NeuroD1 A45T and PAX4 R121W polymorphisms are associated with plasma glucose level of repaglinide monotherapy in Chinese patients with type 2 diabetes

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Repaglinide is an insulin secretagogue agent widely used in the treatment of type 2 diabetes mellitus (T2DM). Obvious interindividual differences in the therapeutic efficacy of and adverse reactions to repaglinide were observed in Chinese T2DM patients.
- There are no reports showing the influence of genetic variations of *NeuroD1/BETA2* A45T and *PAX4* R121W on repaglinide response in Chinese T2DM patients.

WHAT THIS STUDY ADDS

- This study aimed to investigate the association of genetic polymorphisms of *NeuroD1/BETA2* A45T and *PAX4* R121W with T2DM susceptibility and repaglinide therapeutic efficacy in Chinese T2DM patients.
- The *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms were correlated with repaglinide therapeutic efficacy in Chinese T2DM patients.

AIMS

We aimed to determine whether *NeuroD1/BETA2* and *PAX4* polymorphisms were associated with the therapeutic efficacy of repaglinide in Chinese type 2 diabetes mellitus (T2DM) patients.

METHODS

Three hundred and sixty-eight T2DM patients and 132 healthy control subjects were genotyped by restriction fragment length polymorphism. Forty-three patients with various genotypes were randomly selected to undergo 8 weeks of repaglinide treatment (3 mg day⁻¹). Fasting plasma glucose, postprandial plasma glucose, glycated haemoglobin, fasting and postprandial serum insulin (FINS, PINS), homeostasis model assessment for insulin resistance, serum triglyceride, total cholesterol, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol were determined before and after repaglinide treatment.

RESULTS

The allelic frequency of *NeuroD1/BETA2* T45 was higher in T2DM patients than in the control subjects [13.45 vs. 6.82%, $P < 0.01$, odds ratios = 2.342 (1.365, 4.019), $P = 0.002$]. Type 2 diabetes mellitus patients with the mutated allele of *NeuroD1/BETA2* A45T polymorphism showed higher FINS (13.46 ± 12.57 vs. 10.04 ± 7.09 mU L⁻¹, $P < 0.05$) (11.67, 14.83 vs. 8.38, 11.37) and PINS (52.11 ± 40.93 vs. 68.66 ± 43.87 mU L⁻¹, $P < 0.05$) (44.89, 58.35 vs. 55.35, 88.87) than individuals with the T allele. The *PAX4* R121W R allele carriers had higher PINS (52.11 ± 40.93 vs. 68.66 ± 43.87 mU L⁻¹, $P < 0.05$) (44.89, 58.35 vs. 55.35, 88.87) than subjects with the W allele. After repaglinide treatment, patients with the T allele of *NeuroD1/BETA2* A45T polymorphisms had attenuated efficacy on fasting plasma glucose (−2.79 ± 2.14 vs. −0.99 ± 1.80 mmol L⁻¹, $P < 0.01$) (−3.53, −1.84 vs. −1.99, −0.13) and postprandial plasma glucose (−6.71 ± 5.90 vs. −2.54 ± 3.39 mmol L⁻¹, $P < 0.01$) (−9.28, −4.62 vs. −4.34, −0.84). Patients with the RR genotype of *PAX4* R121W showed better efficacy with respect to the level of postprandial plasma glucose than R/W genotypes (−6.53 ± 6.52 vs. −2.95 ± 1.17 mmol L⁻¹, $P < 0.05$) (−8.20, −4.89 vs. −3.92, −1.20).

CONCLUSIONS

The *NeuroD1/BETA2* and *PAX4* polymorphisms were substantially associated with plasma glucose level after repaglinide monotherapy.

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Introduction

Type 2 diabetes mellitus (T2DM) is a multifactorial disease, with both acquired and inherited risk factors. Previous studies showed that genetic mutations associated with insulin secretion and glucose metabolism were involved in susceptibility to and development of T2DM [1–7]. Neurogenic differentiation 1 (*NeuroD1*), also known as *BETA2*, is a transcription factor necessary for development of the pancreatic islets and insulin secretion [8]. It is a tissue-specific member of the basic helix–loop–helix (bHLH) family. *NeuroD1/BETA2* heterodimerizes with bHLH factors E47 and interacts with E-boxes of insulin gene. This complex is known as insulin enhancer factor 1 (IEF-1) and acts as a transcription factor of insulin gene [9]. Insulin enhancer factor 1 activates the insulin gene transcription and promotes the development and function of pancreatic islets cells. The *NeuroD1* gene is located in human chromosome 2q32 [10]. Its polymorphisms in the functional gene regions change the characteristics of translational activator factors and influence the ability of β -cells to differentiate and regenerate [1, 11]. It has been confirmed that mutations of the *NeuroD1/BETA2* gene are associated with maturity-onset diabetes in young people [11]. Recently, some studies have shown that polymorphism A45T of *NeuroD1/BETA2* gene is associated with the development of T2DM in Polish populations [12, 13], however not with Danish and French populations [14, 15]. The mechanisms of these different results remain unclear.

Paired box gene 4 (*PAX4*) belongs to the pax family, which encodes transcription factors characterized by the presence of a highly conserved DNA-binding motif and plays an important role in the growth, differentiation, proliferation and insulin secretion of pancreatic β -cells [16–19]. The *PAX4* gene is expressed during mouse pancreas organogenesis. *PAX4* gene knock-out mice showed a lack of mature insulin-producing β -cells and a severe diabetic syndrome after birth [20]. A transcription-regulated region in the *PAX4* gene that drives pancreas-specific expression also binds with *NeuroD1* [20]. *PAX4* binds with the pancreatic islet cell enhancer sequence elements of insulin, glucagon and somatostatin and promotes β -cell development [21, 22]. Shimajiri *et al.* reported that a nonsynonymous mutation of the *PAX4* gene (R121W) was associated with T2DM in a Japanese population and that patients with T2DM carrying the mutated gene showed less responsiveness to insulin requirements [23]. Type 2 diabetes mellitus patients with a homozygous 121W allele showed defects in the first-phase of insulin secretion [24]. The R121W mutation in the *PAX4* gene was also a predisposing factor for the development of T2DM in a Japanese population [25].

Repaglinide is an insulin secretagogue agent that is widely used in the treatment of T2DM [26–28]. Repaglinide decreases blood glucose and increases blood insulin by inhibiting ATP-sensitive K^+ channels and activating Ca^{2+} channels, as well as stimulating insulin release from pan-

creatic β -cells [29]. However, obvious interindividual differences in the therapeutic efficacy of and adverse reactions to repaglinide have been observed in T2DM patients. The mechanisms are still unclear. It was reported that cytochrome P450 (CYP) 2C8 and CYP3A4 were the principal catalysts involved in the biotransformation of repaglinide *in vitro*, and CYP2C8*3 polymorphism was closely associated with the plasma concentration of repaglinide [30, 31]. Our previous study showed that the inwardly rectifying potassium channel, subfamily J, member 11 gene (*KCNJ11*) Lys23Glu and the transcription factor 7-like 2 gene (*TCF7L2*) rs290487(C/T) polymorphisms were associated with repaglinide therapeutic efficacy [32]. The *KCNJ11* protein plays a crucial role in insulin secretion and glucose metabolism [33], while *TCF7L2* acts in the Wnt signalling pathway and in the growth, differentiation, proliferation and insulin secretion of pancreatic β -cells [34]. Niemi and co-authors suggested that organic anion-transporting polypeptide 1B1 (*OATP1B1*) was a major determinant of repaglinide pharmacokinetics, and *OATP1B1* T521C polymorphism influenced repaglinide disposition *in vivo* [35]. Up to now, there have been no studies reporting the influences of *NeuroD1/BETA2* and *PAX4* polymorphisms on repaglinide response in T2DM patients. We propose that *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms may affect the therapeutic efficacy of repaglinide in T2DM patients. The present study aims to test this hypothesis in Chinese patients undergoing repaglinide monotherapy for 8 weeks.

Methods

Subjects

A total of 368 T2DM patients aged from 25 to 70 years (mean 49.5 ± 10.9 years; 205 male and 163 female) and 132 healthy control subjects aged from 25 to 70 years (mean 47.6 ± 11.1 years; 80 male and 52 female) from Hunan province (China) were recruited in this study. Written informed consent was obtained from all participants before the start of this study. The study was performed in accordance with the Helsinki Declaration and was approved by the Ethics Committee of Xiangya School of Medicine, Central South University (approval number ICPXL-06015). We have applied for a clinical admission from the Chinese Clinical Trial Register (registration number ChiCTR-CCC00000406). The characteristics of the enrolled individuals were evaluated through medical history, physical examination and routine clinical laboratory tests. Type 2 diabetes mellitus was diagnosed in accordance with World Health Organization criteria in 1997 by fasting plasma glucose (FPG) ≥ 7.0 mmol L⁻¹ or 2 h postprandial plasma glucose (2 h PPG) ≥ 11.1 mmol L⁻¹. Only patients with a body mass index between 18.5 and 30 kg m⁻² who had not been treated with any insulin secretagogue, agonist or inhibitor of CYP2C8, CYP3A4 and

OATP1B1 in the last 3 months were recruited. Patients with type 1 diabetes mellitus, a history of ketoacidosis, ischaemic heart disease, congestive heart failure or trauma, kidney or liver diseases, receiving insulin treatment, and pregnant or lactating women were excluded from this study. A total of 43 T2DM patients (26 male and 17 female) with different *NeuroD1/BETA2* A45T and *PAX4* R121W genotypes and the same *CYP2C8**3 139Arg and *OATP1B1* 521TT genotypes (data not shown) were randomly selected to undergo eight consecutive weeks of repaglinide monotherapy (3 mg day⁻¹).

Clinical laboratory tests

After an overnight fast, a 5 ml venous blood sample was collected into an EDTA tube from each subject in the fasting state, with a further sample 2 h after breakfast. This procedure was repeated at the end of the eighth week of repaglinide administration. As previously described [32, 36], the concentrations of plasma glucose, total cholesterol and triglyceride 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. Plasma insulin [fasting serum insulin (FINS) and postprandial serum insulin (PINS)] and glycated haemoglobin (HbA_{1c}) levels were measured using radioimmunoassay kit (BNIBT, Beijing, China) and by high-performance liquid chromatography (HPLC) assay, respectively. The HOMA-IR (homeostasis model assessment for insulin resistance) is given by: HOMA-IR = fasting insulin level (in microunits per millilitre) × fasting glucose level (in millimoles per)/22.5 to measure insulin resistance and β-cell function. Body mass index was calculated as weight (in kilograms)/height (in metres)².

Genotyping procedures for *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms

Genomic DNA was extracted from leukocytes using SQ Blood DNA Kit (Omega, CO, USA). To detect the R121W mutation, a fragment in exon 3 of *PAX4* was amplified by PCR with an annealing temperature of 61°C, and the PCR products were digested for restriction fragment length polymorphism (RFLP) analysis by *Acil* (Fermentas, Glen Burnie, MD, USA). The primer pairs used for *PAX4* R121W locus were as follows: forward 5'-GAGGATTGGGAAGG AGAAAGCAT-3' and reverse 5'-TATTGGGTTGTTGTGAGGG TGAT-3'. To detect the A45T mutation, a fragment in the *NeuroD1/BETA2* gene was amplified by PCR with an annealing temperature of 60°C, and the PCR products were digested for RFLP analysis by *MwoI* (Fermentas). The primer pairs used in amplification of *NeuroD1/BETA2* A45T locus were as follows: 5'-GTAGGAAATCGAAACATGACCAAAT-3' and reverse 5'-GTCTTAGAATAGCAAGGCACCACC-3'. The primer pairs used for *OATP1B1* T521C were as follows: forward 5'-AAAGGAATCTGGGTCATACATGTGGATATACG-3'

and reverse 5'-TTCAAAAGTAGACAAAGGGAAAGTGATC ATA-3'. The restriction-enzyme-digested products of *NeuroD1/BETA2* A45T, *PAX4* R121W and *OATP1B1* T521C polymorphisms were separated on 2% agarose gels and visualized by ethidium bromide staining.

Statistical analysis

Statistical analyses were performed using SPSS software (version 15.0 for Windows; SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium, distribution of genotype and allele frequencies between T2DM patients and healthy control subjects were analysed by a Pearson χ^2 test. Student's unpaired *t*-test was used to compare continuous variables between T2DM patients and healthy control subjects. Student's paired *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. Non-normally distributed data were analysed by Mann-Whitney *U*-test. The association between each SNP and the risk of T2DM was examined using logistic regression. Statistical power was calculated by power calculator software (<http://www.ncss.com>). A value of *P* < 0.05 was considered to be statistically significant for all analyses.

Results

Clinical and biochemical characteristics of subjects

A total of 368 patients and 132 healthy control subjects were genotyped for *NeuroD1/BETA2* and *PAX4* polymorphisms. The basic clinical and biochemical characteristics of subjects are summarized in Table 1. There was no

Table 1

Clinical and biochemical characteristics of type 2 diabetes mellitus (T2DM) patients and healthy control subjects

Parameter	T2DM patients (n = 368)	Healthy control subjects (n = 132)	P value
Sex			
Male	205	80	0.329*
Female	163	52	
Age (years)	49.5 ± 10.9	47.6 ± 11.1	0.097
Body mass index (kg m⁻²)	25.51 ± 3.34	24.95 ± 2.83	0.428
Waist-to-hip ratio	0.91 ± 0.05	0.91 ± 0.06	0.330
FPG (mmol l⁻¹)	9.03 ± 3.33	5.04 ± 0.48	0.000††
Triglyceride (mmol l⁻¹)	2.66 ± 2.62	1.45 ± 0.92	0.000††
Total cholesterol (mmol l⁻¹)	4.94 ± 1.62	4.61 ± 0.85	0.017†
HDL-c (mmol l⁻¹)	1.35 ± 0.82	1.41 ± 0.32	0.497
LDL-c (mmol l⁻¹)	2.74 ± 1.01	2.53 ± 0.82	0.088

Data are given as means ± SD. Abbreviations: FPG, fasting plasma glucose; HDL-c, high-density lipoprotein-cholesterol; LDL-c, low-density lipoprotein-cholesterol. **P* values were determined by Pearson χ^2 test; all other *P* values were assessed by Student's two-sample unpaired *t*-test. †*P* < 0.05, ††*P* < 0.01.

significant difference between T2DM patients and healthy control subjects in age, body mass index, waist-to-hip ratio, HDL-c and LDL-c. However, the concentrations of FPG (9.03 ± 3.33 vs. 5.04 ± 0.48 mmol l⁻¹, $P < 0.01$) ($8.57, 9.27$ vs. $4.98, 5.23$), triglyceride (2.66 ± 2.62 vs. 1.45 ± 0.92 mmol l⁻¹, $P < 0.01$) ($2.42, 3.05$ vs. $1.40, 1.70$) and total cholesterol (4.94 ± 1.62 vs. 4.61 ± 0.85 mmol l⁻¹, $P < 0.05$) ($4.79, 5.16$ vs. $4.49, 4.77$) were significantly higher in T2DM patients than in healthy control subjects.

Genotyping analysis and allelic frequencies

The genotype and allelic frequencies of *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms in T2DM patients and healthy control subjects are shown in Table 2. The allelic frequency of *NeuroD1/BETA2* T45 was higher in T2DM patients than in the control subjects (13.45 vs. 6.82%, $P < 0.01$, 95% CI 0.005, 0.002). There was a significant difference in the TT genotype frequencies between T2DM patients and healthy control subjects (2.72 vs. 0.76%, $P < 0.05$, 95% CI 0.019, 0.025). These data showed that the minor T allele may be a risk factor for the development of T2DM. However, no significant difference of genotype and allelic frequencies of *PAX4* R121W were observed between T2DM patients and healthy control subjects. Genotypes were in Hardy–Weinberg equilibrium for both patients and control groups. Furthermore, we detected that the *NeuroD1/BETA2* A45T polymorphism was significantly associated with T2DM (odds ratios 2.342, 95% CI 1.365, 4.019, $P = 0.002$), while for the *PAX4* R121W polymorphism the odds ratios was 1.151 (95% CI 0.740, 1.788, $P = 0.533$).

Comparisons of baseline parameters of T2DM patients with different *NeuroD1/BETA2* A45T and *PAX4* R121W genotypes

The baseline clinical characteristics of 368 patients with T2DM were determined before repaglinide treatment. As shown in Table 3, there was no significant difference in age,

Table 2

Comparison of distribution frequencies of *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms between T2DM patients and healthy control subjects

Genotype	T2DM patients n = 368 (frequency)	Health control subjects n = 132 (frequency)	P value
NeuroD1/BETA2 A45T			
AA	279 (75.81%)	115 (87.12%)	0.021*
AT	79 (21.47%)	16 (12.12%)	
TT	10 (2.72%)	1 (0.76%)	
Alleles			
A	637 (86.55%)	246 (93.18%)	0.004**
T	99 (13.45%)	18 (6.82%)	
PAX4 R121W			
GG	327 (88.86%)	124 (93.94%)	0.210
GA	39 (10.60%)	8 (6.06%)	
AA	2 (0.54%)	0 (0.00%)	
Alleles			
G	693 (94.16%)	256 (96.97%)	0.075
A	43 (5.84%)	8 (3.03%)	

The frequencies are indicated as absolute values (percentages). *P* values were determined by Pearson χ^2 test. * $P < 0.05$, ** $P < 0.01$.

Table 3

The baseline characteristics of all T2DM patients with various genotypes for the *NeuroD1/BETA2* A45T and *PAX4* R121W loci before the administration of repaglinide

Parameter	<i>NeuroD1/BETA2</i> A45T genotypes		<i>P</i> value	<i>PAX4</i> R121W genotypes		<i>P</i> value
	A/A (n = 279)	A/T+T/T (n = 89)		R/R (n = 325)	R/W+W/W (n = 43)	
Sex						
Male	153	52	0.553*	175	24	0.808*
Female	126	37		150	19	
Age (years)	49.8 $\pm$ 10.5	49.3 $\pm$ 10.4	0.388	48.0 $\pm$ 11.5	48.8 $\pm$ 11.8	0.411
Body mass index (kg m ⁻²)	25.47 $\pm$ 3.42	24.46 $\pm$ 2.42	0.196	25.47 $\pm$ 3.43	25.67 $\pm$ 2.45	0.967
Waist-to-hip ratio	0.91 $\pm$ 0.06	0.90 $\pm$ 0.06	0.144	0.91 $\pm$ 0.06	0.92 $\pm$ 0.06	0.661
FPG (mmol l ⁻¹)	9.13 $\pm$ 3.24	9.36 $\pm$ 4.02	0.360	8.70 $\pm$ 3.15	8.01 $\pm$ 2.56	0.193
PPG (mmol l ⁻¹)	15.71 $\pm$ 5.60	16.19 $\pm$ 6.44	0.903	14.79 $\pm$ 5.95	14.52 $\pm$ 4.40	0.886
FINS (mU l ⁻¹)	13.46 $\pm$ 12.57	10.04 $\pm$ 7.09	0.032†	13.75 $\pm$ 12.03	11.52 $\pm$ 6.30	0.878
PINS (mU l ⁻¹)	54.56 $\pm$ 40.29	40.22 $\pm$ 24.86	0.040†	52.11 $\pm$ 40.93	68.66 $\pm$ 43.87	0.025†
HOMA-IR	5.37 $\pm$ 9.29	3.76 $\pm$ 2.80	0.080	4.79 $\pm$ 4.37	3.93 $\pm$ 2.00	0.946
HbA _{1c} (%)	9.12 $\pm$ 5.32	8.85 $\pm$ 2.62	0.535	8.61 $\pm$ 2.40	8.13 $\pm$ 1.75	0.437
Triglyceride (mmol l ⁻¹)	2.77 $\pm$ 2.80	2.56 $\pm$ 2.30	0.309	2.56 $\pm$ 2.22	2.40 $\pm$ 1.64	0.894
Total cholesterol (mmol l ⁻¹)	5.06 $\pm$ 1.60	5.01 $\pm$ 1.05	0.250	4.96 $\pm$ 1.54	4.75 $\pm$ 1.52	0.934
HDL-c (mmol l ⁻¹)	1.42 $\pm$ 0.98	1.22 $\pm$ 0.34	0.463	1.32 $\pm$ 0.97	1.40 $\pm$ 0.79	0.875
LDL-c (mmol l ⁻¹)	2.64 $\pm$ 0.98	2.68 $\pm$ 0.92	0.549	2.68 $\pm$ 0.87	2.61 $\pm$ 1.28	0.324

Data are given as means $\pm$ SD. Abbreviations: FINS, fasting serum insulin; FPG, fasting plasma glucose; HbA_{1c}, glycated haemoglobin; HDL-c, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; LDL-c, low-density lipoprotein-cholesterol; PINS, postprandial serum insulin; PPG, postprandial plasma glucose.

**P* values were determined by Pearson χ^2 test; all other *P* values were assessed by Student's two-sample unpaired *t*-test. † $P < 0.05$.

body mass index and waist-to-hip ratio in T2DM patients with different *NeuroD1/BETA2* A45T and *PAX4* R121W genotypes. We found that patients with A/A genotype of *NeuroD1/BETA2* A45T polymorphism had higher FINS (13.46 ± 12.57 vs. 10.04 ± 7.09 mU l⁻¹, $P < 0.05$) (11.67 , 14.83 vs. 8.38 , 11.37) and PINS (54.56 ± 40.29 vs. 40.22 ± 24.86 mU l⁻¹, $P < 0.05$) (48.84 , 59.59 vs. 34.42 , 46.02) compared with A/T+T/T genotypes. Furthermore, carriers with the RR genotype of the *PAX4* R121W polymorphism showed higher PINS than individuals with R/W+W/W genotype (52.11 ± 40.93 vs. 68.66 ± 43.87 mU l⁻¹, $P < 0.05$) (44.89 , 58.35 vs. 55.35 , 88.87) (Figure 1).

Effects of *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms on therapeutic efficacy of repaglinide in patients with T2DM

To exclude the influence of *OATP1B1* polymorphisms on repaglinide disposition, 43 T2DM patients (26 male and 17 female) with the same *OATP1B1* T521C genotypes and different *NeuroD1/BETA2* A45T and *PAX4* R121W genotypes were randomly selected to participate in the therapeutic efficacy study. After all patients were treated with 3 mg repaglinide orally daily for eight consecutive weeks, patients with the A/A ($n = 26$) genotype of *NeuroD1/BETA2* A45T polymorphism had better efficacy with respect to the level of FPG (-2.79 ± 2.14 vs. -0.99 ± 1.80 mmol l⁻¹, $P < 0.01$) (-3.53 , -1.84 vs. -1.99 , -0.13) and PPG (-6.71 ± 5.90 vs. -2.54 ± 3.39 mmol l⁻¹, $P < 0.01$) (-9.28 , -4.62 vs. -4.34 , -0.84) than A/T+T/T genotype carriers ($n = 17$). Moreover, patients with the W allele of *PAX4* R121W polymorphism showed an attenuated effect of repaglinide on PPG (-6.53 ± 6.52 vs. -2.95 ± 1.17 mmol l⁻¹, $P < 0.05$) (-8.20 , -4.89 vs. -3.92 , -1.20) (Table 4 and Figure 2).

Discussion

In this case-control study, we identified for the first time that polymorphisms of *NeuroD1/BETA2* A45T and *PAX4* R121W were associated with the susceptibility and development of T2DM and influence the therapeutic efficacy of repaglinide in Chinese T2DM patients. We observed that the *NeuroD1/BETA2* A45T polymorphism was significantly associated with T2DM (odds ratios 2.342, 95% confidence interval 1.365, 4.019, $P = 0.002$), while for the *PAX4* R121W polymorphism the odds ratios was 1.151 (95% confidence interval 0.740, 1.788, $P = 0.533$). We also found that the minor T allele of the *NeuroD1/BETA2* A45T polymorphism may be a risk factor for development of T2DM, whereas T2DM patients with the A allele of *NeuroD1/BETA2* A45T and/or the R allele of *PAX4* R121W had better efficacy of repaglinide treatment.

As mentioned in the introduction, *NeuroD1/BETA2* and *PAX4* are directly or indirectly involved in the function and rebuilding of pancreatic islet cells [8, 11, 16–19], which suggests that genetic polymorphisms of these two genes

Figure 1

Comparison of levels, before repaglinide treatment, of fasting serum insulin (a) and postprandial serum insulin (b) in type 2 diabetes mellitus (T2DM) patients with different *NeuroD1/BETA2* A45T genotypes and postprandial serum insulin (c) in T2DM patients with different *PAX4* R121W genotypes

Table 4

Comparisons of differential values (postadministration minus pre-administration) in T2DM patients with different *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms before and after repaglinide treatment

Parameter	A45T genotype A/A (n = 26)	A/T+T/T (n = 17)	P value	R121W R/R (n = 36)	R/W+W/W (n = 7)	P value
Sex						
Male	14	12	0.272*	24	4	0.629*
Female	12	5		12	3	
FPG (mmol l ⁻¹)	-2.79 ± 2.14	-0.99 ± 1.80	0.009††	-2.56 ± 2.00	-0.98 ± 1.28	0.053
PPG (mmol l ⁻¹)	-6.71 ± 5.90	-2.54 ± 3.39	0.006††	-6.53 ± 6.52	-2.95 ± 1.17	0.046†
FINS (mU l ⁻¹)	2.99 ± 5.49	1.60 ± 6.28	0.737	3.15 ± 6.45	1.19 ± 3.56	0.451
PINS (mU l ⁻¹)	20.21 ± 28.01	15.00 ± 36.46	0.669	18.54 ± 24.92	3.39 ± 42.38	0.571
HbA _{1c} (%)	-3.05 ± 2.89	-2.15 ± 2.47	0.377	-2.73 ± 2.80	-1.31 ± 1.12	0.282
HOMA-IR	-0.08 ± 2.50	0.26 ± 2.73	0.456	0.12 ± 2.48	-0.05 ± 1.03	0.862
Triglyceride (mmol l ⁻¹)	0.14 ± 1.22	-0.27 ± 0.40	0.474	-0.02 ± 1.08	0.06 ± 0.22	0.212
Total cholesterol (mmol l ⁻¹)	-0.41 ± 0.78	-0.28 ± 1.35	0.239	-0.49 ± 1.18	-0.34 ± 1.01	0.693
HDL-c (mmol l ⁻¹)	0.18 ± 0.32	0.12 ± 0.16	0.603	0.15 ± 0.29	0.21 ± 0.15	0.531
LDL-c (mmol l ⁻¹)	-0.52 ± 0.47	-0.09 ± 1.11	0.324	-0.43 ± 0.85	-1.09 ± 1.14	0.119

Data are given as means ± SD. Abbreviations are as in the footnote to Table 3. *P values were determined by Pearson χ^2 test; all other P values were assessed by Student's two-sample unpaired t-test. †P < 0.05, ††P < 0.01.

may be involved in the interindividual differences of repaglinide therapeutic efficacy. In the present study, we showed that the T45 allelic frequency of *NeuroD1/BETA2* in T2DM patients was significantly higher than that in healthy control subjects ($P < 0.01$), which suggest individuals carrying this allele may be more likely to suffer from T2DM. Our findings are in accordance with the results of Iwata *et al.* [37]. In T2DM patients, we also found that patients with the A/A genotype of *NeuroD1/BETA2* A45T polymorphism had higher FINS and PINS values compared with A/T+T/T genotypes.

Shimajiri and co-authors reported that the R121W mutation in the *PAX4* gene was a predisposing factor for the development of T2DM in a Japanese population [25]. However, we did not find significant differences in the genotype and allelic frequencies of *PAX4* R121W polymorphism between T2DM patients and healthy control subjects. The data from the present study showed that T2DM patients with the R/R genotype of *PAX4* R121W polymorphism had a higher PINS value compared with the R/W+W/W genotype, which suggested that genetic polymorphism of *PAX4* R121W may also contribute to interindividual differences in repaglinide response. However, more investigations are required to explore the potential mechanism underlying the increased effects of repaglinide in patients with R/R genotypes for *PAX4* R121W in the future.

Repaglinide inhibits ATP-sensitive K⁺ (K_{ATP}) currents and enhances the Ca²⁺ concentration in pancreatic β -cells, which results in an increase of insulin secretion [38]. Repaglinide is metabolized in the liver through CYP2C8 and CYP3A4 enzymes [30]. Hepatic uptake by OATP1B1 is an important step of repaglinide metabolism. In the present study, we selected patients with the same OATP1B1 T521C genotypes in order to avoid any possible influences of

OATP1B1 polymorphism on the change of repaglinide pharmacokinetics.

In summary, the *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms are associated with the therapeutic efficacy of repaglinide in Chinese T2DM patients. Patients with at least one T allele of the *NeuroD1/BETA2* A45T polymorphism appear to be insensitive to repaglinide in comparison to patients with the A/A genotype. Additionally, the R/R homozygote of *PAX4* R121W polymorphism is more sensitive to repaglinide compared with carriers with R/W or W/W genotypes. Finally, our findings suggest that the T allele of *NeuroD1/BETA2* A45T polymorphism may be a risk factor for T2DM development and an unbeneficial factor to repaglinide treatment. Therefore, we suggest that prior genotyping analysis for *NeuroD1/BETA2* A45T and *PAX4* R121W polymorphisms may be beneficial for T2DM repaglinide monotherapy. 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 78 to 98%. Therefore, the result of this study needs to be confirmed in a larger population.

Competing Interests

There are no competing interests to declare.

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Figure 2

Comparison of differential values (postadministration minus preadministration) of fasting plasma glucose (a) and postprandial plasma glucose (b) between A/A genotype and A/T+T/T genotypes of *NeuroD1*/*BETA2* A45T polymorphism and postprandial plasma glucose (c) between R/R genotype and R/W+W/W genotypes of *PAX4* R121W polymorphism in T2DM patients after repaglinide treatment

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