

Evaluation of the effect of MTNR1B rs10830963 gene polymorphism on the therapeutic efficacy of nateglinide in treating type 2 diabetes among Chinese Han patients

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Abstract

Background: Several studies have shown the association of polymorphisms in the MTNR1B gene with type 2 diabetes mellitus (T2DM). However, there is no evidence about the impacts of its genetic polymorphism on the therapeutic efficacy of nateglinide. Therefore, this prospective case-control study was designed to investigate the effect of MTNR1B rs10830963 gene polymorphism on the therapeutic efficacy of nateglinide in treating T2DM.

Methods: We genotyped 200 healthy subjects using the method of the high resolution of melting curve (HRM). A total of 60 T2DM patients were enrolled and given nateglinide (360 mg/d) for 8 weeks orally who had the same genotypes CYP2C9*1 and SLC01B1 521TT respectively. The outcome was measured by collecting the venous blood samples before and at the 8th week of the treatment. Also, anthropometric measurements, glucose, and lipid metabolism were determined before and after the nateglinide treatment.

Results: It was found that the risk G allelic frequency of MTNR1B rs10830963 was higher in T2DM patients when compared with the healthy subjects ($P < 0.05$). 60 newly diagnosed patients with type 2 diabetes after completing the eight weeks treatment came for the follow-up visit and showed a reduction in fasting plasma glucose (FPG) levels with an increase in homeostasis model assessment for β cell HOMA- β in the carriers of genotype CG + GG at rs10830963, when compared with the wild-type CC ($P < 0.05$).

Conclusion: Thus, it was found that the MTNR1B rs10830963 polymorphism was associated with the therapeutic efficacy of nateglinide in T2DM patients. Also, the CC homozygotes had a better effect than G allele carriers. Trial registration: This study was registered in the Chinese Clinical Trial Register (No. ChiCTR-CCC13003536).

Background

Type 2 diabetes mellitus (T2DM) is a chronic genetic heterogeneous disease with multiple genes and multiple environmental factors ^[1]. In order to effectively control the glycemic level and to reduce the risk of secondary damage to cardiovascular, cerebrovascular, kidney and other organs, most patients with T2DM require treatment with hypoglycemic drugs ^[1]. Oral hypoglycemic therapy helps in achieving glycemic control in patients with type 2 diabetes. Clinical practices have found that the same drug regimen used on different patients showed significant individual differences in the efficacy of hypoglycemic. The genetic variation of the genes involved in insulin resistance and islet beta-cell functioning which may ultimately affect the susceptibility and disease progression of T2DM. This will result in further affecting the patient's responsiveness to drug therapy.

Nateglinide is a novel non-sulfonylurea oral hypoglycemic agent that improves blood glucose levels by promoting insulin secretion from pancreatic islet beta cells. However, the efficacy of the drug varies from each individual ^[2, 3]. The mechanism of the difference in the efficacy and adverse effects of nateglinide

remains unclear. It is thought to vary based on the genetic conditions of the drug transporters, drug-metabolizing enzymes, drug receptors, and T2DM susceptibility genes [3]. There are a few types of research that attribute this difference may be due to the genetic polymorphisms of cytochrome P450 2C9 (CYP2C9) and organic anion transporting polypeptide 1B1 (OATP1B1). It is found that due to the genetic polymorphism of the enzymes mentioned above and their impact on the pharmacokinetic process of nateglinide might contribute to the difference in the efficacy. But, this could not elucidate the complete mechanism of action by which the same nateglinide therapy results in various therapeutic responses [4-6].

In pharmacodynamics, the onset of nateglinide is rapid but with a shorter duration of action, compared with sulfonylureas. Thus it is able to significantly reduce postprandial hyperglycemia as well as improve glycemic control in T2DM [7,8]. In addition, other reports also showed that nateglinide had a good effect on improving insulin resistance [9-11]. Therefore, it is understood that genetic polymorphisms affecting insulin resistance or islet cell function may impact the efficacy of nateglinide treatment on T2DM patients. Hence it is important to study the effect of genetic variation on individual differences in the efficacy of nateglinide drugs for guiding clinical rational drug use and optimizing T2DM treatment strategies.

Three genome-wide association studies (GWAS) among the European populations conducted during the recent years have found that *MTNR1B* gene mutations have an association with the increased risk of T2DM, increased fasting plasma glucose (FPG), and decreased insulin secretion [12-14]. Among the observed risk variants, *MTNR1B* rs10830963(C>G) was the most strongly associated one with FPG [14]. Subsequently, it was also confirmed that *MTNR1B* rs10830963 had an association with increased FPG and increased risk of T2DM in Chinese Han population [15,16]. Further, pharmacogenomic studies have shown that polymorphisms in genes associated with insulin secretion and insulin sensitivity, such as *KCNQ1*, *SLC30A8*, and *NOS1AP* gene polymorphisms, may affect T2DM patients' responsiveness to hypoglycemic drugs [17,18].

As aforementioned, the biological function of the *MTNR1B* gene and the therapeutic effect of nateglinide are mainly focused on the regulation of insulin secretion and resistance. However, whether the efficacy of nateglinide gets affected by the *MTNR1B* gene polymorphism remains unclear. Thus, in this study, *MTNR1B* rs10830963 gene was selected as a genetic marker and the effect of *MTNR1B* gene polymorphism on the therapeutic efficacy of nateglinide in Chinese type 2 diabetes patients is determined.

Aim of the study

This study aimed to investigate the effects of *MTNR1B* rs10830963 gene polymorphism on the efficacy of nateglinide in the treatment of type 2 diabetes.

Ethics approval and consent to participate

The study was registered in the Chinese Clinical Trial Register (No. ChiCTR-CCC13003536), in which the protocol used was approved by the ethics committee of the Affiliated Hospital of Xuzhou Medical College and followed the Helsinki Declaration II. Written informed consent was obtained from each participant before the study.

Methods

Participants

This prospective case-control study included 200 unrelated T2DM patients (111 men and 89 women) and 200 healthy controls (105 men and 95 women) for analysis of *MTNR1B* rs10830963 polymorphism. The T2DM patients and the healthy subjects were enrolled from the Department of Endocrinology and the Health Screening Center of the Affiliated Hospital of Xuzhou Medical College respectively. Diagnosis of T2DM was carried out based on the 1997 World Health Organization (WHO) criteria for hyperglycemia under the following conditions: FPG >7.0 mmol/l or postprandial plasma glucose (PPG) >11.1 mmol/l. The patients receiving insulin treatment, pregnant women, lactating mother, those who have a history of ketoacidosis, ischemic heart disease, congestive heart failure, trauma, kidney and liver diseases were excluded from the study. A total of 60 unrelated T2DM patients (36 men and 24 women) with the same *CYP2C9**1 and *OATP1B1* 521TT genotypes were recruited for analysis of *MTNR1B* rs10830963 polymorphism. All patients were asked to take 360mg nateglinide per day (120 mg before each meal) orally for eight consecutive weeks. They were also advised of the same standard of diet control and exercise therapy. Patients with a body mass index (BMI) of 18.5–30 kg/m² and who were not treated with any insulin secretagogues, agonists or inhibitors of *CYP2C8*, *CYP2C9*, *CYP3A4* and *OATP1B1* in the past 3 months were included for the study.

This study was registered in the Chinese Clinical Trial Register (No. ChiCTR-CCC13003536) and obtained approval from the ethics committee of the Affiliated Hospital of Xuzhou Medical College and followed the Helsinki Declaration II. Written informed consent was obtained from each participant before the study.

Genotyping analysis

SiMax Genome DNA Kit (Sbsbio, Shanghai, China) was used to isolate the genomic DNA from the peripheral blood leucocytes. High resolution of melting curve (HRM) method was used to analyze the *MTNR1B* rs10830963 polymorphism. Following primer pairs were used for the analyses: 5'-GAGGATTTGCTTGCTGAACA-3' (forward) and 5'-CCCAGGCAGTTACTGGTTCT-3' (reverse). The total HRM reaction system for detecting *MTNR1B* gene mutation was 20 μ L, including 10 μ L of HRM MasterMix buffer, 2.4 μ L of Mg²⁺, 0.4 μ L of each of the forward and reverse primers, and 5 μ L of DNA and water was added to 20 μ L. Cycle parameters: 95 ° C for 10 min, 95 ° C for 10 s, 65 ° C for 15 s, 72 ° C for 15 s, a total of 55 cycles. Melting: 95 ° C 1 min, 40 ° C 1 min, 70 ° C 1 s, 95 ° C 1 min. Cooling: 40 ° C 30s.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for genotyping of *CYP2C9* polymorphism and the four primer pairs used include forward primer: 5'-TGCACGAGGTCCAGAGATGC-3', reverse primer: 5'-CTATGAATTTGGGACTTCG-3'. Amplification refractory mutation system (ARMS) was used to detect the *OATP1B1* T521C genotypes and the four primer pairs used include: forward primer: 5'-AAGTAGTTAAATTTGTAATAGAAATG C-3', reverse primer: 5'-GTAGACAAAGGGAAAGTGATCATA-3'; forward primer for wild-type genotype: 5'-GGGTCATACATGTGGATATAAGT-3', reverse primer for mutant variants: 5'-AAGCATATTACCCATGAACG-3'. 2% agarose gel electrophoresis was used to separate the obtained DNA fragments followed by ethidium bromide staining and visualization with UV transillumination.

Clinical laboratory tests

Blood samples were collected from participants in fasting state (fasting for more than 8 hours) and 2 h after breakfast respectively. 100 g of sugar-free steamed bread was provided for standard breakfast. Body parameters that included body height, body mass index (BMI), waist circumference, hip circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured before and at 8 weeks of treatment respectively. BMI was calculated as weight (kg) / height (m)². Waist-to-Hip Ratio (WHR) was calculated as waistline (cm) / hipline (cm). Clinical indicators were also detected before and at 8 weeks after the administration of nateglinide. Roche Cobas8000 analyzer (Roche, Basel, Switzerland) was used to detect the plasma glucose, serum lipids triglycerides (TG), total cholesterol (TC), low-density cholesterol (LDL-c) and high-density cholesterol (HDL-c) with standard laboratory methods. Electro-chemiluminescence assay (Roche, Shanghai China) was used to measure insulin levels. High-performance liquid chromatography (HPLC) was used to determine the amounts of glycated hemoglobin (HbA_{1c}). Homeostasis model assessment for insulin resistance (HOMA-IR) and islet β cell function (HOMA- β) was calculated using the formula: HOMA-IR = fasting insulin (mU/l) \times fasting plasma glucose (mmol/l) / 22.5 and HOMA- β = 20 \times FINS(mU/l) / [FPG(mmol /l) - 3.5] respectively.

Statistical analysis

Statistical analyses were performed with spss 18.0 software (SPSS, Chicago, IL, USA). Gene calculation was used for determining the allele frequencies and Hardy-Weinberg equilibrium tests were used to confirm the population representativeness of the samples. All continuous variables were expressed as mean \pm standard deviation (mean \pm SD). The paired Student's *t*-test was used to compare all the parameters between the two groups before and after nateglinide treatment. The two-sample *t*-test or one-way ANOVA test and non-parametric test were used for comparison between the two groups for the parameters of normal and abnormal distribution respectively. A value of $P < 0.05$ was considered statistically significant.

Results

Allelic frequency analysis

A total of 200 T2DM patients (111 men and 89 women) and 200 healthy subjects (99 men and 101 women) were genotyped for *MTNR1B* rs10830963 polymorphism. The genotype distribution in each group was consistent with the Hardy-Weinberg equilibrium ($P > 0.05$). The allele frequencies of the *MTNR1B* rs10830963 polymorphism in T2DM patients and healthy subjects are given in Table 1. The frequency of the *MTNR1B* rs10830963 G allele was higher in T2DM patients when compared to the healthy subjects (42.50% vs 34.50%, $P < 0.05$).

Assessment of baseline parameters with different *MTNR1B* rs10830963 genotypes in T2DM patients

The baseline clinical characteristics of 200 T2DM patients with different *MTNR1B* rs10830963 genotypes were analyzed in table 2. There was no association between *MTNR1B* rs10830963 polymorphism and sex, age, BMI, WHR, PPG, fasting serum insulin (FINS), postprandial serum insulin (PINS), HOMA-IR, HbA1c, TG, TC, HDL-c and LDL-c were observed. However, FPG, (9.61 ± 2.01 mmol/l for CC genotype, 9.91 ± 2.79 mmol/l for CG and 10.82 ± 1.79 mmol/l for GG, respectively; $P < 0.05$, Fig. 1) showed significant differences.

Effects of *MTNR1B* rs10830963 polymorphism on the efficacy of nateglinide in T2DM patients

60 T2DM patients with different *MTNR1B* rs10830963 but the same *OATP1B1* 521TT and *CYP2C9**1 genotypes were randomly selected to participate in our study to avoid the potential impacts of *OATP1B1* and *CYP2C9* genetic polymorphisms. It was observed that these patients responded to nateglinide therapy. After 8 weeks of treatment, they showed a remarkable decline in the amounts of FPG, PPG, HbA1c and TC (all $P < 0.05$), but significant increase in the levels of FINS, PINS and HOMA- β (all $P < 0.05$). The comparison with the pretreatment values was tabulated in Table 3.

Since the GG genotype frequency was lower in the selected population, we combined the CG genotype (26 cases) and the GG genotype (8 cases) for analysis and compared with the CC genotype (26 cases). After nateglinide treatment, the FPG value of the patients with genotypes CG and GG was higher, when compared with the carriers of genotype CC. PINS and HOMA- β values were lower, when compared with the CC genotype carriers ($P < 0.05$). T2DM patients with genotype CC at *MTNR1B* rs10830963 had a significant decrease in FPG (mmol/l) when compared with the genotypes CG and GG (-3.75 ± 1.68 vs -2.87 ± 1.32 ; $P < 0.05$) respectively. In addition, the carriers of genotype CC at *MTNR1B* rs10830963 had higher differential values of HOMA- β , when compared with the genotypes CG and GG (40.87 ± 23.52 vs 25.13 ± 19.21 ; $P < 0.05$) respectively. (Table 4, figure 2).

Discussion

A significant basis for clinical individualized drug delivery is provided by a relatively new subject called pharmacogenomics. Presently, the pharmacogenomics researches of oral hypoglycemic drugs mainly focus on the study of classic sulfonylurea oral hypoglycemic agents, thiazolidinedione insulin sensitizers and biguanide hypoglycemic drugs. There are few reports on the pharmacogenomics of the novel insulin secretagogues of glinide, but no evidence related to *MTNR1B* gene is found.

Studies conducted with the Chinese Han population have confirmed that single nucleotide polymorphisms (SNPs) of *MTNR1B* are associated with T2DM susceptibility [19–20]. Among the SNP sites involved in *MTNR1B*, the rs10830963 locus is a functional polymorphic locus and is closely related to glucose metabolism and islet β cell function. Further, it is highly correlated with the pathogenesis of T2DM. The variation of the *MTNR1B* gene is consistent with the FPG level; rs10830963 is the most relevant, and each G allele increases the FPG level by 0.07mmol/l. The homeostasis model assessment (HOMA- β) analysis showed impaired beta-cell function [14]. In this study, we found that the risk G allelic frequency of *MINR1B* rsl0830963 was significantly higher in T2DM patients, when compared to the healthy subjects ($P < 0.05$) and remains consistent with previous studies [14–15]. Also, it is found that patients with T2DM carrying the GG genotype had higher FPG levels when compared with the CC and CG genotypes. The difference being statistically significant, suggest that the G allele has an association with the elevated FPG levels.

Our study has identified that genetic polymorphisms of *MTNR1B* rs10830963 influences the therapeutic efficacy of nateglinide in patients with T2DM. We observed this in T2DM patients with G allele of *MTNR1B* rs10830963 decreased the efficacy of nateglinide. Consequently, *MTNR1B* gene represents a susceptibility target for T2DM and affects the response to nateglinide.

The pineal gland releases the circulating hormone melatonin (MLT) and its action is mediated by melatonin receptor 1 and 2 (MT1, MT2) respectively [12]. MT2 is encoded by the *MTNR1B* gene and is expressed in the islet beta cells of both animals and human beings [12]. Multiple GWAS studies conducted in European populations found that *MTNR1B* rs10830963 is associated with FPG, insulin secretion, and T2DM susceptibility [13, 14]. Subsequently, it was also found that *MTNR1B* rs10830963 has an association with FPG and islet β -cell function in Chinese Han population [15, 16]. However, the molecular mechanism by which the *MTNR1B* gene polymorphism increases T2DM susceptibility remains unclear. Studies have reported that after MLT activates MT2, MT2 gets coupled with the inhibitory G protein, mediating cAMP and cGMP signal transduction pathways and inhibits insulin release from islet beta cells [19–20]. In addition to MLT in MT2 knockout mice, islet β cells release insulin increases [21] and therefore *MTNR1B* gene polymorphism increased T2DM susceptibility relating to its influence on insulin secretion.

Next to repaglinide, nateglinide is the new non-sulfonylurea oral hypoglycemic agent. It is more commonly used in clinical practices, but the difference in efficacy and adverse reactions is significant. The main mechanism of action of nateglinide is to close the ATP-dependent K^+ channel on the islet β -cell membrane to cause the depolarization of the cell membrane and open the Ca^{2+} channel to lead to Ca^{2+}

influx and thus promote insulin secretion [22]. Therefore, the *MTNR1B* gene polymorphism plays a role in the hypoglycemic effect of nateglinide.

The purpose of this study was to analyze the effect of *MTNR1B* rs10830963 gene polymorphism on the efficacy of nateglinide in treating the newly diagnosed type 2 diabetes patients. Previous studies have reported that *CYP2C9* and *SLCO1B1* gene polymorphisms may affect the pharmacokinetics of nateglinide [23–26]. Hence we decided to retain the same patients with the *CYP2C9**1 and *SLCO1B1* 521TT genotypes as subjects to rule out interference. After 8 consecutive weeks of nateglinide monotherapy, patients with FPG, PPG, FINS, PINS, HOMA-IR, HOMA-β, HbA1c, and TC showed significant improvement. Thus indicating that nateglinide has a good therapeutic effect on patients with type 2 diabetes. There are literatures reporting the nateglinide effect on improving insulin resistance [9, 10]. Our research results were found to be consistent with the literature results. But, there was no evidence to find the relationship between *MTNR1B* rs10830963 gene polymorphism and nateglinide efficacy. Therefore, in our study, we compared the difference between the clinical indicators before and after nateglinide treatment. The decrease of FPG and the increase of HOMA-β in *MTNR1B* rs10830963 risk gene G carriers were lower when compared with the CC wild type patients ($P < 0.05$). These results indicated that the risk gene G carriers had a worse response to nateglinide when compared with the wild type patients. Also, the clinical treatment showed that the GG genotype patient had poor nateglinide treatment. Prokopenko et al [14] reported that calculation of islet beta-cell function using the homeostasis model showed that, *MTNR1B* rs10830963 risk gene G carriers had lower islet function. Lys-senko et al [13] found GG genotype carriers, oral or intravenous glucose stimulation early-phase insulin release was impaired. Previous reports results were consistent with the results of this study. After nateglinide treatment, risk gene G may further reduce the efficacy of nateglinide by affecting FPG and HOMA-β. The exact mechanism by which the *MTNR1B* gene polymorphism affects the efficacy of nateglinide requires further investigation.

However, this study does have some shortcomings as the sample size is not large enough, and the frequency of *MTNR1B* rs10830963 GG genotype is low. Therefore this study might miss some meaningful results. Hence we recommend further detailed study with expanded sample size. Glinide drugs are mealtime blood glucose regulators and are characterized by rapid but short-acting insulin secretion with weak hypoglycemic effect and good safety. Therefore, this study neither focused on the clinical adverse events during nateglinide monotherapy nor did it receive reports of adverse events in the subjects. T2DM is a multi-gene metabolic disease and in this study we found that the *MTNR1B* gene polymorphism has a certain effect on the efficacy of nateglinide. But the individual difference in the efficacy of hypoglycemic drugs is caused by the accumulation of multiple gene polymorphisms as well as the changes in the environmental factors and lifestyles. The results of a single genetic polymorphism study could not fully explain the individual differences in drug efficacy. Therefore, in order to apply this research results to clinical practice, it requires collaboration among researchers from different regions.

In summary, the results of this study suggest that the *MTNR1B* rs10830963 polymorphism is associated with the efficacy of nateglinide in the treatment of type 2 diabetes, and also has a certain role in

promoting clinical individualized drug delivery. But, this study is only an exploratory trial of glinide pharmacogenomics research. We recommend exploration with more extensive and comprehensive clinical research along with an in-depth mechanism to confirm its relevance.

Limitations of the study

This study is only an exploratory trial of glinide pharmacogenomics research, and more extensive and comprehensive clinical research and more in-depth mechanism exploration are needed to confirm its relevance.

Conclusion

In conclusion, we report for the first time that *MTNR1B* rs10830963 polymorphism might influence the incidence of T2DM among the Chinese Han population and the efficacy of nateglinide monotherapy. The CC homozygotes had a better effect than G allele carriers.

Abbreviations

High resolution of melting curve (HRM)

Type 2 diabetes mellitus (T2DM)

Fasting plasma glucose (FPG)

Genome-wide association studies (GWAS)

World Health Organization (WHO)

Body mass index (BMI)

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

Amplification refractory mutation system (ARMS)

Systolic blood pressure (SBP)

Diastolic blood pressure (DBP)

Waist-to-Hip Ratio (WHR)

Triglycerides (TG)

Total cholesterol (TC)

Low-density cholesterol (LDL-c)

High-density cholesterol (HDL-c)

High-performance liquid chromatography (HPLC)

Glycated hemoglobin (HbA1c)

Homeostasis model assessment for insulin resistance (HOMA-IR) and islet β cell function (HOMA- β)

Fasting serum insulin (FINS)

Postprandial serum insulin (PINS)

Declarations

Availability of data and materials

All data have been available from the corresponding author upon request.

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Contributions

All authors contributed substantially to the work presented in this paper, read and approved the final manuscript. JS designed all the work under the supervision of YZ. YZ designed the research, contributed substantially with data analysis, results interpretations and manuscript editing and approval. MZ, JN and TW collected the patients' data and did Gene analysis.

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Ethics declarations

Ethics approval and consent to participate

The study was registered in the Chinese Clinical Trial Register (No. ChiCTR-CCC13003536), in which the protocol used was approved by the ethics committee of the Affiliated Hospital of Xuzhou Medical College and followed the Helsinki Declaration II. Written informed consent was obtained from each participant before the study. As our study contains the data obtained from the hospital record.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1: Comparison of genotype and frequencies of *MTNR1B* rs10830963 polymorphism between T2DM patients and healthy subjects

Genotypes	Healthy subjects	T2DM patients	P values
	(n =200)	(n =200)	
MTNR1B rs10830963			
CC	82(41.00%)	70(35.00%)	0.001 ^{a*}
CG	98(49.00%)	90(45.00%)	
GG	20(10.00%)	40(20.00%)	
Alleles			
C	262(65.50%)	230(57.50%)	0.001 ^{a*}
G	138(34.50%)	170(42.50%)	

The allelic frequencies are indicated in absolute values (percentage). ^aP values are determined by the Pearson chi-square test. *P<0.05.

Table 2: The baseline characteristics in T2DM patients with various *MTNR1B* rs10830963 genotypes before treatment with nateglinide (n=200)

Parameters	MTNR1B rs10830963 genotype			P value
	CC	CG	GG	
N(male/female)	70(40/30)	90(48/42)	40(23/17)	0.85 ^{aΔ}
Age (years)	47.81±10.82	48.01±12.04	47.09±13.92	0.921
BMI (kg/m ²)	26.41±3.24	25.43±3.31	26.59±4.11	0.104
WHR	0.92±0.06	0.91±0.06	0.92±0.08	0.552
FPG (mmol/L)	9.61±2.01	9.91±2.79	10.82±1.79	0.034*
PPG (mmol/L)	15.36±2.46	14.21±4.39	14.69±5.71	0.224
FINS (mU/L)	8.56±5.39	7.37±6.99	7.53±6.39	0.477
PINS (mU/L)	30.01±17.10	28.11±20.51	33.51±17.49	0.320
HOMA-IR	3.27±1.30	3.09±3.21	4.01±2.47	0.160
HbA _{1c} (%)	9.71±1.97	9.11±2.62	9.95±2.04	0.098
TG (mmol/L)	2.25±1.38	2.31±2.13	1.97±1.52	0.596
TC (mmol/L)	5.21±1.29	4.99±1.31	5.49±1.51	0.143
HDL-c (mmol/L)	1.28±0.95	1.33±0.59	1.41±0.29	0.645
LDL-c (mmol/L)	3.45±0.68	3.61±1.19	3.80±0.93	0.199

BMI = body mass index; WHR = waist to hip ratio; FPG = fasting plasma glucose; PPG = postprandial plasma glucose; FINS = fasting serum insulin; PINS = postprandial serum insulin; HOMA-IR = homeostasis model assessment for insulin resistance; HbA_{1c} = hemoglobin A_{1c}; TG = triglyceride; TC = total cholesterol; HDL-c = high-density lipoprotein-cholesterol; and LDL-c = low-density lipoprotein-cholesterol.

Data are given as (mean ± SD). P values represent the statistical difference between the three different genotypes assessed by one-way ANOVA. ^aP values are determined by the Pearson chi-square test. *P<0.05.

Table 3: Clinical characteristics of T2DM patients before and after nateglinide treatment (n=60)

Parameters	Before treatment	After treatment	P values
	n=60	n=60	
BMI (kg/m ²)	25.41±3.54	25.33±3.11	0.896
WHR	0.92±0.06	0.91±0.06	0.363
FPG (mmol/L)	10.41±1.23	7.25±1.21	0.000*
PPG (mmol/L)	14.56±2.76	9.38±2.61	0.000*
FINS (mU/L)	7.56±4.39	9.51±3.46	0.008*
PINS (mU/L)	30.31±17.10	47.21±15.16	0.000*
HOMA-IR	3.57±1.30	2.72±1.41	0.001*
HOMA-β	25.73±14.5	60.32±21.15	0.000*
HbA1c (%)	9.31±1.87	7.89±0.81	0.000*
TG (mmol/L)	2.25±1.38	1.86±1.23	0.105
TC (mmol/L)	5.21±1.29	4.31±1.09	0.000*
HDL-c (mmol/L)	1.38±0.75	1.45±0.59	0.571
LDL-c (mmol/L)	3.85±1.78	3.42±1.07	0.111

BMI = body mass index; WHR = waist to hip ratio; FPG = fasting plasma glucose; PPG = postprandial plasma glucose; FINS = fasting serum insulin; PINS = postprandial serum insulin; HOMA-IR = homeostasis model assessment for insulin resistance; HOMA-β= Homeostasis model assessment for islet β cell function; HbA1c = hemoglobin A1c; TG = triglyceride; TC = total cholesterol; HDL-c = high-density lipoprotein-cholesterol; and LDL-c = low-density lipoprotein-cholesterol.

Data are expressed as (mean ± SD). P values are determined by the Student's t-test. *P<0.05.

Table 4: Effects of different *MTNR1B* rs10830963 genotypes in T2DM patients on clinical traits determined before and after nateglinide treatment.

Parameters		CC(n=26)	CG(n=26)+GG(n=8)	P value
N(male/female)		26 (16/10)	34(20/14)	0.902 ^a
FPG (mmol/L)	Before	10.73±2.05	10.81±1.92	0.877
	After	6.98±1.35	7.94±1.23	0.006*
	DV	-3.75±1.68	-2.87±1.32	0.027*
PPG (mmol/L)	Before	14.51±4.31	13.04±4.72	0.220
	After	9.34±3.24	9.27±3.49	0.937
	DV	-5.17±4.08	-3.77±3.19	0.141
FINS (mU/L)	Before	8.82±6.81	7.80±4.20	0.478
	After	10.18±6.60	9.32±3.64	0.522
	DV	1.36±4.10	1.84±3.80	0.641
PINS (mU/L)	Before	31.42±20.47	27.47±16.43	0.410
	After	48.41±19.78	37.13±20.87	0.038*
	DV	16.99±17.82	9.66±19.37	0.138
HOMA-IR	Before	4.48±2.88	3.24±1.73	0.066
	After	3.39±2.12	2.43±1.18	0.072 c
	DV	-0.89±1.93	-0.79±1.31	0.704 c
HOMA-β	Before	27.75±16.03	24.08±17.32	0.405
	After	68.62±45.21	49.21±24.36	0.037*
	DV	40.87±23.52	25.13±19.21	0.006
HbA1c (%)	Before	9.79±1.86	9.15±2.26	0.246
	After	7.00±0.84	7.01±1.05	0.968
	DV	-2.79±1.53	-2.14±1.74	0.137
TG (mmol/L)	Before	2.46±1.96	2.31±1.90	0.766
	After	2.21±1.91	1.79±1.23	0.306
	DV	-0.25±1.95	-0.52±1.58	0.096
TC (mmol/L)	Before	5.27±1.69	5.22±1.15	0.892
	After	4.84±1.12	4.89±1.37	0.880
	DV	-0.43±1.53	-0.33±1.09	0.769
HDL-c (mmol/L)	Before	1.42±0.51	1.47±0.47	0.695
	After	1.35±0.55	1.43±0.51	0.563
	DV	-0.07±0.68	-0.04±0.46	0.839 c
LDL-c (mmol/L)	Before	3.28±1.19	3.18±1.05	0.731c
	After	3.19±1.20	3.11±1.07	0.786
	DV	-0.09±1.26	-0.07±1.08	0.948

BMI = body mass index; WHR = waist to hip ratio; FPG = fasting plasma glucose; PPG = postprandial plasma glucose; FINS = fasting serum insulin; PINS = postprandial serum insulin; HOMA-IR = homeostasis model assessment for insulin resistance; HOMA-β= Homeostasis model assessment for islet β cell function; HbA1c = hemoglobin A1c; TG = triglyceride; TC = total cholesterol; HDL-c = high-density lipoprotein-cholesterol; and LDL-c = low-density lipoprotein-cholesterol.

Data are given as (mean ± SD). P values represent the statistical difference between the three different genotypes assessed by one-way ANOVA. ^aP values are determined by the Pearson chi-square test. ^cP values are determined by the Kruskal-Wallis test. *P<0.05.

DV, differential values (post-administration minus pre-administration).

Figures

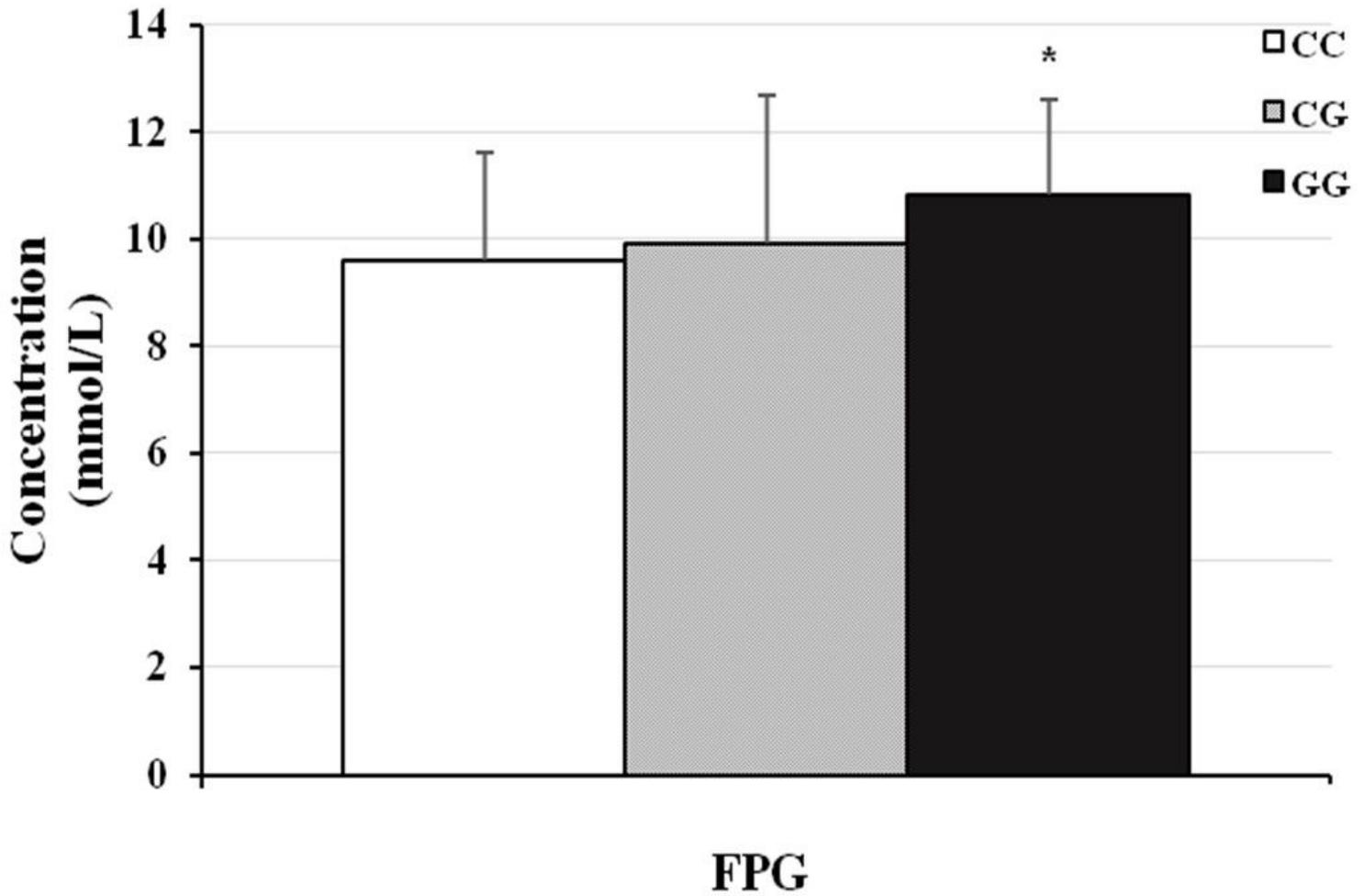


Figure 1

Baseline levels of FPG in T2DM patients with different MTNR1B rs10830963 genotypes. Data are expressed as (mean \pm SD). *P<0.05 compared with CC genotype group.

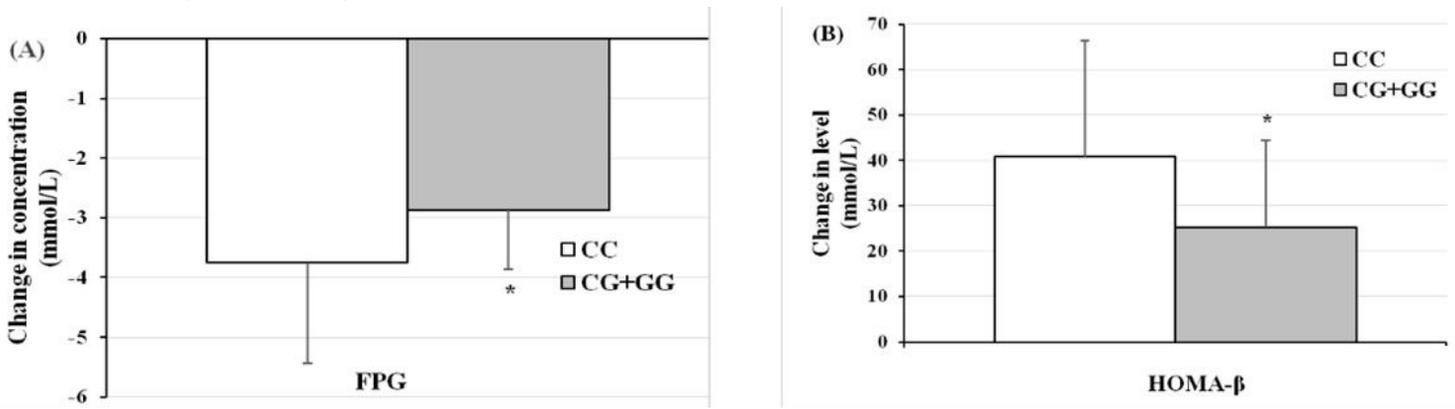


Figure 2

Comparisons of differential values (pre-administration levels subtracted from the post-administration levels) of FPG (A) and HOMA- β (B) among different MTNR1B rs10830963 genotypes in T2DM patients before and after treatment of nateglinide. Data are expressed as (mean \pm SD). *P<0.05 compared with CC genotype group respectively.