Department of Endocrinology[1], Xiang-Ya Hospital; Department of Pharmaceutical Engineering[2], College of Chemistry and Chemical Engineering; Institute of Clinical Pharmacology[3], Hunan Key Laboratory of Pharmacogenetics, Central South University; Institute of Clinical Medicine[4], People's hospital of Hunan province, the first affiliated hospital of Hunan Normal University; Public Health School[5], Central South University, Changsha, China

Effect of genetic polymorphism of UCP2-866 G/A on repaglinide response in Chinese patients with type 2 diabetes

Shan Wang[1,2], Yan-Mei Se[1], Zhao-Qian Liu[3], Ming-Xiang Lei[1], Hao-BoYang[1], Zhi-Xiang Sun[1], Sheng-Dan Nie[4], Xiao-Min Zeng[5], Jing Wu[1]

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Jing Wu, MD, PhD, Department of Endocrinology, Xiang-Ya Hospital, Central South University, 87 Xiang-Ya Road, Changsha, Hunan, 410008, P. R. China
wujing0731@hotmail.com

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The aim of the present study was to evaluate the impact of the UCP2-866 G/A polymorphism on the efficacy of repaglinide in treating patients with diabetes mellitus type 2 (T2DM). 370 patients with T2DM and 166 healthy volunteers were enrolled to identify UCP2-866 G/A genotypes. 16 patients with GG genotype, 14 with GA genotype and 11 with AA genotype of UCP2-866 G/A underwent an 8-week repaglinide treatment regimen. There were no differences in allele frequency of UCP2-866 G/A between T2DM patients and control subjects. The patient with AA genotype of UCP2-866 G/A had higher levels of fasting plasma glucose (FPG), 30-min and 2-h postload plasma glucose, glycated haemoglobin (HbA_{1c}), and lower concentrations of 30-min and 2-h postload plasma insulin, homeostasis model assessment of beta cell function (HOMA-β), $\Delta I_{30}/\Delta G_{30}$ compared with GG genotype. After repaglinide treatment for 8 consecutive weeks, we found that A allele carriers of UCP2 in the T2DM patients had smaller decrease in FPG ($P < 0.05$) and HbA_{1c} ($P < 0.05$), and smaller increase in 30-min postload plasma insulin ($P < 0.01$) compared with GG genotypes. We demonstrated that UCP2-866 G/A polymorphism is associated with the therapeutic efficacy of repaglinide in Chinese T2DM patients.

1. Introduction

Repaglinide, an insulin secretagogue, can increase the release of insulin from pancreatic β-cells by inhibiting the adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channel and activating the Ca^{2+} channel (Gribble and Reimann 2003; Gromada et al. 1995). It is used in the treatment of type 2 diabetes mellitus (T2DM). However, marked interindividual differences in therapeutic response to repaglinide in T2DM patients have been reported. It is essential to investigate the possible mechanisms of and reasons for the interindividual variation in therapeutic efficacy of repaglinide.

Uncoupling protein 2 (UCP2), one of the mitochondrial inner membrane carriers, is highly expressed in pancreatic islets (Chan et al. 2001; Esterbauer et al. 2001). UCP2 can catalyze the mitochondrial inter-membrane H^+ leak that bypasses ATP synthase, resulting in the decrease in cellular ATP production (Chan et al. 2001). Therefore, UCP2 is a key modulator of energy metabolism, including pancreatic β-cells energy metabolism. In pancreatic β-cells, glucose increased the ATP/ADP ratio through its oxidative metabolism, resulting in increased insulin secretion by causing the closure of the membrane K_{ATP} channel, subsequent membrane depolarization, influx of Ca^{2+} (Sesti et al. 2003). It is well documented that UCP2 negatively regulates glucose-stimulated insulin secretion (GSIS) by reducing ATP content, subsequently increasing activity of K_{ATP} channel and decreasing Ca^{2+} influx (Chan et al. 2001; Hong et al. 2001; O'Rahilly 2001). The UCP-866 G/A is a common polymorphism in the promoter region of the UCP2 gene. Giorgio et al. found that the common-866 G/A polymorphism in the UCP2 gene may contribute to the biological variation of insulin secretion in humans (Krempler et al. 2002; Sesti et al. 2003). Until now, there have been no studies reporting the influence of genetic UCP2 on repaglinide response in T2DM patients.

In view of the fact that UCP2 plays an important role in insulin secretion, we aimed in this study to evaluate the association of UCP2-866 G/A polymorphism with susceptibility to development of T2DM and to identify the effect of the polymorphism on repaglinide efficacy in Chinese patients with T2DM.

2. Investigations and results

2.1. Clinical characteristics of subjects

The genotypes of UCP2-866 G/A polymorphisms were determined in 370 T2DM patients (185 male, 185 female) and 166 healthy controls (95 male, 71 female). The basic clinical characteristics of the subjects are listed in Table 1. There were significant differences between T2DM patients and healthy controls in waist measurement ($P < 0.01$), hip measurement ($P < 0.05$), fasting plasma glucose (FPG) ($P < 0.001$), 2-h postload plasma glucose ($P < 0.001$), triglycerol (TG) ($P < 0.001$) and total cholesterol (CHO) ($P < 0.001$) level. However, no

Table 1: Clinical characteristics of T2DM patients and healthy controls

Parameter	Healthy controls (n = 166)	T2DM patients (n = 370)	*P value*
Sex (male/female)	95/71	185/185	0.204
Age (year)	48.6 ± 11.3	49.8 ± 10.7	0.248
Height (cm)	162.5 ± 8.0	161.3 ± 8.2	0.139
Weight (kg)	64.1 ± 13.2	64.6 ± 11.5	0.654
BMI (kg/m^2)	24.22 ± 4.35	24.73 ± 3.38	0.207
Waist (cm)	85.66 ± 11.9	$88.4 \pm 9.2^{**}$	0.006
Hip (cm)	94.6 ± 7.8	$96.2 \pm 7.1^{*}$	0.021
Waist-to-hip ratio	0.91 ± 0.07	0.92 ± 0.06	0.071
SBP (mmgH)	122.51 ± 14.24	123.86 ± 17.02	0.487
DBP (mmgH)	76.98 ± 10.15	77.64 ± 10.09	0.374
FPG (mmol/L)	5.11 ± 0.42	$9.14 \pm 3.55^{***}$	< 0.001
2-h glucose (mmol/L)	6.12 ± 1.07	$15.78 \pm 6.37^{***}$	< 0.001
TG (mmol/L)	1.51 ± 0.85	$2.58 \pm 2.63^{***}$	< 0.001
Total-CHO (mmol/L)	4.40 ± 0.80	$5.04 \pm 1.4^{***}$	< 0.001
HDL-c (mmol/L)	1.39 ± 0.32	1.34 ± 0.75	0.474
LDL-c (mmol/L)	2.35 ± 0.67	2.64 ± 0.91	0.332

Continuous variables were expressed as $\overline{X} \pm SD$ and compared using Student t-test; categorical variables were compared by χ^2 test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. healthy controls

significant differences between the two groups were seen with respect to gender distribution, age, height, weight, body mass index (BMI), waist-to-hip ratio, systolic and diastolic blood pressure (SBP and DBP), high density lipoprotein-CHO (HDL-c) or low density lipoprotein-CHO (LDL-c).

2.2. Genotype analysis and allelic frequencies

The allelic frequencies of the UCP2-866 G/A polymorphism in the T2DM patients and healthy controls are shown in Table 2. The minor A allelic frequencies of the UCP2-866 G/A polymorphism were 46.6% and 45.5% in T2DM patients and healthy controls, respectively. In this study, there were no significant differences in allelic frequencies for UCP2-866 G/A between T2DM patients and control subjects.

2.3. Comparison of baseline parameters of UCP2-866 G/A genotypes among T2DM patients

Table 3 summarizes the clinical characteristics of different genotypes of UCP2-866 G/A in 370 T2DM patients before repaglinide therapy. There were no statistical differences in gender distribution, age, course of disease, BMI, waist, hip, waist-to-hip ratio, SBP, DBP, TG, total CHO, HDL-c and LDL-c among three UCP2-866 G/A genotypes. However, patients with the AA genotype had significantly higher FPG, 30-min and 2-h postload plasma glucose, and lower 30-min and 2-h postload plasma insulin compared with GG genotype ($P < 0.05$). Patients carrying AA genotype had higher glycated haemoglobin (HbA_{1c}) and lower HOMA-β compared with the G allele carriers ($P < 0.05$). The A allele carriers had lower $\Delta I_{30}/\Delta G_{30}$ (the ratio of the incremental insulin to glucose responses over the first 30 minutes during the OGTT) compared with GG genotype ($P < 0.05$).

Table 2: Comparison of distributive frequencies of UCP2-866 G/A polymorphisms between T2DM patients and healthy controls

Genotypes	Healthy controls (n = 166)	T2DM patients (n = 370)	*P*
-886 G/A genotypes			
GG	55(33.1%)	113(30.5%)	
GC	71(42.8%)	169(45.7%)	
AA	40(24.1%)	88(23.8%)	0.792
-886 G/A alleles			
G	181(54.5%)	395(53.4%)	
A	151(45.5%)	345(46.6%)	0.729

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

2.4. Influence of UCP2-866 G/A polymorphisms on therapeutic efficacy of repaglinide in T2DM patients (n = 41)

To exclude the influence of OATP1B1 polymorphism on repaglinide disposition, 41 T2DM patients with various UCP2-866 G/A genotypes but with the same OATP1B1 T521C genotype were randomly selected to participate in this study. Repaglinide significantly decreased the values of FPG ($P < 0.001$), 2-h postload plasma glucose ($P < 0.001$), HbA_{1c} ($P < 0.001$), total-CHO ($P < 0.01$) and LDL-c ($P < 0.001$), while markedly increased fasting plasma insulin (FINS) ($P < 0.01$), 2-h postload plasma insulin ($P < 0.001$), HOMA-β ($P < 0.001$), $\Delta I_{30}/\Delta G_{30}$ ($P < 0.001$) and HDL-c ($P < 0.01$) in T2DM patients after 8 weeks of repaglinide treatment (Table 4). Moreover, we found that the A allele carriers of UCP2 in the T2DM patients had smaller decrease in FPG ($P < 0.05$) and HbA_{1c} ($P < 0.05$), and smaller increase in 30-min postload plasma insulin ($P < 0.01$) compared with GG genotype of UCP2-866 G/A ($P < 0.05$) (Table 5, Fig. 1).

3. Discussion

UCP2 plays an important role in the regulation of human energy metabolism and insulin secretion (Wang et al. 2004). The -866 G/A is a common polymorphism in the promoter region of the UCP2 gene. Recently, this variant of UCP2-866 G/A related to the susceptibility of diabetes has gained more attention. Our present study has shown for the first time that a genetic polymorphism of UCP2-866 G/A may influence the therapeutic efficacy of repaglinide in Chinese patients with T2DM.

In the present study, we found no difference in distribution frequencies of UCP2-866 G/A between T2DM patients and healthy controls, which is consistent with previous results in a Japanese population and Pima Indians (Kovacs et al. 2005; Sasahara et al.

Table 3: Baseline characteristics of all T2DM patients with various genotypes of the UCP2-866 G/A before the administration of repaglinide

	-866 G/A			
	GG (n = 113)	GA (n = 169)	AA (n = 88)	*P*
Sex (male/female)	47/66	92/77	46/42	0.095
Age (year)	48.63 ± 11.04	49.89 ± 10.53	54.34 ± 9.45	0.190
course of disease (year	1.51 ± 2.26	1.74 ± 2.90	1.36 ± 2.13	0.491
BMI (kg/m^2)	24.84 ± 3.72	24.21 ± 2.80	24.53 ± 2.00	0.915
Waist (cm)	88.12 ± 10.31	88.12 ± 9.30	89.15 ± 8.35	0.661
Hip (cm)	96.18 ± 7.62	96.19 ± 7.00	96.41 ± 6.87	0.969
Waist-to-hip ratio	0.92 ± 0.07	0.92 ± 0.06	0.93 ± 0.05	0.492
SBP (mmgH)	122.13 ± 17.52	123.47 ± 17.42	126.82 ± 15.30	0.142
DBP (mmgH)	76.65 ± 10.76	77.12 ± 9.60	79.91 ± 9.88	0.052
FPG (mmol/l)	8.53 ± 3.52	9.02 ± 3.49	10.15 ± 3.59*	0.005
30-min glucose (mmol/l)	12.28 ± 4.19	13.21 ± 4.01	14.07 ± 3.90*	0.008
2-h glucose (mmol/l)	14.55 ± 6.92	15.84 ± 5.99	17.01 ± 5.98*	0.018
HbA_{1c}(%)	8.15 ± 2.54†	8.36 ± 2.40†	9.39 ± 2.73	0.001
FINS (mU/l)	9.71 ± 5.95	9.39 ± 5.66	9.13 ± 4.81	0.755
30-min insulin (mU/L)	40.45 ± 51.15	24.25 ± 19.79	20.39 ± 14.69*	0.000
2-h insulin (mU/L)	53.71 ± 48.58	48.19 ± 44.09	38.31 ± 37.81*	0.048
Ln HOMA-IR	1.05 ± 0.65	1.10 ± 0.62	1.22 ± 0.62	0.171
Ln HOMA-β	3.65 ± 0.94†	3.56 ± 0.93†	3.32 ± 0.82	0.032
Ln $\Delta I_{30}/\Delta G_{30}$	1.42 ± 1.42	0.88 ± 1.31*	0.57 ± 1.33*	0.000
TG (mmol/l)	2.39 ± 1.96	2.79 ± 3.31	2.43 ± 1.77	0.368
Total-CHO (mmol/l)	4.77 ± 1.16	5.17 ± 1.63	5.11 ± 1.23	0.051
HDL-c (mmol/l)	1.31 ± 0.51	1.33 ± 0.58	1.41 ± 1.16	0.614
LDL-c (mmol/l)	2.55 ± 0.94	2.66 ± 0.92	2.74 ± 0.86	0.305

Continuous variables were expressed as $\overline{X} \pm SD$ and compared using ANOVA, categorical variables were compared by χ^2 test. $^*P < 0.05$ vs. -866 G/G; $^\dagger P < 0.05$ vs. -866 A/A genotype after Bonferroni correction.

2004; Yang et al. 2009). Interestingly, the allelic frequency of UCP2-866 G/A in Chinese population is different from the Caucasus and the European whites (Bulotta et al. 2005; D'Adamo et al. 2004; Krempler et al. 2002). Gable et al. (2006) indicated that the -866 A/A genotype be associated with the risk of T2DM in British Caucasian male. However, in Caucasians from Italy, a reduced risk of T2DM was observed in -866 G/A and -866 A/A carriers (Bulotta et al. 2005). It is very likely that ethnic differences contribute to these differences, and other genetic factors may play a critical role in the development of T2DM in the Chinese population.

To investigate whether the common -866 G/A polymorphism in the promoter of the UCP2 gene affects insulin secretion in T2DM patients, we estimate insulin secretion using the HOMA-β index and $\Delta I_{30}/\Delta G_{30}$. Our data have shown that patients with T2DM carrying AA genotype of UCP2-866 G/A had lower HOMA-β compared with the G allele carriers and the A allele carriers had lower $\Delta I_{30}/\Delta G_{30}$ compared with GG genotype. It suggests that the carriers of AA genotype had lower β-cell insulin secretion compared with GG genotype. This is consistent with other evidence that the UCP2-866 G/A polymorphism may contribute to the biological variation of insulin secretion in glucose-tolerant

Table 4: Clinical characteristics of all T2DM patients before and after repaglinide treatment (n = 41)

	Before	After	P value
BMI	23.57 ± 2.31	23.67 ± 2.84	0.633
SBP (mmgH)	127.63 ± 13.57	129.83 ± 1.25	0.344
DBP (mmgH)	80.27 ± 8.27	81.07 ± 7.21	0.600
FPG (mmol/l)	8.87 ± 1.76	6.68 ± 1.27***	0.000
30-min glucose (mmol/l)	13.11 ± 3.16	9.29 ± 1.75***	0.000
2-h glucose (mmol/l)	15.89 ± 3.07	11.03 ± 2.66***	0.000
HbA_{1c} (%)	8.54 ± 1.32	6.70 ± 0.94***	0.000
FINS (mU/l)	8.33 ± 5.36	11.04 ± 5.33**	0.001
30-min insulin (mU/l)	17.45 ± 9.58	34.26 ± 18.54***	0.000
2-h insulin (mU/l)	35.33 ± 27.45	58.27 ± 25.97***	0.000
HOMA-IR	0.85 ± 1.01	1.03 ± 0.67	0.186
HOMA-β	3.17 ± 1.08	4.14 ± 0.71***	0.000
$\Delta I_{30}/\Delta G_{30}$	0.51 ± 1.29	2.11 ± 0.80***	0.000
TG (mmol/l)	2.15 ± 1.75	2.13 ± 1.38	0.879
Total-CHO (mmol/l)	5.23 ± 1.23	4.83 ± 0.93**	0.006
HDL-c (mmol/l)	1.27 ± 0.38	1.36 ± 0.38**	0.005
LDL-c (mmol/l)	2.71 ± 0.74	2.35 ± 0.51***	0.000

Continuous variables were expressed as $\overline{X} \pm SD$ and compared using Student t-test. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. before repaglinide treatment.

Table 5: Comparisons of differential values (postadministration minus preadministration) in T2DM patients with different UCP2-866 G/A polymorphisms before and after repaglinide treatment

Index	-866 G/A		*P* value
	GG (n = 16)	GA + AA (n = 25)	
Sex			0.228
male	11	13	
female	5	12	
FPG (mmol/l)			
Before treatment	9.47 ± 1.57	8.48 ± 1.80	0.080
After treatment	6.73 ± 0.87	6.81 ± 1.49	0.845
DV	−2.74 ± 1.70	−1.67 ± 1.38*	0.033
30-min glucose (mmol/l)			
Before treatment	13.00 ± 2.13	13.18 ± 3.72	0.863
After treatment	9.57 ± 1.48	9.11 ± 1.91	0.419
DV	−3.43 ± 2.46	−4.07 ± 3.07	0.488
2-h glucose (mmol/l)			
Before treatment	16.29 ± 2.68	15.63 ± 3.33	0.507
After treatment	11.22 ± 2.14	10.91 ± 2.98	0.717
DV	−5.07 ± 3.28	−4.72 ± 3.44	0.749
FINS (mU/l)			
Before treatment	10.36 ± 6.42	7.03 ± 4.18	0.051
After treatment	13.01 ± 4.76	9.77 ± 5.38	0.057
DV	2.65 ± 4.66	2.75 ± 5.24	0.952
30-min insulin (mU/l)			
Before treatmen	17.58 ± 8.89	17.38 ± 10.17	0.948
After treatment	44.20 ± 21.55	27.90 ± 13.22	0.005
DV	26.63 ± 20.01	10.52 ± 10.34**	0.002
2-h insulin (mU/l)			
Before treatment	32.85 ± 26.67	36.93 ± 29.49	0.648
After treatment	59.69 ± 25.42	57.35 ± 26.81	0.782
DV	26.85 ± 24.51	20.42 ± 22.13	0.390
HbA_{1c} (%)			
Before treatment	8.78 ± 1.38	8.39 ± 1.29	0.367
After treatment	6.40 ± 0.66	6.89 ± 1.06	0.104
DV	−2.38 ± 1.46	−1.49 ± 0.91*	0.022
Ln HOMA-IR			
Before treatment	1.08 ± 1.16	0.70 ± 0.89	0.237
After treatment	1.26 ± 0.53	0.88 ± 0.72	0.075
DV	0.18 ± 0.85	0.18 ± 0.90	0.991
Ln HOMA-β			
Before treatmen	3.21 ± 1.19	3.15 ± 1.03	0.869
After treatment	4.35 ± 0.51	4.01 ± 0.80	0.135
DV	1.14 ± 1.05	0.86 ± 1.17	0.432
Ln ($\Delta I_{30}/\Delta G_{30}$)			
Before treatment	0.77 ± 0.99	0.33 ± 1.42	0.291
After treatment	2.25 ± 0.83	2.02 ± 0.78	0.378
DV	1.49 ± 0.73	1.68 ± 1.17	0.568
TG			
Before treatment	2.03 ± 0.77	2.23 ± 2.17	0.717
After treatment	2.04 ± 1.16	1.18 ± 1.53	0.764
DV	0.02 ± 0.85	−0.05 ± 1.12	0.833
CHO			
Before treatment	4.82 ± 1.04	5.50 ± 1.28	0.086
After treatment	4.50 ± 0.65	5.06 ± 1.03	0.060
Change	−0.33 ± 0.80	-0.44 ± 0.91	0.683
HDL			
Before treatmen	1.35 ± 0.41	1.22 ± 0.36	0.303
After treatment	1.38 ± 0.38	1.35 ± 0.38	0.817
DV	0.03 ± 0.19	0.13 ± 0.20	0.120
LDL			
Before treatment	2.65 ± 0.64	2.74 ± 0.81	0.705
After treatment	2.29 ± 0.61	2.39 ± 0.44	0.561
DV	−0.36 ± 0.32	−0.35 ± 0.73	0.982

Continuous variables were expressed as $\overline{X} \pm SD$ and compared using ANOVA, categorical variables were compared by χ^2 test. *$P < 0.05$, **$P < 0.01$ vs. -866 G/G genotype after Bonferroni correction.

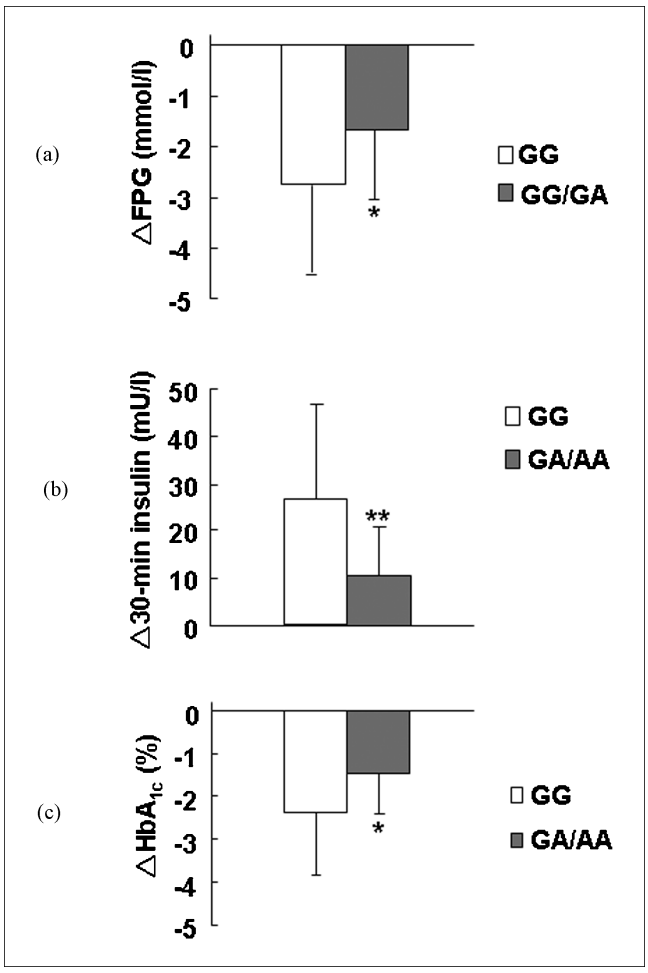

Fig. 1: Comparisons of differential values (postadministration minus preadministration) of (a) FPG, (b) 30-min postload plasma insulin, and (c) HbA_{1c} between the GG genotype and the GA or AA (GA/AA) genotypes of the UCP2 polymorphism in T2DM patients after treatment with repaglinide. $^{*}P < 0.05$, $^{**}P < 0.01$

subjects (Sesti et al. 2003). In the present study, we also found that patients with T2DM carrying the AA genotype of UCP2-866 G/A had lower postload plasma insulin and higher fasting and postload plasma glucose and HbA_{1c} compared with -866 G/G carriers, which coincides with the decreased insulin secretion in -866 A/A carriers. Several studies have provided strong evidence that UCP2 is an important negative regulator of β-cell insulin secretion and the A allele of UCP2-866 G/A is associated with higher promoter activity of UCP2 in β-cells (Krempler et al. 2002; Sasahara et al. 2004). Thus, we speculate that the higher promoter activity of UCP2 in the A allele carriers may contribute to the suppressed β-cell insulin secretion in T2DM patients, and thus subsequently increased plasma glucose. In French Caucasian subjects with T2DM, increased TG, total CHO and LDL-c levels were significantly less frequent in homozygous carriers of the G-allele than in homozygous carriers of the A-allele (Reis et al. 2004). In our research, the patients with GG genotype had lower CHO levels than the A allele carriers, but the comparison of this DV did not reach statistical significance. Larger samples and multicentre studies may be needed to confirm the relationship between UCP2-866 G/A polymorphism and lipid levels in Chinese patients with T2DM.

It was reported that the OATP1B1 T521C polymorphism might be a main factor for the variation in the pharmacokinetics of repaglinide in Chinese population (Yu et al. 2010). In order to investigate the impact of the UCP2-866 G/A polymorphism on therapeutic efficacy of repaglinide in the Chinese population, we firstly need to exclude the effect of OATP1B1 T521C polymorphism on the pharmacokinetics of repaglinide. Therefore, in this study, we selected patients with the same OATP1B1 T521C genotype to research.

After the treatment with repaglinide for 8 consecutive weeks, fasting and 2-h postload plasma glucose, HbA_{1c}, TC, and LDL-c decreased significantly, and fasting and 2-h plasma insulin, HOMA-β, $\Delta I_{30}/\Delta G_{30}$ and HDL-c increased markedly in all T2DM patients. Also, our results showed that the UCP2-866 G/A polymorphism was associated with an enhanced repaglinide response in Chinese patients with T2DM, and that subjects with the GG genotype of UCP2-866 G/A polymorphism showed a greater increase in 30-min postload plasma insulin and also a greater decrease in FPG and HbA_{1c} level than those with the GA or AA genotypes did. It is suggested that the carriers of GG genotype of UCP2-866 G/A had a better response to repaglinide monotherapy compared with other genotypes. It has been demonstrated that the A allele of UCP2-866 G/A polymorphism showed increased promoter activity of UCP2 (Krempler et al. 2002) and UCP2 overexpression impaired GSIS of pancreatic β-cells by increasing the activity of K_{ATP} channel and decreasing the Ca^{2+} influx (Chan et al. 2001). Interestingly, repaglinide stimulates the release of insulin through inhibiting the K_{ATP} channel and activating the Ca^{2+} channel. We therefore speculate that in the A allele carriers, the increased activity of K_{ATP} channel and decreased Ca^{2+} influx induced by increased promoter activity of UCP2 may contribute to the reduced response to repaglinide.

In summary, in this study we observed that the genetic polymorphism of UCP2-866 G/A appears to be associated with the therapeutic efficacy of repaglinide in Chinese patients with T2DM. Patients with GG genotype of UCP2-866 G/A polymorphism seem to be more sensitive to repaglinide than individuals with the GA or AA genotypes. These data suggest that prior genotyping for UCP2-866 G/A single-nucleotide polymorphism may be beneficial for the repaglinide therapy in Chinese patients with T2DM.

4. Experimental

4.1. Subjects

A total of 370 patients with newly diagnosed T2DM (185 male patients and 185 female patients, mean age: 48.6 ± 11.3 years) and 166 healthy volunteers (95 male subjects and 71 female subjects, mean age: 49.8 ± 10.7 years) were recruited from department for endocrine of XiangYa Hospital of Central South University in China. The diagnosis criteria of T2DM patients were made according to the World Health Organization in 1999, namely, FPG ≥ 7.0 mmol/L or random plasma glucose level ≥ 11.1 mmol/L or 2-h OGTT ≥ 11.1 mmol/L. The age and BMI of patients were in the range of 25–70 years and 18.5–30 kg/m^2, respectively. The patients have not received any medication treatment in the last 2 months. The patients were excluded if they were pregnant or lactating women or had acute or severe chronic diabetic complications or serious disease such as acute myocardial infarction, cerebral vascular accident, trauma, and kidney disease or liver disease. Patients with positive glutamic acid decarboxylase antibodies or with FPG > 15mmol/L were also excluded. The study was designed in compliance with the ethics regulations set out by the Helsinki Declaration and was approved by the Ethics Committee of Xiangya Hospital, Central South University. All subjects provided written informed consent.

4.2. Study design

A total of 41 T2DM patients with various UCP2-866 G/A genotypes and the same OAP1B1 T521C genotype were randomly selected from the 370 T2DM patients. After a 7 days run-in period of diet, the patients took repaglinide (NovoNorm, NovoNordiskA/S, Denmark) 1 mg three times a day before breakfast, lunch and dinner for 8 consecutive weeks.

4.3. Clinical laboratory tests

Anthropometric measurements included height, weight, BMI, waist to hip ratio, SBP and DBP. After an overnight fast by the study subjects, blood

samples for measurements of plasma glucose and insulin were collected from them in the fasting state and at 30-min and 2-h during the OGTT. These parameters were measured again at the end of weeks 0 and 8. Plasma glucose, insulin, HbA_{1c} and serum lipids, including TG, total CHO, HDL-c and LDL-c were measured as previously described (Sun et al. 2008). BMI was calculated as weight (kg)/height(m)^2. For the quantification of insulin resistance (IR), the homeostasis model assessment of IR (HOMA-IR) index was calculated as fasting plasma insulin (FINS) × FPG/22.5 (Matthews et al. 1985). Insulin secretion was assessed using the HOMA-β index ($20 \times$ FINS/[FPG $-$ 3.5]) (Matthews et al. 1985) and $\Delta I_{30}/\Delta G_{30}$ (Chen et al. 2010; Wareham et al. 1995).

4.4. Genotyping procedures for UCP2-866 G/A polymorphism

DNA of T2DM patients and controls were isolated from peripheral blood leukocytes. The gene polymorphisms were genotyped using polymerase chain reaction (PCR)-restriction fragment-length polymorphism assay. The primer pairs used in the amplification of the UCP2-866 G/A were sense primer: 5′-CTTTGGGACTCCGTTTCCTCATTG-3′, antisense primer: 5′-TGGAGCGGCCTGGCGTTTAG-3′ (reverse). The primer pairs used for OATP1B1 T521C were sense primer: 5′-AAAGGAATCTGGGTCATACATGTGGATATACG-3′, antisense primer: 5′-TTCAAAAGTAGACAAAGGGAAAGTGATCATA-3′. The PCR products for analysis of UCP2-866 G/A and OATP1B1 T521C polymorphisms were digested by Mlu I.

4.5. Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) and compared using Student t-test and ANOVA. Sex and allelic frequencies in different groups were compared using Pearson's χ^2 test. The allelic distribution of each single nucleotide polymorphism (SNP) was verified using Hardy-Weinberg equilibrium. Non-normally distributed variables (HOMA-IR, acute insulin response, HOMA-β, $\Delta I_{30}/\Delta G_{30}$) was log-transformed before analysis. Significance was defined as ≤ 0.05.

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References

Bulotta A, Ludovico O, Coco A, Di Paola R, Quattrone A, Carella M, Pellegrini F, Prudente S, Trischitta V (2005) The common -866 G/A polymorphism in the promoter region of the UCP-2 gene is associated with reduced risk of type 2 diabetes in Caucasians from Italy. J Clin Endocrinol Metab 90: 1176–1180.

Chan CB, De Leo D, Joseph JW, McQuaid TS, Ha XF, Xu F, Tsushima RG, Pennefather PS, Salapatek AM, Wheeler MB (2001) Increased uncoupling protein-2 levels in beta-cells are associated with impaired glucose-stimulated insulin secretion: mechanism of action. Diabetes 50: 1302–1310.

Chen G, Liu C, Yao J, Jiang Q, Chen N, Huang H, Liang J, Li L, Lin L (2010) Overweight, obesity, and their associations with insulin resistance and beta-cell function among Chinese: a cross-sectional study in China. Metabolism 59: 1823–1832.

D'Adamo M, Perego L, Cardellini M, Marini MA, Frontoni S, Andreozzi F, Sciacqua A, Lauro D, Sbraccia P, Federici M, Paganelli M, Pontiroli AE, Lauro R, Perticone F, Folli F, Sesti G (2004) The -866 A/A genotype in the promoter of the human uncoupling protein 2 gene is associated with insulin resistance and increased risk of type 2 diabetes. Diabetes 53: 1905–1910.

Esterbauer H, Schneitler C, Oberkofler H, Ebenbichler C, Paulweber B, Sandhofer F, Ladurner G, Hell E, Strosberg AD, Patsch JR, Krempler F, Patsch W (2001) A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. Nat Genet 28: 178–183.

Gable DR, Stephens JW, Cooper JA, Miller GJ, Humphries SE (2006) Variation in the UCP2-UCP3 gene cluster predicts the development of type 2 diabetes in healthy middle-aged men. Diabetes 55: 1504–1511.

Gribble FM, Reimann F (2003) Differential selectivity of insulin secretagogues: mechanisms, clinical implications, and drug interactions. J Diabetes Complications 17: 11–15.

Gromada J, Dissing S, Kofod H, Frokjaer-Jensen J (1995) Effects of the hypoglycaemic drugs repaglinide and glibenclamide on ATP-sensitive potassium-channels and cytosolic calcium levels in beta TC3 cells and rat pancreatic beta cells. Diabetologia 38: 1025–1032.

Hong Y, Fink BD, Dillon JS, Sivitz WI (2001) Effects of adenoviral overexpression of uncoupling protein-2 and -3 on mitochondrial respiration in insulinoma cells. Endocrinology 142: 249–256.

Kovacs P, Ma L, Hanson RL, Franks P, Stumvoll M, Bogardus C, Baier LJ (2005) Genetic variation in UCP2 (uncoupling protein-2) is associated with energy metabolism in Pima Indians. Diabetologia 48: 2292–2295.

Krempler F, Esterbauer H, Weitgasser R, Ebenbichler C, Patsch JR, Miller K, Xie M, Linnemayr V, Oberkofler H, Patsch W (2002) A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces type 2 diabetes risk in obese middle-aged humans. Diabetes 51: 3331–3335.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419.

O'Rahilly S (2001) Uncoupling protein 2: Adiposity angel and diabetes devil? Nat Med 7: 770–772.

Reis AF, Dubois-Laforgue D, Bellanne-Chantelot C, Timsit J, Velho G (2004) A polymorphism in the promoter of UCP2 gene modulates lipid levels in patients with type 2 diabetes. Mol Genet Metab 82: 339–344.

Sasahara M, Nishi M, Kawashima H, Ueda K, Sakagashira S, Furuta H, Matsumoto E, Hanabusa T, Sasaki H, Nanjo K (2004) Uncoupling protein 2 promoter polymorphism -866 G/A affects its expression in beta-cells and modulates clinical profiles of Japanese type 2 diabetic patients. Diabetes 53: 482–485.

Sesti G, Cardellini M, Marini MA, Frontoni S, D'Adamo M, Del Guerra S, Lauro D, De Nicolais P, Sbraccia P, Del Prato S, Gambardella S, Federici M, Marchetti P, Lauro R (2003) A common polymorphism in the promoter of UCP2 contributes to the variation in insulin secretion in glucose-tolerant subjects. Diabetes 52: 1280–1283.

Sun H, Gong ZC, Yin JY, Liu HL, Liu YZ, Guo ZW, Zhou HH, Wu J, Liu ZQ (2008) The association of adiponectin allele 45T/G and -11377C/G polymorphisms with Type 2 diabetes and rosiglitazone response in Chinese patients. Br J Clin Pharmacol 65: 917–926.

Wang H, Chu WS, Lu T, Hasstedt SJ, Kern PA, Elbein SC (2004) Uncoupling protein-2 polymorphisms in type 2 diabetes, obesity, and insulin secretion. Am J Physiol Endocrinol Metab 286: E1–7.

Wareham NJ, Phillips DI, Byrne CD, Hales CN (1995) The 30 minute insulin incremental response in an oral glucose tolerance test as a measure of insulin secretion. Diabet Med 12: 931.

Yang M, Huang Q, Wu J, Yin JY, Sun H, Liu HL, Zhou HH, Liu ZQ (2009) Effects of UCP2-866 G/A and ADRB3 Trp64Arg on rosiglitazone response in Chinese patients with Type 2 diabetes. Br J Clin Pharmacol 68: 14–22.

Yu M, Xu XJ, Yin JY, Wu J, Chen X, Gong ZC, Ren HY, Huang Q, Sheng FF, Zhou HH, Liu ZQ (2010) KCNJ11 Lys23Glu and TCF7L2 rs290487(C/T) polymorphisms affect therapeutic efficacy of repaglinide in Chinese patients with type 2 diabetes. Clin Pharmacol Ther 87: 330–335.