer: 5′- TTCAAAAGTAGACAAAGGGAAAGTGA-3′. The PCR products were digested by Mlu I (Fermentas). The genotype of *CYP2C8* Arg139Lys was determined by

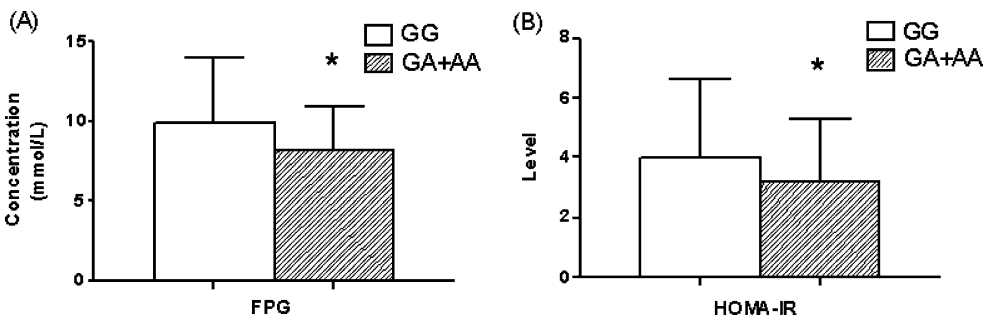

Fig. 2 Baseline levels of FPG (**a**) and HOMA-IR (**b**) in T2DM patients with different of rs16889462 genotypes. Data are expressed with mean ± standard deviation (SD). $^{*}P < 0.05$ compared with GG genotype group (n=443). (See "Abbreviations")

Table 3 Clinical characteristics of type 2 diabetes mellitus (T2DM) patients before and after repaglinide treatment (n=48)

Parameters (see "Abbreviations")	Before	After	P values
FPG (mmol/l)	8.87±2.00 (8.24, 9.51)	6.84±2.48 (6.34, 7.35)	0.000***
PPG (mmol/l)	15.92±4.47 (14.49, 17.35)	11.57±3.07 (10.59, 12.55)	0.000***
FINS (mU/l)	7.98±6.27 (5.97, 9.98)	11.41±6.66 (9.28, 13.54)	0.002**
PINS (mU/l)	36.73±25.96 (28.43, 45.03)	59.94±30.47 (50.20, 69.69)	0.000***
HOMA-IR	3.17±2.61 (2.33, 4.00)	3.43±2.14 (2.74, 4.11)	0.513
HbAlc (%)	8.67±1.72 (8.12, 9.22)	6.84±1.10 (6.49, 7.19)	0.000***
triglyceride (mmol/l)	2.30±1.78 (1.73, 2.87)	2.34±1.49 (1.86, 2.81)	0.940
TC(mmol/l)	5.33±1.22 (4.94, 5.72)	4.84±0.97 (4.53, 5.15)	0.017*
HDL-c (mmol/l)	1.32±0.36 (1.21, 1.44)	1.41±0.37 (1.29, 1.53)	0.014*
LDL-c (mmol/l)	3.10±1.13 (2.74, 3.46)	2.39±0.95 (2.08, 2.69)	0.000***

Data are expressed as mean ± standard deviation (95% confidence interval). P values are determined by paired Student's t test. *P<0.05, **P<0.01, ***P<0.001

direct sequencing with primers as follow: forward primer: 5′- AGGCAATTCCCCAATATCTC -3′, reverse primer: 5′-ACTCCTCCACAAGGCAGTGA -3′.

Statistical analysis

Statistical analyses were performed with SPSS software (Version 15.0 for Windows; SPSS Inc, Chicago, IL, USA). All continuous variables were expressed as means ± standard deviation (SD) [95% confidence interval (CI)]. Hardy-Weinberg equilibrium and allelic frequencies in different groups were assessed with a Pearson χ^2 test of goodness-of-fit in the study sample. Paired Student's t test was used to compare the differences in the degree of reduction or enhancement in plasma concentrations among the different genotypic groups before and after repaglinide treatment. Normally distribution data were analyzed by two-sample t test. Nonnormally distribution data were determined by Mann–Whitney test. A two-sided test with alpha type error level set at 5% was used in all statistical analyses. Haplotype frequencies were determined with the PHASE software package [26]. Statistical power was calculated by power calculator software PASS (www.ncss.com). P value <0.05 was considered to be statistically significant for all analyses.

Results

Genotyping analysis and allelic frequencies

A total of 443 T2DM patients (233 men, 210 women) and 229 healthy volunteers (129 men, 100 women) were

Table 4 Comparisons of differential values (DV) in type 2 diabetes mellitus (T2DM) patients with different rs13266634 and rs16889462 genotypes before and after repaglinide treatment

Parameters (see "Abbreviations")	rs13266634 genotype		P value	rs16889462 genotype		P value
N(male/female)	CC 26 (11/15)	CT+TT 22 (14/8)		GG 38 (20/18)	GA 10 (5/5)	
DV FPG (mmol/l)	−1.87±1.83 (−2.71, −1.04)	−2.21±2.02 (−3.18, −1.24)	0.582	−1.57±1.60 (−2.16, −0.98)	−3.62±2.10 (−5.23, −2.01)	0.003**
DV PPG (mmol/l)	−4.16±4.14 (−6.05, −2.28)	−4.55±5.21 (−7.06, −2.04)	0.798	−3.18±3.96 (−4.63, −1.72)	−8.38±4.63 (−11.94, −4.82)	0.002**
DV FINS(mU/l)	1.28±5.54 (−1.24,3.80)	5.81±6.64 (2.61, 9.02)	0.024*	3.10±6.93 (0.56, 5.64)	4.58±4.44 (1.16, 7.99)	0.551
DV PINS (mU/l)	15.67±14.92 (8.88,22.46)	31.56±20.76 (21.55,41.56)	0.008**	22.13±19.94 (14.81,29.44)	26.97±18.13 (13.03,40.90)	0.518
DV HbA1c (%)	−1.57±1.93 (−2.45, −0.69)	−2.11±1.60 (−2.88, −1.34)	0.071[a]	−1.43±1.11 (−1.84, −1.03)	−3.19±2.86 (−5.39, −0.99)	0.040*[a]
DV HOMA-IR	−0.09±2.37 (−1.17, 0.99)	0.66±2.68 (−0.63, 1.95)	0.353	0.25±2.77 (−0.77, 1.27)	0.31±1.44 (−0.80, 1.42)	0.952
DV triglyceride (mmol/l)	−0.27±2.61 (−1.41, 0.92)	0.38±0.99 (−0.10, 0.86)	0.062[a]	−0.09±2.22 (−0.90, 0.72)	0.48±1.04 (−0.31, 1.28)	0.225[a]
DV TC (mmol/l)	−0.73±1.27 (−1.31, −0.15)	−0.24±1.22 (−0.83, 0.35)	0.220	−0.66±1.22 (−1.11, −0.21)	0.08±1.27 (−0.90, 1.05)	0.122
DV HDL-c (mmol/l)	0.07±0.25 (−0.34, 0.19)	0.11±0.19 (0.02, 0.20)	0.641	0.08±0.22 (−0.00, 0.16)	0.12±0.22 (−0.04, 0.29)	0.600
DV LDL-c (mmol/l)	−0.74±0.83 (−1.12, −0.36)	−0.68±0.70 (−1.02, −0.35)	0.818	−0.72±0.84 (−1.03, −0.41)	−0.70±0.38 (−0.98, −0.41)	0.943

Data are given as mean ± standard deviation (95% confidence interval). P values represent statistical difference between CC and CT+TT (GG and GA) groups as assessed by two-sample t-test. *P values are determined by Mann-Whitney test. *P<0.05, **P<0.01.

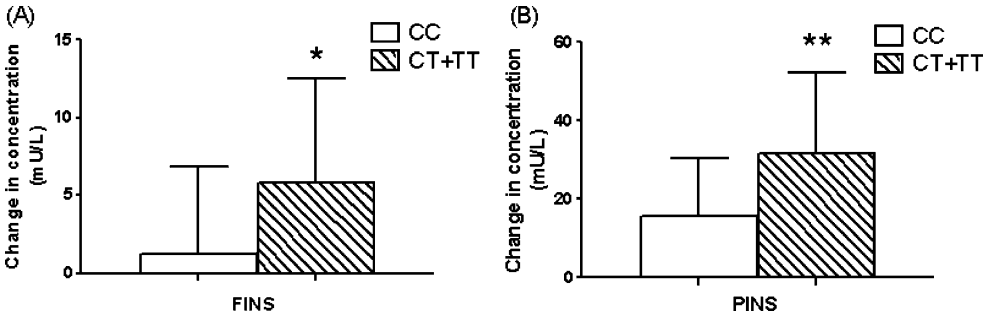

Fig. 3 Change levels of FINS (**a**) and PINS (**b**) between CC genotype and CT+TT genotypes of SLC30A8 rs13266634 polymorphism in T2DM patients after the treatment of repaglinide. Data are expressed with mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$ compared with CC genotype group (n=48) (See "Abbreviations")

genotyped for *SLC30A8* rs13266634 and rs16889462 polymorphisms. There were no statistical differences in age, BMI and waist-to-hip ratio (WHR) values between patients and controls. The genotypic distributions were in agreement with Hardy–Weinberg equilibrium (P>0.05). The frequency of rs13266634 risk C allele was significantly higher in T2DM patients than that in controls (54.06% vs 48.25%, P<0.05). The rs16889462 A allelic frequency was 6.55% in T2DM patients and 4.80% in controls; however, there was no significant difference between them. Furthermore, we found a significant linkage disequilibrium between rs13266634 locus and rs16889462 locus (|D′|= 0.928, P<0.05). *SLC30A8* rs13266634 and rs16889462 polymorphism distribution in patients and controls is summarized in Table 1.

Comparisons of baseline characteristics of T2DM patients with different rs13266634 and rs16889462 genotypes

The baseline clinical characteristics of 443 T2DM patients with different rs13266634 and rs16889462 genotypes are summarized in Table 2. There were no significant differences in age, BMI, and WHR between different genotype groups. Patients with rs13266634 CC genotype show higher FPG (mmol/l) (9.92±4.38 vs 8.42±2.78, P< 0.001), PPG (mmol/l) (16.56±6.94 vs 14.48±5.13, P< 0.01), and HbAlc (%) (9.87±6.58 vs 8.36±2.19, P<0.01) compared with CT+TT genotype (Table 2, Fig. 1). On the other hand, the FPG concentration (mmol/l) (9.78±4.04 vs 8.22±2.71, P<0.05) and HOMA-IR level (4.03±2.72 vs 3.17±2.13, P<0.05) in rs16889462 GG carriers were higher than in GA+AA genotype individuals (Table 2, Fig. 2). There was no significant difference of all tested clinical parameters among different haplotype groups (data not shown).

Influence of rs13266634 and rs16889462 polymorphisms on therapeutic efficacy of repaglinide in T2DM patients

A total of 48 T2DM patients (25 men, 23 women) were treated with 3 mg repaglinide daily for 8 weeks. Repaglinide significantly decreased the concentrations of FPG (P< 0.001), PPG (P<0.001), HbA1c (P<0.001), TC (P<0.05) ,

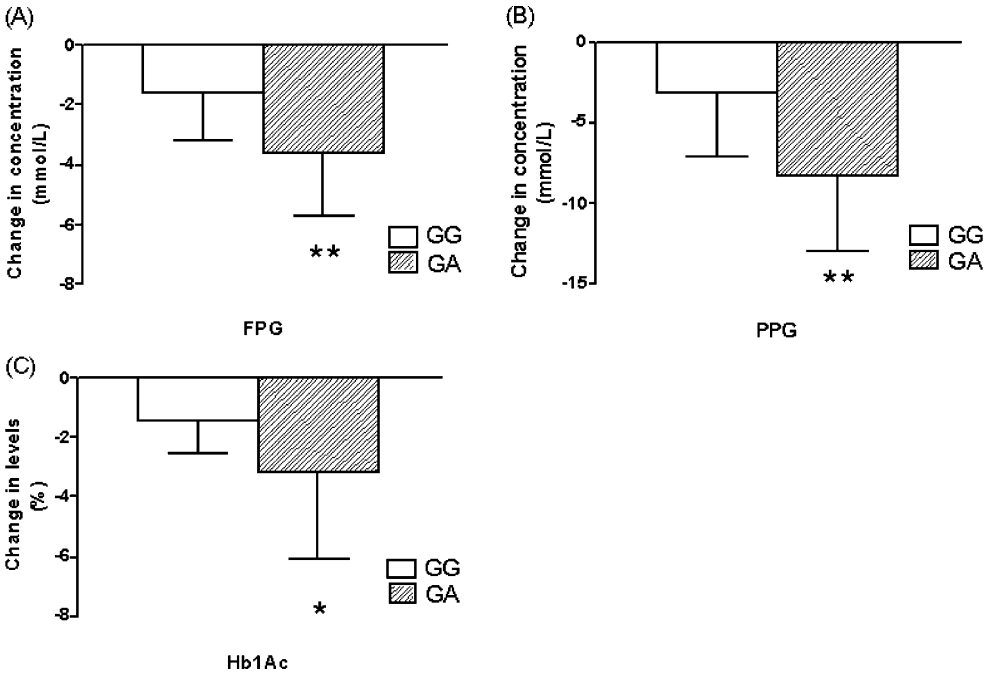

Fig. 4 Change levels of FPG (**a**), PPG (**b**), and HbA1c (**c**) between GG genotype and GA genotype of SLC30A8 rs16888462 polymorphism in T2DM patients after the treatment of repaglinide. Data are expressed with mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$ compared with GG genotype group (n=48) (See "Abbreviations")

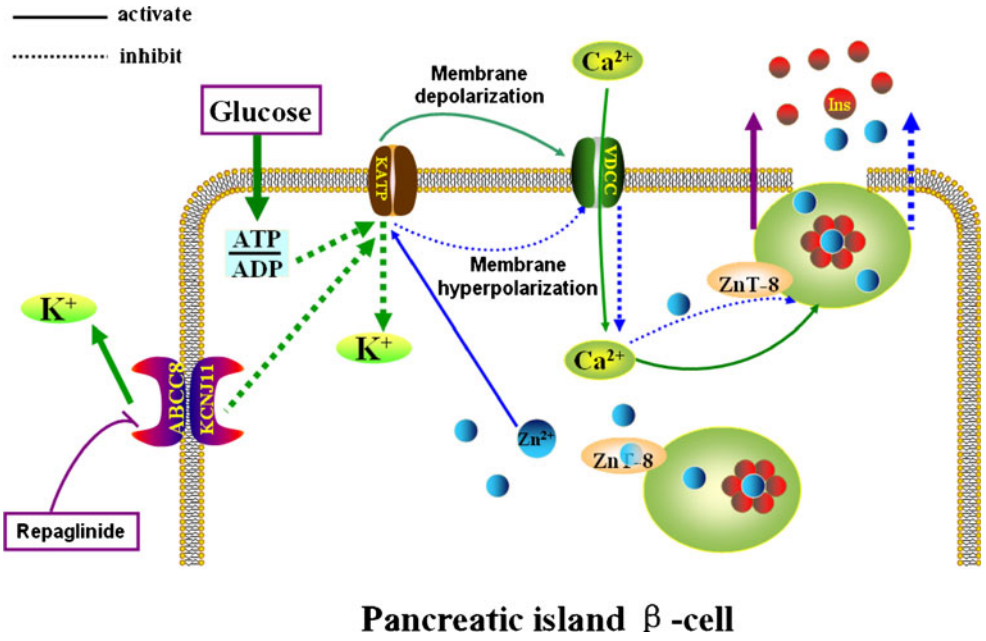

Fig. 5 Role of ZnT-8 and zinc ions in the insulin secretion in pancreatic β cell. Repaglinide combines with ABCC8 and blocks KATP, this stimulates Ca^{2+}i influx and causes insulin release. Conversely, zinc activates KATP and blocks VDCC, which shows a negative control on insulin secretion. Mutated ZnT-8 could accumulate more zinc into insulin secretion vesicles and decrease the concentration of zinc in cytoplasm, thus increasing insulin secretion (See "Abbreviations")

and LDL-c ($P<0.001$), whereas it increased the levels of FINS ($P<0.01$), PINS ($P<0.001$), and HDL-c ($P<0.05$) in T2DM patients (Table 3). Patients with rs13266634 CT+TT genotypes showed augmented repaglinide effects compared with CC genotype patients. This was shown by the decrease value of FINS (mU/l) (5.81±6.64 vs 1.28±5.54, $P<0.05$) and PINS (mU/l) (31.56±20.76 vs 15.67±14.92, $P<0.01$) (Table 4 and Fig.3). On the other hand, there were also significant difference of the decrease value of FPG (mmol/l) (–3.62±2.10 vs –1.57±1.60, $P<0.01$), PPG (mmol/l) (–8.38± 4.63 vs –.18±3.96, $P<0.01$), and HbAlc (%) (–3.19±2.86 vs –1.43±1.11, $P<0.05$) between GA and GG genotype patients (Table 4, Fig. 4). This indicates that GA genotype T2DM patients have better repaglinide response.

Discussion

We explored the effects of rs13266634 and rs16889462 polymorphisms of *SLC30A8* on repaglinide therapeutic efficacy with 3 mg repaglinide daily for 8 consecutive weeks in Chinese T2DM patients. There were significantly augmented repaglinide effects in patients with rs13266634 CT+TT genotypes on FINS and PINS compared with rs13266634 CC genotype ($P<0.05$, $P<0.01$, respectively). Moreover, patients with rs16889462 GA genotype showed enhanced repaglinide effects on FPG, PPG, and HbAlc compared with GG genotype ($P<0.01$, $P<0.01$ and $P<0.05$, respectively). Our study suggests that *SLC30A8* is one susceptibility gene for T2DM and influences response to repaglinide.

In β-cells, zinc binds with insulin and forms a solid hexamer, which is stored in secretory vesicles. Insulin secretion is accompanied by zinc release, a very important mechanism of insulin secretion regulation [11, 27]. Zinc homeostasis is regulated by two main molecules: metallothionein and zinc transporters – the latter being encoded by two solute-linked carrier (SLC) genes: Zip (SLC39) and ZnT (SLC30) [28]. The Zips control the intracellular uptake of zinc [29], and the ZnTs control the cellular efflux of zinc into the extracellular matrix or intracellular vesicles. ZnT-8 is a 369 amino acid protein exclusively expressed in pancreas β-cells. It locates in insulin secretory granules and plays an important role in mediating insulin trafficking, maturation, storage, and secretion [27, 30]. It facilitates zinc accumulation from the cytoplasm into intracellular vesicles and circulation, and its function on zinc homeostasis is altered in the pathogenic state of diabetes [11, 31].

Based on this study, rs13266634 T allele frequency in controls was higher than that in T2DM patients ($P<0.05$), and it is a protective allele for T2DM. However, the allelic frequency of rs16889462 polymorphism in the Chinese population showed no significant difference between T2DM patients and health controls. *SLC30A8* gene polymorphisms also showed ethnic differences. In this study, we found that the rs13266634 T allelic frequency was higher in Chinese than in Japanese T2DM patients ($P<0.05$). The T allelic frequencies of rs13266634 in the Asian population were much higher than that in the French (34.66%), Austrian (25.97%), Israeli Ashkenazi (23.84%), Moroccan (16.34%), African Americans (8.41%), and Pima Indian (8.73%) populations [6, 33]. To date, allelic frequency differences of rs16889462 polymorphism in other ethnic populations remain unclear and need further study.

It has been reported that the genetic polymorphism of *SLC30A8* was associated with impaired proinsulin conversion

involved in the production and secretion pathway [32]. Fu et al. found that reduced ZnT-8 expression in cultured pancreatic β cells gives rise to reduced insulin response to hyperglycemia and that *SLC30A8* polymorphism could affect insulin secretion and glycemic response [34]. Another two studies indicate that patients with the rs13266634 C allele showed decreased first-phase insulin release following an intravenously administered glucose load [12, 13]. Furthermore, it has been found that the C alleles of rs13266634 at *SLC30A8* were associated with increased FPG and decreased insulin during the oral glucose tolerance test. An investigation also showed SNP rs13266634 increased the risk for T2DM by 1.24-fold in Chinese Han population [12]. Our study was consistent with the above reports. These studies taken together indicate that the rs13266634 polymorphism is associated with T2DM in the Chinese population.

Repaglinide is an inhibitor of pancreatic β cells KATP. It can inhibit KATP and stimulate $[Ca^{2+}]i$, which in turn promotes insulin secretion [35]. After KATP is blocked by repaglinide, voltage-dependent calcium channels (VDCC) are opened, which results in the increase of $[Ca^{2+}]i$, which is the main trigger of fusion of insulin vesicles to the membrane. This causes insulin secretion (Fig. 5). During this pathway, zinc can activate KATP and negatively regulate insulin secretion [36]. Many studies demonstrated that zinc regulates this pathway at many levels, including the KATP channel [23], alpha cell, and insulin synthesis and storage level [37, 38]. Therefore, we speculate that *SLC30A8* polymorphisms influence the zinc disposition and that KATP function, in turn, affects the therapeutic efficacy of repaglinide. The rs13266634 and rs16889462 SNPs are located in the C-terminal region of ZnT-8 protein. Kang et al. demonstrated that rs13266634 polymorphism might be a functional SNP that increases expression of ZnT-8 protein [39]. This SNP changed the posttranslational modification process in the ZnT-8 C-terminus, and this might be another possible mechanism of insulin secretion change. The rs13266634 and rs16889462 polymorphisms could disrupt the protein kinase A and protein kinase C recognition motif, which might result in transporter function change [39]. However, the exact molecular mechanisms remain to be investigated.

Repaglinide is metabolized in the liver through cytochrome P450 (CYP) 2C8 and 3A4 enzymes [19]. Hepatic uptake by OATP1B1 is another important step during the metabolism of repaglinide. Thus, their genetic polymorphisms may affect the pharmacokinetics of repaglinide [20, 21]. In this study, to avoid the effect of pharmacokinetic factors of repaglinide to its therapeutic efficacy, we selected patients with the same *CYP2C8**3 139Arg and *OATP1B1* 521TT genotypes (data not show).

To the best of our knowledge, this study is the first to address the influences of the *SLC30A8* gene rs13266634 and rs16889462 polymorphisms on repaglinide therapeutic efficacy in Chinese T2DM patients. This study showed that the genetic polymorphisms of *SLC30A8* rs13266634 and rs16889462 are associated with therapeutic efficacy of repaglinide in Chinese T2DM patients. However, it should be noted that the relatively small sample size is the limitation of this study. Although we calculated the power values of each analysis, and they are from 72% to 98%, we still think further study in a large sample of T2DM patients is necessary.

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Conflict of interest The authors declare that there is no conflict of interest associated with this manuscript.

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